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The assessment of maternal haemodynamic profile via transthoracic bioreactance as a screening tool for the early prediction of preeclampsia (PE) and normotensive fetal growth restriction (FGR).

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“The assessment of maternal haemodynamic profile via transthoracic bioreactance as a screening tool for the early prediction of preeclampsia (PE) and normotensive fetal growth restriction (FGR).”

Volume 1/1

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Doctorate of Philosophy

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CANDIDATE THESIS DECLARATION

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of Doctor of Philosophy, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work.

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ABBREVIATIONS

2D	Two dimensional
a`	Late diastolic velocities of the LV lateral wall
AC	Abdominal Circumference
ACOG	American Congress of Obstetricians and Gynecology
AEK	Afif EL-Khuffash
AFP	Alpha fetoprotein
AJ	Adam James
AJOG	American Journal of Obstetrics and Gynaecology
AoCSA	Aortic cross sectional area
APJ	Apelin receptor
AUC	Area under curve
BMFMS	British Maternal Fetal Medicine Society
BMI	Body Mass Index
BP	Blood pressure
BPD	Biparietal diameter
BPD	Bronchopulmonary dysplasia
BPP	Biophysical profile
BPM	Beats per minute
BRT-CO	Bioreactance obtained cardiac output
BRT-HR	Bioreactance obtained heart rate
BRT-SV	Bioreactance obtained stroke volume
BSA	Body surface area
CE	Coefficient of error
CI	Confidence interval
CL	Confidence limits
cm	Centimetres
CM	Cathy Monteith
COI	Cardiac output index
CO	Cardiac output
CO2	Carbon dioxide
CP	Cardiac power,

CPAP	Continuous positive airway pressure
CPI	Cardiac power index
CPR	Cerebro-placental ratio
CRB	Colm R Breatnach
CS	Caesarean Section
CV	Coefficient of variation
CTG	Cardiotocograph
DA	Discriminant Analysis
DBP	Diastolic blood pressure
DIT	Dublin Institute of Technology
DM	Diabetes Mellitus
e`	Early diastolic velocities of the LV lateral wall
ECG	Electrocardiograph
ECHO	Echocardiography
ECHO-CO	Echocardiography obtained cardiac output
ECHO-HR	Echocardiography obtained heart rate
ECHO-SV	Echocardiography obtained stroke volume
ECLIA	Electro-chemiluminescence immunoassay
EF	Ejection fraction
EFW	Estimated fetal weight
EJOGRB	European Journal Obstetrics and Gynaecology & Reproductive Biology
ELISA	Enzyme Linked Immunosorbent Assay
EOPE	Early onset preeclampsia
EU	European Union
FAU	Fetal Assessment unit
FAC	Fractional area change
FBC	Full blood count
FGR	Fetal growth restriction
FHx	Family history
FL	Femur length
FR	Frame rate
FTOE	Fractional tissue oxygen extraction
FWV	Flow wave volume

GA	Gestational age
GDM	Gestational diabetes
GH	Gestational hypertension
GLS	Global longitudinal strain
GTT	Glucose tolerance test
HANDLE	HAEMODYNAMIC Assessment iN pregnancy and neonatal Echocardiography assessment
HC	Head circumference
HDU	High dependency unit
HIE	Hypoxic-ischaemic encephalopathy
HIPE	Hospital in-patient enquiry
HR	Heart rate
HR	Hazard ratio
HRB	Health research board
HSE	Health Service Executive
HTN	Hypertension
ICC	Intra-class correlation coefficient
INAB	Irish National Accreditation Board
IOG	Institute of Obstetricians and Gynaecologists
IOL	Induction of Labour
iPIMS	Integrated patient information management systems
ISSHP	International Society for the study of Hypertension in pregnancy
IQR	Interquartile range
IVH	Intraventricular haemorrhage
FHX	Family history
JOGS	Junior Obstetrics & Gynaecology Society
KE	Karl Egan
kHz	Kilohertz
kg	Kilogram
LA:Ao	Left Atrium : Aorta
LOA	Limits of agreement
LOS	Length of stay
LSCS	Lower segment caesarean section

LV	Left ventricle
LVEDD	Left ventricle end diastolic diameter
LVEDV	Left ventricular end-diastolic volume
LVTR	Left ventricle twist rate
LVUTR	Left ventricle untwist rate
µL	Microlitre
MAP	Mean airway pressure
MAP	Mean arterial pressure
MBP	Mean blood pressure
MCA	Middle cerebral artery
MCRG	Medical Charities Research Group
MHz	Megahertz
mg	Milligram
ml	Millilitre
MoM	Multiples of the median
MPV	Mean platelet volume
Multip	Multiparous
MV	Mitral valve
N/A	Not applicable
NEC	Necrotising enterocolitis
NICE	National Institute of Clinical Excellence
NICU	Neonatal intensive care
NICOM	Non invasive cardiac output monitor
nm	Nanometre
NND	Neonatal death
NO	Nitric oxide
NS	Not significant
Nullip	Nulliparous
OR	Odds ratio
PAPP-A	Pregnancy associated plasma protein A
PBS	Phosphate buffered saline
PDA	Patent ductus arteriosus
PE	Preeclampsia
pg/ml	picograms per millilitre

PI	Pulsatility index
PLGF	Placental growth factor
PPH	Postpartum haemorrhage
PSV	Peak systolic velocity
PVL	Periventricular leucomalacia
QC	Quality control
REC	Research Ethics Committee
RCOG	Royal College of Obstetricians and Gynaecologists
RCPI	Royal College of Physicians in Ireland
RCSI	Royal College of Surgeons in Ireland
ROC	Receiver Operating characteristic
ROI	Region of interest
RR	Relative risk
RV	Right ventricle
s'	Mitral valve annular systolic velocity
SBP	Systolic blood pressure
SD	Standard deviation
Sec	Second
SFH	Symphysio-fundal height
SGA	Small for gestational age
s-flt-1	Soluble fms-like tyrosine kinase 1
s-flt-1: PLGF	Soluble fms-like tyrosine kinase 1: Placental growth factor ratio
SMFM	Society of Maternal Fetal Medicine
SNRI	Serotonin norepinephrine reuptake inhibitor
SOAP	Society for Obstetric Anaesthesia and Perinatology
STE	Speckle tracking echocardiography
SV	Stroke volume
SVD	Spontaneous vaginal delivery
SVI	Stroke volume index
SVV	Stroke volume variation
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler imaging
TFC	Thoracic fluid content

TGF	Transforming growth factor
TORCH	Toxoplasmosis, Rubella, CMV, Herpes
TPA	Tissue plasminogen activator
TPR	Total peripheral resistance
TPRI	Total peripheral resistance index
TV	Tricuspid valve
TTE	Transthoracic echocardiography
UA	Umbilical artery
UK	United Kingdom
US	Ultrasound
USCOM	Ultrasound cardiac output monitor
USPSTF	US preventative strategies task force
UtPI	Uterine artery pulsatility index
VEGF	Vascular endothelial growth factor
VTI	Velocity Time Index
WHO	World Health Organisation

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THESIS SUMMARY

Preeclampsia (PE) and Fetal Growth Restriction (FGR) are common complications of pregnancy. Data presented in this thesis, which mainly originate from the single centre prospective **HAEMODYNAMIC Assessment iN pregnancy and neonatal Echocardiography assessment (HANDLE)** study, consolidate knowledge of the haemodynamics of normal pregnancy and the changes and predictive capability in those affected by PE & FGR.

The HANDLE study recruited 422 low risk nulliparous women at their first antenatal visit. Following exclusion and patient self-withdrawal a total of 366 women completed the study; 1.6% (n=6) had a pregnancy complicated by PE; 4.9% (n=18) by gestational hypertension (GH) and 6.6% (n=24) by FGR.

The objective of the primary analyses of this study was to assess the haemodynamics of pregnancy and the postpartum using NICOM® and the ability of these profiles to predict disease states. Presented in this thesis I have detailed four different haemodynamic profiles in pregnancy. In pregnancies complicated by preeclampsia, HR and SVi when combined with BP became statistically significant predictors at 14 weeks' gestation (AUC=0.75, p=0.01 and AUC=0.77, p=0.009 respectively). In the postpartum, comparison between non-pregnant controls and those with GH demonstrated persistence of elevated DBP (p=0.01), TPRi (p=0.01) and lower SVi (p=0.03).

Secondary analyses were to validate the use of NICOM® in the obstetric population and correlation of biomarkers to haemodynamic variables. In keeping with findings in the adult and neonatal population I have shown that NICOM® is an acceptable alternative method of CO measurement in pregnant women. Evaluation of the biomarkers and haemodynamics at 14 weeks' showed a weak positive correlation between Apelin 13 and SV ($r=0.29$; $p=0.005$) and a weak inverse correlation with TPRi ($r=-0.29$; $p=0.004$).

In conclusion, uteroplacental disease is a multifactorial complication of pregnancy. This thesis further highlights the need for the development of prediction models especially in the case of FGR.

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Dedicated to My family, my husband Ian and my two boys-
Bobby and Ollie

In memory of Bobby Campbell

PUBLICATIONS AND PRESENTATIONS

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1. C Monteith, S Mullers, J Unterscheider, K Flood, F Breathnach, S Daly, MP Geary, MM Kennelly, FM McAuliffe, K O'Donoghue, A Hunter, JJ Morrison, G Burke, P Dicker, EC Tully, FD Malone **Is a normalizing Cerebro-Placental Ratio (CPR) a potential predictor for adverse outcome in Intrauterine Growth Restriction: results of the multicenter PORTO Study.** American Journal of Obstetrics and Gynecology November 2016; Doi:10.1016/j.ajog2016.11.1008

2. Colm R. Breatnach; Eva Forman; Adrienne Foran; Cathy Monteith; Lisa McSweeney; Fergal D. Malone; Naomi McCallion; Orla Franklin; Afif EL-Khuffash. **Left Ventricular Rotational Mechanics in Infants with Hypoxic Ischaemic Encephalopathy and Preterm Infants at 36 Weeks Post Menstrual Age: a Comparison with Healthy Term Controls** Echocardiography December 2016. Doi : 10.1111/echo.13421

3. Anne Doherty, Afif EL-Khuffash, Cathy Monteith, Lisa McSweeney, Colm R. Breatnach, Etaoin Kent, Elizabeth C. Tully, Fergal D. Malone, Patrick Thornton **Non-Invasive Cardiac Output Monitoring and Myocardial Function Assessment in Nulliparous Women: A Comparison between Two Methods.** British Journal of Anaesthesia April 2017; Doi: 10.1093/bja/aex045

4. C Monteith, L McSweeney, CR Breathnach, A Doherty, L Shirren, E Tully, P Dicker, F Malone, A EL-Khuffash, E Kent. **Non-Invasive Cardiac Output Monitoring (NICOM®) can predict the evolution of uteroplacental disease – Results of the Prospective HANDLE Study** –EJOGRB July 2017, DOI: 10.1016/j.ejogrb.2017.07.018

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3. C Monteith, R Horgan, L McSweeney, A EL-Khuffash, FD Malone, E Kent. **A 13-year review of the falling prevalence of Preeclampsia in a large tertiary maternity unit in Ireland.** The Journal of maternal-fetal and Neonatal medicine.

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Oral Presentations

1. JOGS Spring meeting Travel Bursary awards 22nd April 2016
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Cathy Monteith

3. INFANT Research study day, Cork 9th September 2016
Postnatal Breastfeeding rates in low risk Nulliparous women in a tertiary hospital. Lisa McSweeney

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Pregnancies complicated by Hypertensive Disease demonstrate a high resistance vasculature and blunted adaptation in the postpartum period – Results of the prospective HANDLE Study. &

Abnormal haemodynamic parameters in pregnancy complicated by uteroplacental disease obtained by Non-Invasive cardiac output monitor (NICOM)- results of the prospective HANDLE study.

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1. SOAP 2016

2. SMFM 2017, Caesar's Palace, Las Vegas, 23-28th January 2017

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Left Ventricular Rotational Mechanics in Infants with Hypoxic Ischaemic Encephalopathy and Preterm Infants at 36 weeks Post Menstrual Age: A Comparison with Healthy Term Controls
Colm R. Breathnach ; Eva Forman ; Adrienne Foran ; Cathy Monteith; Fergal Malone; Naomi McCallion; Orla Franklin; Afif EL-Khuffash.

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Abnormal hemodynamic parameters in pregnancy complicated by utero-placental disease obtained by Non-Invasive Cardiac Output Monitoring (NICOM) – Results of the Prospective HANDLE Study C Monteith, L McSweeney, L Shirren, A Doherty, CR Breathnach, E Tully, P Dicker, F Malone, A EL-Khuffash, E Kent.

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An 11 year review of the changing prevalence of Pre-eclampsia in the East of Ireland. R Horgan, C Monteith, L McSweeney, E Kent

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Postnatal Breastfeeding rates in low risk Nulliparous women in a tertiary hospital. L McSweeney, C Monteith, C R Breatnach, A James, P Dicker, A El-Khuffash, E Kent

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A comparison of different formulations of Prostaglandin given during induction of labour in a low risk Nulliparous population
L McSweeney, C Monteith, P Dicker, FD Malone, A El-Khuffash, E Kent

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Non-invasive Cardiac Output Monitoring (NICOM) – A Novel Technique for Measurement of Cardiovascular Status in Healthy Nulliparous Pregnancy. C Monteith, L McSweeney, L Shirren, A Doherty, CR Breatnach, E Tully, P Dicker, F Malone, A EL-Khuffash, E Kent.

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Pregnancies complicated by Hypertensive Disease demonstrate a high resistance vasculature and blunted adaptation in the postpartum period – Results of the prospective HANDLE Study. C Monteith, L McSweeney, CR Breatnach, A James, P Dicker, F Malone, A EL-Khuffash, E Kent.

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First trimester prediction of uteroplacental disease- results of the prospective HANDLE study. Cathy Monteith, Lisa McSweeney, Colm R. Breatnach, Lucy Shirren, Pat Dicker, Elizabeth C. Tully, Fergal D. Malone, Etaoin Kent, Afif EL-Khuffash, Anne Doherty.

Chapter 1 INTRODUCTION

1.1 Background of hypertensive disorders and fetal growth restriction in pregnancy.

Preeclampsia (PE) and fetal growth restriction (FGR) account for a significant proportion of perinatal and maternal morbidity and mortality currently encountered in obstetric practice. PE is thought to complicate up to 10% of all pregnancies world-wide.(1) PE remains one of the leading direct causes of maternal death and has a recently reported maternal mortality rate of 0.25/100,000 pregnancies (95% CI 0.09 -0.55/ 100,000).(2) One primary goal of antenatal care is the early recognition of such conditions to allow treatment and optimization of both maternal and fetal outcomes. This can prove challenging for obstetricians as the clinical manifestation of disease can often appear abruptly and late in the course of the disease.

Both PE and FGR are most commonly diagnosed in the low risk nulliparous population.(3, 4) Consequently research has focused on factors, which may identify those women who are at an increased risk of PE and FGR. The National Institute of Clinical Excellence (NICE) in the UK has prioritised the need for further research to identify those at risk PE.(1) To date, this has involved serum biomarkers and maternal uterine artery velocimetries which have predictive capabilities but, have not been adopted into routine practice.(5) Better identification of those women at an increased risk for PE and FGR would enable tailoring their antenatal care with increased surveillance to potentially allow earlier detection of these conditions.

Management of pregnancies complicated by PE and/or FGR remains one of the greatest challenges in obstetrics. The morbidity, both maternal and fetal, associated with these conditions is

considerable. (6) Provision of antenatal care is centred on regular maternal and fetal surveillance in order to enable timely identification of these complications. Frequently, however, clinical evidence of underlying uteroplacental dysfunction only emerges at a late stage in the disease process. With advanced disease the only therapeutic intervention is delivery of the fetus and placenta. Prolonging the pregnancy is associated with the risk of increasing maternal morbidity, maternal mortality and in utero fetal demise.(7) However, early delivery is associated with an increased risk of perinatal mortality and lifelong neurodevelopmental complications for the fetus. Current guidelines recommend expectant management for PE if both mother and fetus remain stable until 34 weeks. However, the commonly used criteria for severity (including hypertension and proteinuria) correlate poorly with maternal and fetal outcome.(8)

Traditionally, pregnant populations are screened for the known clinical risk factors, which increased their relative risk of developing PE. The World Health Organisation (WHO) recommends that all pregnant women should have a blood pressure (BP) and urine screened at each antenatal visit in an effort to allow early detection of PE.(9) Identifying mothers at risk of developing PE early in their pregnancy, prior to the emergence of clinical disease, has several advantages. It would allow tailored antenatal care with patients engaging in intensive antenatal surveillance of both maternal and fetal well-being and additionally would allow for preventative strategies such as the administration of low dose aspirin before 16 weeks' gestation when it demonstrates greatest success.(10-12)

The challenge in the identification of this high-risk cohort of patients is to identify a screening tool that is effective, reproducible and safe with good patient acceptability. It must be a test that is easy to perform and therefore can be employed on a large scale. Non-invasive assessment of maternal haemodynamics represents

a novel screening strategy to the evaluation of maternal cardiovascular parameters during pregnancy. Profiling the changes that occur in these parameters across pregnancy gives the potential to identify a unique haemodynamic profile associated with underlying uteroplacental dysfunction. If this hypothesis is proven, the potential exists to use this test on a global scale in pregnancy.

1.2 Aetiology and Pathophysiology of hypertensive disorders in pregnancy.

The Institute of Obstetricians and Gynaecologists (IOG), Royal College of Physicians of Ireland (RCPI) and the International Society for the Study of Hypertension in Pregnancy (ISSHP) defines a chronic or pre-existing hypertension as that which predates the pregnancy or appears before 20 weeks gestation. A diagnosis of gestational hypertension (GH) in pregnancy is defined as a systolic blood pressure greater or equal to 140mmHg, or a diastolic blood pressure of greater than or equal to 90mmHg. This should be on the basis of at least two measurements, taken in the same arm with appropriately sized blood pressure cuff, several hours apart. The diagnosis of preeclampsia (PE) is made when there is maternal hypertension and the presence of clinically significant proteinuria. The upper limit of a normal 24 hour urine protein excretion is 0.3g/24 hours (based on a confidence interval of 95% for urinary protein in pregnancy). (13, 14) The timing of the onset of preeclampsia can also impact on both maternal and fetal outcomes. Early onset preeclampsia has previously demonstrated an increased risk of maternal mortality than late onset and additionally has been identified as a contributing factor for the development of FGR.(3)

1.2.1 Fetal factors

Multiple pregnancies are associated with increased risk of complication and adverse perinatal outcome. Higher order pregnancies and molar pregnancies typically have a larger placental mass and a reduction trophoblast perfusion allowing for an increased potential for development of PE.(4, 15, 16) In addition to a larger placental mass molar pregnancies have demonstrated increased levels of vascular endothelial growth factor (VEGF), a biomarker linked to the development of PE.(17) In cases of fetal structural abnormalities such as male genitalia malformations and pregnancies classified as “multiple congenital abnormalities” where there is decreased perfusion of the trophoblast there is also a positive correlation with PE.(18)

There have been some small case series assessing the link between preeclampsia and trisomy 13.(19-22) In the UK, Tuohy et al identified 25 women who delivered an infant affected by trisomy 13 between 1971 and 1989. Through this study he demonstrated a greater incidence of preeclampsia in the trisomy 13 group when compared with both trisomy 18 infants and normal controls. They hypothesized that an alteration in genetic coding of trisomy 13 infants resulted in the increased risk of the development of preeclampsia.(23) Interestingly the gene responsible for soluble-fms like tyrosine-1 (s-flt-1) is carried on chromosome 13q 12 and as expected trisomy 13 fetuses have a higher concentration of circulating s-flt-1 than their normal karyotype counterparts.(24, 25)

1.2.2 Maternal factors

There have been many observational studies assessing the risk factors associated with the development of PE. Many of these risk factors are non-modifiable such as nulliparity,(4, 26-28) maternal

extremes of age and also paternal age.(29, 30) Maternal ethnicity in particular non-white mothers such as those of African American, Pacific islands or Filipino origin demonstrate an increased risk of PE.(3, 31, 32) There is evidence that a long inter-pregnancy interval or a pregnancy with a new partner are at increased risk of PE.(33, 34) In addition women with a positive personal or family history of hypertensive disease in pregnancy are at risk of recurrence.(35) Assisted reproductive techniques, especially those with donor sperm are associated with increased risk of PE. However, multiple assisted reproductive cycles have been shown to be protective.(27, 36-38)

Modifiable clinical risk factors include maternal weight, obesity, a body mass index (BMI) >25,(4, 36, 39-41) endothelial dysfunction,(42-45) dyslipidaemia,(41) medications such as tricyclic or Serotonin- norepinephrine reuptake inhibitor (SNRI) anti-depressants or prolonged use of oral contraceptives correlate to an increased risk of PE.(46, 47) Pre-existing medical conditions have been demonstrated to increase the risk of hypertensive disease in pregnancy. These include renal disease,(35, 48) anti-phospholipid syndrome,(49, 50) vascular and connective tissue disease(51) and diabetes.(52-55) The positive correlation between these conditions and PE has led to the suggestion that the development of PE is of immunological origin and subsequent investigation of complement activation.(56-59)

1.2.3 Environmental factors

Both the external and in utero environment have an impact on the development of PE. Preeclampsia is one of the most common complications of pregnancy in women who are living at high altitude with reported rates of 16%.(60) The aetiology is unclear but is likely secondary to the chronic hypoxia and resultant impaired

placentation. In addition seasonal variations of temperature and humidity have been suggested to influence the incidence of PE but this has not been confirmed.(61) Environmental exposures such as moderate alcohol consumption and in-utero exposure to Diethylstilbestrol are also linked to increased risk of PE.(62, 63) In direct contrast to the usual positive correlation between smoking and disease states, a maternal smoking history is protective against the development of PE.(4)

1.2.4 Placental factors

Preeclampsia has long been described as a disease of placental hypoperfusion with disease resolution following delivery of the placenta. The two-stage model of PE proposes that stage one is the placental hypoperfusion secondary to failure of remodelling of the maternal vessels such as the spiral arteries occupying the intervillous space.(64, 65) This hypoperfusion is not sufficient alone to cause Stage 2 (maternal disease syndrome) but its development is dependent on other factors such as maternal, fetal and environmental as detailed in section 1.2.1- 1.2.3.(66, 67) Structural factors such as a placental mass <10th centile is associated with an increased risk of both preterm and term PE.(68) Similarly an anomalous cord insertion e.g. velamentous cord insertion is a risk factor for term but not preterm PE.(69) Histological findings such as maternal vasculopathy is associated with hypertensive disease in pregnancy. However, disease correlation with this histological finding is reported as low as one in five.(70)

1.2.5 Treatment and prevention of preeclampsia

The mainstay of treatment of preeclampsia is early recognition and prevention of progression to eclampsia and end organ disease.

However, the definitive treatment is delivery of the fetus and the placenta. As a result potential treatment plans involve weighing up the risks of iatrogenic preterm delivery and potential consequences to the fetus versus prolongation of pregnancy and the potential associated maternal morbidity. The aim of treatment is to achieve blood pressure control to allow for advancement of gestation closer to term. This is usually achieved via mono- or polypharmacy with the use of Labetalol (β -blocker), Nifedipine (calcium channel blocker) or Methyldopa (dopamine antagonist). In instances of fulminating PE or eclampsia the use of parenteral Labetalol (β -blocker) or Hydralazine (a peripheral arterial vasodilator) may be required to obtain blood pressure control prior to emergent delivery.(14) The addition of parenteral magnesium sulphate has the additive benefit of halving the number of fulminating PE that progress to eclampsia.(71, 72) The risk of eclampsia does not always dissipate following delivery with one third of seizures occurring postpartum. Continued anti-hypertensive treatment should continue if required in the postpartum.(73)

A benefit of first trimester screening for patients at increased risk of PE is that they are identified at a gestational age where preventative measures are possible. This can be achieved by optimization of modifiable risk factors e.g. reducing maternal BMI or changes to pharmacological management of dyslipidaemia or psychological disease.

In addition to the above measures there is also the potential for the use of anti-thrombotic or antiplatelet medications in women who are deemed to be at high risk of PE. A recurring placental pathology in the setting of PE is placental villous infarction secondary to thromboembolic occlusive disease of the spiral arteries. The use of anti-thrombotic medication is employed on the

assumption that reducing the risk of spiral artery occlusion will consequently reduce the risk of PE.(74)

There has been conflicting evidence regarding the benefit of prophylactic administration of aspirin in pregnancy. However, the evidence supporting the use of antithrombotic agents in the first trimester of women who are increased risk of uteroplacental disease is expanding.(11, 75-82) The largest trial to date, from the CLASP group involved more than 9000 women at high risk for uteroplacental disease. Their study demonstrated no difference in incidence of uteroplacental disease with either aspirin 60mg or placebo.(83) A meta-analysis by Askie et al in 2007 involved 31 studies and 32,000 participants showed reduced incidences of uteroplacental disease secondary to the use of antiplatelet agents.(84) More recently Rolnik et al published their findings in the New England Journal of Medicine detailing the Fetal Medicine Foundation algorithm for the prediction of PE was superior to NICE screening and that use of 150mg aspirin vs placebo in 1700 identified high risk women resulted in reduced incidence of PE.(85, 86)

As a result of these positive findings both the US Preventative Strategies Task Force (USPSTF) and the American College of Obstetricians and Gynecologists advocate the use of aspirin for the prevention of recurrent PE.(87) This is consistent with the NICE Guideline Quality standard for antenatal care (QS22) that women at high risk of PE should be offered aspirin between 12- 36 weeks' gestation. In contrast, recent publications from Werner et al and the TEST group are advocating for universal prescribing of aspirin in the pregnant population as the universal approach appears to be more cost-effective and has additional gains relative to reductions in overall preterm delivery and life years gained.(88, 89)

1.2.6 Long-term maternal consequences of preeclampsia

There is a growing body of evidence supporting PE as an independent risk factor for the development of cardiovascular disease in later life.(90) Data from the Central Statistics Office in Ireland show that the leading cause of death in women is from vascular occlusive disease.(91) Women whose pregnancies have been complicated by PE are at an increased risk of death from myocardial infarction with an increased hazard ratio (HR) 2.16 – 2.22 as early as 11 years after the index pregnancy. Similar increases were observed with regard to death by cerebrovascular accident with HR 1.81 – 1.92 after 10 years from the index pregnancy.(92, 93) This mirrors results of the Lancet study by Ray et al who demonstrated an average age of only 38 years at the time of first cardiac event in women with previous uteroplacental disease.(94)

This increased risk demonstrates a dose-response effect with increasing severity of hypertensive disease translating to increasing cardiovascular disease risk. There is a progressive increase in risk of cardiovascular disease, which mirrors the progression from gestational hypertension to mild PE to moderate PE to severe PE. Furthermore, there is an additive effect if concomitant FGR and problems such as intra uterine demise, preterm birth or cardiac risk factors such as tobacco use or metabolic syndrome are involved.(94-96)

It is well established that women with pregnancies complicated by PE are at increased risk of chronic hypertension with a reported relative risk (RR) of 3.70 after 14 years.(92, 97, 98) The PE cohort has higher rates of insulin resistance and progression to Diabetes Mellitus with a RR of 3.97 in comparison to 2.21 in uncomplicated pregnancies.(99) This increased risk of Diabetes remains after

adjusting for pregnancies with coexisting gestational diabetes and results in a risk twice that of unaffected pregnancies with diagnosis usually occurring by eight years following the affected pregnancy.(100) With the individual increased risk of chronic hypertension and impaired glucose tolerance it is not surprising that PE is also associated with increased rates up to nine fold of Metabolic syndrome.(101, 102)

The underlying haemodynamic aberrations in this cohort of patients may be a factor influencing their long-term risk. Assessing the maternal haemodynamic profile in the postnatal period will enable us to study whether or not haemodynamic changes identified during pregnancy persist following delivery. This may further our understanding of the enhanced cardiovascular risk seen in these women.

1.2.7 Paediatric consequences of hypertensive disease

Abnormalities in utero-placental function during pregnancy result in maternal and fetal complications including preeclampsia, (PE), gestational hypertension (GH) and fetal growth restriction (FGR). These pathological processes increase neonatal morbidity and mortality as they frequently lead to delivery at preterm gestational ages. In addition, an abnormal intrauterine environment can have a direct effect on the cardiovascular wellbeing of infants in the immediate postnatal period.(103) Maternal PE results in increased natriuretic peptide and homocysteine in neonates (suggesting abnormal myocardial function),(104) and children born to mothers with PE are also noted to have altered cardiac profiles compared with controls when assessed with echocardiography at 5-8 years of age.(105) In addition, FGR infants demonstrate altered cardiac morphology and reduced myocardial function over the first week of life.(106)

Studies on the effect of GH, and maternal antihypertensive therapy on myocardial performance in neonates in the immediate postnatal period are lacking. Drugs commonly used to treat GH include labetalol and nifedipine. Beta receptor and calcium channel blockade in the mother may have an impact on cardiac performance in the neonate and studies have demonstrated neonatal effects of antenatal labetalol exposure including hypoglycaemia, bradycardia and altered cerebral auto-regulation.(107, 108) However, the myocardial effects of such exposure remain unexplored. With the emergence of new echocardiography modalities including deformation and rotational characteristics of the left ventricle (LV), and right ventricle (RV) specific measurements, detailed characterisation of myocardial performance in various disease states and physiological milieus is now possible. (109, 110) Those measurements are feasible in the neonatal population and demonstrate excellent intra- and inter-rate reproducibility. (111, 112) The newer measures of myocardial performance have the ability to detect subtle preclinical cardiac dysfunction. (113, 114)

In addition to the immediate neonatal cardiac implications, a maternal diagnosis of uteroplacental disease is associated with an increased risk in a range of diagnoses in adulthood. Adults born from a pregnancy affected by uteroplacental disease have increased rates of adult hypertension, dyslipidaemia, metabolic syndrome and increased mortality from cardiovascular diseases such as ischaemic cardiac disease and stroke. (103, 115-119)

1.3 Aetiology & pathophysiology of fetal growth restriction

The Institute of Obstetricians and Gynaecologists (IOG), Royal College of Physicians of Ireland (RCPI) and the Royal College of Obstetricians and Gynaecology (RCOG) defines fetal growth restriction (FGR) as an abdominal circumference (AC) or estimated

fetal weight (EFW) less than the 10th centile for gestational age. (120, 121) As a result FGR is expected to affect approximately 10% of obstetric populations with increasing levels of clinical concern when the EFW is less than the 5th centile or there is additional evidence of poor placental function e.g. reduced amniotic fluid indices, abnormal uterine artery Doppler indices and evidence of fetal brain sparing.(122-125)

Fetal growth restriction accounts for a significant proportion of perinatal morbidity and mortality currently encountered in obstetric practice.(126) The primary goal of antenatal care is the early recognition of such conditions, in order to allow treatment and optimization of both maternal and fetal outcomes. Management of pregnancies complicated by FGR remains one of the greatest challenges in obstetrics. The morbidity, both maternal and fetal, associated with FGR is considerable.(126-131) However, the antenatal detection of FGR via clinical examination is suboptimal with a reported detection rate of one in three.(132-134) As a result many pregnancies complicated by FGR remain undetected and this translates to a greater than an eight-fold increased risk of stillbirth when compared to normal controls 19.8 vs. 2.4 /1000.(135)

1.3.1 Fetal factors

Where there is suspicion of FGR a methodical evaluation of the fetal anatomy is important when determining the underlying cause. A diagnosis of FGR is often present in association with fetal structural abnormalities, polyhydramnios, fetal infection (TORCH) or fetal chromosomal abnormality.(136-142) Table 1.1 further details fetal conditions with an increased risk of FGR. Multiple gestations are also at greater risk of FGR with either disparity of fetal growth or progression to twin – to – twin transfusion in cases of monochorionic diamniotic twin gestations.

Table 1-1 Fetal risk factors for FGR.(136-142)

Chromosomal

Trisomy 21

Trisomy 18

Trisomy 13

Achondroplasia

Chromosomal deletions

Parental disomy

Confined placental mosaicism

Structural malformations

Anencephaly

Omphalocele

Gastroschisis

Congenital diaphragmatic hernia

Renal agenesis

Cardiac malformations

Fetal infection

Toxoplasmosis

Rubella

Cytomegalovirus

Herpes

Syphilis

Recent media reports have highlighted the disparity in Ireland's maternity services in relation to universal access to a routine anatomical survey. If such cases of FGR are identified in units where a routine anatomical survey is not performed, they should have a tertiary referral to out rule an underlying structural or chromosomal cause.

1.3.2 Maternal factors

There are many maternal/ parental factors, which can have a negative effect on the fetal growth potential. When addressing these risk factors it is important to look at both those which are modifiable, whereby early intervention may offer the possibility of risk reduction and non-modifiable risk factors. Some non-modifiable factors including maternal demographics and medical history are detailed further in Table 1.2. (143-148) In addition to the parental physical and medical attributes factors such as lower income, lower social economic group and fewer years in education are risk factors for FGR.(144-146) Interestingly the family environment can also influence the fetal growth potential as Sundquist et al demonstrated that those who lived in deprived neighbourhoods, even after adjustment for social economic grouping were at an increased risk of FGR.(149)

Modifiable maternal risk factors for FGR include a low maternal body mass index (BMI); and a rise in BMI with subsequent pregnancies has been reported to lower the subsequent FGR risk.(144, 147, 150) Women who reported low activity levels in pregnancy are at a greater risk of a pregnancy complicated by FGR.(151, 152) In a similar trend, improving a suboptimal maternal diet may have a role to play in reducing the risk of FGR. Women who reported a high first trimester fatty acid intake, low folic acid intake, low intake of vitamin D and diets high in caffeine have been reported to demonstrate an increased risk of FGR.(153-157) Maternal substance use/abuse from tobacco or illegal drugs such as cocaine are also independent risk factors for the development of FGR.(146, 147, 158)

Table 1-2 Non-modifiable risk factors.(136, 138, 145, 147, 149, 154, 159-161)

Maternal characteristics

Extremes of age

Nulliparity

Short stature

Ethnicity, in particular Asian

Paternal characteristics

Short stature

Paternal history of FGR infant

New partner

Maternal History

Malaria

Antiphospholipid syndrome

Lupus

HIV

Hypertension

Renal Disease (glomerulonephritis/ renal transplant/ chronic renal failure)

Maternal history of being an FGR infant

Obstetric History

Placental abruption

Preeclampsia

Gestational hypertension

Previous FGR

Stillbirth

Short inter-pregnancy interval

Long inter-pregnancy interval

1.3.3 Placental factors

Macroscopic findings such as small placenta volume, placental abruption, circumvallate placenta demonstrated as early as the first trimester are associated with suboptimal fetal and placental growth.(162-164) The presence of an anomalous cord insertion such as a marginal or velamentous cord insertion is associated with an increased risk of having a small placenta and FGR.(69) A small placenta in combination with maternal serum alpha fetoprotein and pregnancy associated plasma protein A (PAPP-A) has been shown to identify women at high risk of FGR.(165) Toal et al also described a high proportion of pregnancies with evidence of placental dysfunction (defined as combinations of abnormal pregnancy screening tests, abnormal uterine artery Doppler indices and small placenta) were subsequently found to have evidence of placental infarction following delivery.(74) Microscopic placental lesions of decidual vasculopathy or chronic inflammation are common in cases of both normotensive and hypertensive FGR.(166) Histological studies have described abnormalities of the maternal spiral arterioles, dysregulated villous vasculogenesis and fibrin deposition within the placentae of FGR pregnancies.(167, 168)

1.3.4 Environmental factors

The clinical significance of high-altitude hypoxia for pregnancy outcome is best characterised by the threefold greater incidence of fetal growth restriction (FGR) at high (≥ 2500 m) altitude in comparison to low altitude.(169-172) A study by Jensen et al demonstrated that high altitude reduces birth weight by an average of 120 g per 1000 m elevation gain.(170) Other environmental exposures, which have a negative effect on fetal growth include cigarette smoking, substance misuse and teratogenic drug

exposure. The prevalence of cigarette smoking in Ireland is highest in young adults of childbearing age at 27.3% of 25 -34 year olds.(173) This represents an important modifiable risk factor whereby intervention in smoking cessation either in the primary care setting or in antenatal clinic is shown to have a positive impact on fetal growth.(174) A prospective study by Bernstein et al, recruited 160 women assessing the impact of smoking on fetal growth. They demonstrated that smoking in the third trimester had a significant negative effect on fetal growth but that smoking reduction or cessation in pregnancy had a positive impact on fetal growth.(175)

1.3.5 Treatment and prevention of FGR

An accurate first trimester dating scan is key to detection as this provides a baseline for comparison with expected fetal growth. Currently in Ireland there is no universal schedule in the sonographic assessment of both fetal growth or anatomical assessment. For the detection of FGR a first trimester dating scan followed by anatomical survey and a further third trimester biometry is felt to be the optimal management for screening. Currently routine third trimester biometry for fetal growth assessment is not provided and instead relies on recognition by measurement of symphysiofundal height (SFH), which is known to be suboptimal.(132-134) Further evaluation by ultrasound is indicated when a suspicion of FGR has been raised from the clinical examination. However, measurement of fetal biometry can be influenced by ultrasound equipment, sonographer training, sonographer experience and technically challenging assessments such as in the setting of increased maternal adiposity. The poor antenatal detection leaves a deficit, resulting in the need for development of prediction tools for FGR.

Frequently, clinical evidence of underlying uteroplacental dysfunction may only emerge at a late stage in the disease process and with advanced disease the only therapeutic intervention is delivery of the fetus and placenta. Prolonging the pregnancy is associated with the increasing risk of in utero fetal demise. Gardosi et al demonstrated that in the setting of diagnosed FGR patients were delivered on average 10 days earlier and this resulted in a reduction in the overall stillbirth rate.(135)

At present there are no specific interventions shown to improve fetal growth. Optimization of maternal health and the in utero environment through smoking cessation, nutritional advice and pregnancy health education have had modest improvements.(150, 175) There is current research being undertaken by the international STRIDER group. This group are investigating the use of sildenafil, a vasodilator, which acts by relaxation of arterial smooth muscle, which can improve blood flow.(176) The STRIDER group hypothesises that the use of sildenafil in pregnancies with a diagnosis of early-onset extreme FGR may result in prolongation of the pregnancy secondary to improved placental blood flow and as a direct consequence improved fetal growth. However, results from this trial are not expected until after 2020.(177)

The EVERREST group in the UK, are currently undertaking two large trials in the area of early-onset extreme FGR. The Clinical trial arm was commenced in 2014 and is investigating the use of directed gene therapy to the uterine arteries with the aim of increasing maternal VEGF and ultimately increasing uterine blood flow and fetal growth. This clinical trial is due to complete the recruitment phase in early 2019 with results likely also available in 2020. In addition the EVERREST group have launched a large European multicentre prospective study. Over the next 6 years this prospective study aims to better delineate the natural history of

early-onset extreme FGR and attempt to identify a biomarker which can predict adverse fetal outcome.(178)

1.3.6 Long term sequelae of FGR

In addition to the immediate neonatal morbidity often secondary to iatrogenic preterm delivery, infants affected by FGR have been shown to have lasting effects throughout childhood and into adult life. The use of the cerebroplacental ratio (CPR) in assessing the “at-risk” fetus is gaining interest.(122, 125, 179, 180) With respect to the antenatal management of FGR pregnancies, an abnormal CPR (value <1.0) demonstrates “brain-sparing” which is associated with adverse perinatal outcome. There has been an increase in the reporting of long-term neurological sequelae in the FGR fetus.(181) Leitner et al previously found that FGR children were significantly delayed in growth parameters and neurocognitive performance at 9-10 years.(182) Meher et al have also proposed a hypothesis of neurological injury occurring prior to an abnormal CPR as a response to the altered fetal haemodynamic adaption to hypoxia.(129) Their review also suggested that an abnormal MCA Doppler may in fact be a late event in the overall fetal brain redistribution of blood flow. The TRUFFLE group have also demonstrated that cerebral redistribution has a strong association with neurodevelopmental impairment at two years of age.(183, 184) The PANDA study group, a three year follow-on to the Perinatal Ireland PORTO study are also due to publish their neurodevelopmental findings later this year.

Infants affected by FGR demonstrate altered feeding behaviours, which are believed to be secondary to altered programming and persist through development and into adult life.(185-187) Some of the sequelae following a diagnosis of FGR include short stature, premature adrenarche and polycystic ovarian syndrome.(188) A

diagnosis of obesity is more prevalent in this population and a consequence of which is a greater risk in adulthood of diseases such as metabolic disease, diabetes mellitus and cardiovascular disease. (189) As in preeclampsia (another uteroplacental disease), infants affected by FGR have evidence of fetal cardiovascular programming, which remains in early childhood. This is evident even when prenatal severity predictors are reassuring and the neonatal outcome has been normal. This primary cardiovascular programming has been postulated as a cause for the increased cardiovascular mortality seen in adults of FGR affected pregnancies.(128)

1.4 Maternal haemodynamic adaption to normal pregnancy

The physiological changes associated with pregnancy begin to be apparent from as early as 6 gestational weeks (190) when there is peripheral vasodilation, secondary to progesterone and Nitric Oxide and a significant increase in maternal heart rate which translates to an increase in Cardiac output (Cardiac Output= stroke volume x heart rate)(191-193). Maternal HR continues to rise until the third trimester when HR is typically 10-15bpm greater than in the non-pregnant state.

In addition to an increased HR there is a large fluid volume shift associated with pregnancy beginning around the 6th week and plateauing between 32-34 weeks.(190, 194) There is a moderate increase in the intracellular water and a large expansion of the extracellular volume signifying an increase of up to 1.5 litres of plasma volume by term. Despite the increased production of erythrocytes (20-30%) associated with pregnancy, it is outweighed by the up to 45% increase in plasma volume.(195) This increase in plasma volume results in a physiological haemodilution of red blood cells resulting in anaemia.(196, 197)

A combination of hypertrophy of the ventricular wall musculature and an increase in end diastolic volume (secondary to expanded blood volume) causes a net increase in the SV and subsequently the CO ($CO = SV \times HR$)(192). The combination of increases in both maternal HR and SV results in an overall increased CO by 30-50% usually peaking by 20 completed gestational weeks. Following this, during the third trimester the gravid uterus can impede venous return to the heart via aortocaval compression while in the supine position. This is easily reversible by placing the woman in the left lateral position usually by placing a wedge or pillow under their right side.(198)

The increased CO in pregnancy is necessary to meet the increased needs of the uteroplacental circulation. With increasing gestation the volume of the uteroplacental circulation increases markedly, and circulation within the intervillous space acts partly as an arteriovenous shunt. As both the placenta and fetus develop, blood flow to the uterus must increase to about 1 L/min (20% of normal CO) at term. This increased uterine blood flow equates to approximately 10 times that of a non-pregnant woman. After delivery, the uterus contracts, and CO drops rapidly to about 15 to 25% above normal, then gradually decreases until it reaches the pre-pregnancy level at about 6 week postpartum.(199)

These changes are accompanied by normal adaptive changes in the maternal blood pressure. As blood pressure usually falls in pregnancy the increase in CO is also associated with a reduction in peripheral vascular resistance.(200, 201) The systemic vascular adaption begins with a fall in vascular tone, resulting in approximately 30% decrease in systemic vascular resistance.(202)

Following delivery and the shift of uterine blood flow to the maternal circulation (autotransfusion) there is a resultant increase in the CO by 10-20%. SV, HR and CO remains elevated for the first

2 days postpartum. Within the first two weeks after delivery the cardiac output falls rapidly, at six weeks postpartum it is halfway between pregnant and non-pregnant values and at 24 weeks postpartum it falls to pre-pregnancy values.(199)

During the 2nd trimester, BP usually drops (and pulse pressure widens), even though CO and renin and angiotensin levels increase, because uteroplacental circulation expands (the placental intervillous space develops) and systemic vascular resistance decreases. Systemic vascular resistance decreases secondary to decreased blood viscosity, a decrease in sensitivity to angiotensin and in addition the relaxing effect of progesterone on vascular smooth muscle. During the 3rd trimester, BP may return to normal and subsequently rise until term.(201) With twins, CO increases more and diastolic BP is lower at 20 week than with a single fetus.

1.5 Maternal haemodynamic adaption to pregnancy complicated by uteroplacental disease

There have been many studies over the years detailing the altered cardiovascular profile of pregnancy in the co-existing presence of uteroplacental disease.(203-207) Previous longitudinal studies have described a unique central haemodynamic profile with hypertension in pregnancy -specifically that of a lower Cardiac output (CO) and increased total vascular resistance (TPR) when compared to unaffected pregnant controls. In the setting of fetal growth restriction a second haemodynamic profile of uteroplacental disease exists as affected pregnancies are found to have a CO lower than the hypertensive counterparts and a moderate elevation in TPR when compared to pregnant controls and not reaching that of the hypertensive group.(207-213)

The majority of previous studies have employed transthoracic maternal echocardiography (TTE) as the methodology of choice for

evaluation of the cardiac profile in the presence of uteroplacental disease. However, TTE is both technically challenging and time consuming requiring highly skilled personnel to perform and evaluate the studies. Advances in technology have provided alternative non-invasive methodologies such as the Arteriograph, (214-216) Phonocardiograph, (217) Ultrasound Cardiac output monitor (USCOM)(216, 218) and Non-invasive cardiac output monitor (NICOM®)(208, 218-220) which are now being employed within the obstetric population.

In a pilot study published by my Co-Investigators Dr. A. Doherty and Prof A. EL-Khuffash longitudinal studies via NICOM® demonstrated the haemodynamic profile of uteroplacental disease in a high risk population.(208) The herald of these advances in medical technology has enabled researchers to assess the changing haemodynamic profiles in pregnancy with relative ease. Validation studies within this unique population have confirmed its application to the obstetric cohort.(221-223)

It has been recognized that both PE and FGR are associated with unique maternal haemodynamic changes, which predate clinical emergence of disease.(224, 225) Interrogation of maternal haemodynamic profile, therefore, represents a potential tool for prediction of these disease states.(208) Traditional methods of assessing the maternal haemodynamic outputs included invasive pulmonary artery catheterization or non-invasive but labour intensive maternal echocardiography. The use of maternal echocardiography has demonstrated increased cardiac output and mean arterial pressure in the first trimester of women, who go on to develop PE.(209) Invasive haemodynamic techniques have identified significant increases in heart rate (HR), blood volume, left ventricular end-diastolic volume (LVEDV), stroke volume (SV) and

cardiac output (CO) during the first and second trimesters of pregnancy.

Despite these changes, maternal blood pressure (BP) falls due to a large reduction in total peripheral resistance (TPR) from systemic vasodilatation and the formation of a low-resistance utero-placental circulation.(226) PE and FGR are diseases of placental origin with consequent wide-ranging maternal haemodynamic effects. Both have been associated with an elevated systemic vascular resistance and low cardiac output as compared to normal pregnancy. As a result these disorders have been regarded as part of the same disease spectrum. There has been limited study on the maternal cardiovascular changes associated with FGR occurring in the absence of maternal hypertensive disease. A recent pilot study, using NICOM® in a high-risk obstetric cohort, has identified differing haemodynamic profiles as early as 22 weeks' gestation between women who subsequently develop PE and those that develop normotensive FGR.(208) This evidence would suggest that PE and FGR may require different interventions to optimise maternal cardiac output in order to resuscitate the feto-placental unit and limit the severity of associated fetal growth restriction; however, this remains to be clarified.

1.6 Non Invasive Cardiac monitoring in pregnancy

Accurate monitoring of Haemodynamic outputs has traditionally been performed using invasive methods such as pulmonary artery catheterization or minimally invasive methods such as an arterial catheter for pulse contour analysis, intra-tracheal tube for partial CO₂ rebreathing, or continuous Doppler velocity flow assessment via a supra-sternal transthoracic ultrasound beam or an oesophageal probe.(227, 228) Measurement of the cardiac output with a pulmonary artery catheter using the bolus thermodilution has

been the clinical gold standard for central haemodynamic monitoring and is the reference standard used to compare non-invasive technologies.(229, 230) However, it has been shown to have disadvantages and associated complications.(231) Non-invasive monitoring of haemodynamic changes has the advantages of being easy-to-use, safe and cost effective.(219, 232-235) Echocardiography has been used in many studies evaluating cardiac output in pregnancy.(236-238) However, Doppler echocardiography is technically demanding, time-consuming and requires a skilled operator. Accuracy also depends upon the signal quality during recording.(231)

Transthoracic bioreactance is a new technique of non-invasive continuous cardiac output monitoring based on analysis of relative phase shifts of oscillating currents occurring when current traverses the thoracic cavity.(239) Bioreactance technology is performed by placing 4 dual sensors on the patient. The NICOM® monitor then sends a signal at 75kHz to the outer portion of the dual sensor and the known frequency is received via the inner portion of the dual sensor and a comparison is made to the original signal. The NICOM® then observes the extent of time delay/ or phase shift which has occurred. It then determines how much blood would have had to exit the left ventricle and enter the base of the aorta to cause the specific time delay (aka stroke volume). The ECG element of the NICOM® sensors detects the patients heart rate and as a results enables the NICOM® to calculate the cardiac output ($CO = HR \times SV$)

Measurements derived from bioreactance-based non-invasive cardiac output monitor (NICOM®) assessment correlate well with results derived from pulmonary artery catheterization and NICOM® has recently been validated against transthoracic echocardiography in the obstetric population.(221, 222, 239) The NICOM® system has acceptable accuracy, precision and

responsiveness for CO monitoring in patients in a wide range of circulatory situations.(233, 240) Its use in the obstetric population has gained recent interest. (208, 214, 216, 218, 240-243)

1.7 Previous research in predicting PE

1.7.1 Imaging methods in the prediction of preeclampsia

In recent years with advances in medicine more modern methods have been employed in an effort to predict which women will develop PE. One form of screening involves the use of sonographic measurement of maternal uterine artery Doppler velocimetries. This is performed in either the first or second trimester via a trans-abdominal ultrasound in the sagittal plane. Following the identification of the internal cervical os the ultrasound probe is panned laterally to the left and right with colour flow to identify the right and left uterine arteries. Once identified the sampling gate on pulse wave velocimetry is set to 2mm with the angle of insonation $<30^\circ$. When these pre-requisites are met 3 consecutive waveforms are recorded in both the right and left uterine artery and the mean pulse index (PI) is calculated.(74) The finding of an abnormal Uterine artery Doppler PI and its association with the subsequent development of PE further supports the theory that PE is a disorder caused by abnormal trophoblast invasion of the small spiral arteries.

A meta-analysis performed in 2014 of 18 studies assessed the predictive use of first trimester uterine artery Doppler. The demonstration of an abnormal flow velocity waveform was predictive of early onset PE (EOPE, a diagnosis at <34 weeks gestation) with sensitivity 47.8% (95%CI 39-56.8%) and specificity of 92.1% (95%CI 88.6 -94.6%). When applied to a diagnosis of either EOPE or late onset PE the value of this screening test was

reduced to sensitivity 26.4% (95%CI 22.5 -30.8%) and specificity 93.3% (95%CI 90.9- 95%).(244) Although current research in this isolated modality has been positive, it has not yet been introduced into routine practice.(245-248) It is also noteworthy that the measurement of Uterine artery Doppler can be affected by gestational age, maternal weight, ethnicity, a history of pre-existing diabetes and should be presented as multiples of the median (MOM) after adjustment for these factors.

Nicolaides' group performed a study of 534 women involving a first trimester (11-14 week) scan involving uterine artery Doppler assessment in association with maternal echocardiography. A multiple logistic regression demonstrated significant independent contribution in the prediction of PE by mean arterial pressure (MAP), uterine artery PI and SV. In this study the predictive capabilities reached a probability of >5.4 with a sensitivity of 77.8%, specificity of 79.1% and a false positive rate of 20%. Women who had pregnancies complicated by PE and no FGR had increased SV, CO and cardiac index suggestive of enhanced left ventricular function.(212)

1.7.2 Haemodynamics used in the prediction of preeclampsia

The measurement of mean arterial pressure (MAP) in the first and second trimester has predictive capabilities in the development of hypertensive disease in pregnancy. This demonstrates a dose-response effect with the greatest predictive capabilities seen in those developing EOPE. When compared to maternal demographic risk factors (47%) the addition of MAP improved the prediction to 76-84%. Whereas for GH, a similar improvement in prediction from maternal demographic risk factors (31%) was observed as the addition of MAP improved the prediction to 48%.(249, 250) A study by Poon et al detailed that measurement of MAP from an average

of two measurements from both arms had a strong predictive value with an area under the receiver operator curve of 0.77 in the prediction of PE.(251)

The measurement of MAP in the first trimester is affected by the following variables: maternal weight, maternal height, age, ethnicity, smoking, family history of PE, personal history of PE in a previous pregnancy and history of chronic hypertension and should be adjusted for these factors. In addition, there is a reported inter-arm difference of measured blood pressure in pregnant women, the prevalence of which is increased with a coexisting diagnosis of hypertension. The consequence of which can lead to a resultant under-reporting of PE by as much as one third.(252)

Preeclampsia has traditionally been regarded as a condition of hypoperfusion with increased total peripheral resistance (TPR) resulting in hypertension. An elevated cardiac output (CO) is associated with the development of PE with changes apparent as early as the first trimester.(209, 253)

1.7.3 Biomarkers used in the prediction of preeclampsia

A large number of biochemical biomarkers have been investigated in the prediction of PE see Table 1.3.(5) Changes in angiogenesis, fibrinolytic and complement pathways have been described in preeclampsia and alterations in these individual pathways are emerging as being of potential diagnostic and predictive value.(254-265) Many of these represent measurable manifestations of impaired placentation due to inadequate trophoblastic invasion of maternal spiral arteries and decreased placental perfusion. The result of which causes placental ischaemia related damage with release of inflammatory factors, platelet activation, endothelial dysfunction, maternal renal dysfunction or

oxidative stress. In this thesis I have examined the roles of soluble fms-like tyrosine-1 (s-flt-1), placental growth factor (PLGF), Apelin 13 and mean platelet volume (MPV).

Table 1.3 Current proposed Biomarkers .(5)

A disintegrin and metalloprotease 12 (ADAM 12)	Endothelin	L-arginine	Pregnancy associated plasma protein-A
Activin A	Estriol	L-homoarginine	Prostacyclin
Adiponectin	Ferritin	Leptin	Relaxin
Adrenomedullin	Foetal DNA	Magnesium	Resistin
Alpha fetoprotein	Foetal RNA	Matrix metalloproteinase-9	Serum Lipids
Alpha-1-microglobulin	Free fetal haemoglobin	Microalbuminuria	Soluble endoglin
Ang-2 angiopoietin-2	Fibronectin	Microtransferrinuria	Soluble fms-like tyrosine kinase
Antiphospholipid antibodies	Genetic markers	N-acetyl- β -glucosaminidase	Thromboxane
Antithrombin III	Haptoglobin	Neurokinin B	Thyroid function
Atrial natriuretic peptide	Haematocrit	Neuropeptide Y	Total proteins
Beta2-microglobulin	Homocysteine	Neutrophil gelatinase-associated lipocalin	Transferrin
C-reactive protein	Human chorionic gonadotropin	P-selectin	Tumour necrosis factor receptor-1
Calcium	Human placental growth hormone	Pentraxin 3	Uric acid
Cellular adhesion molecules	Inhibin A	Placenta growth factor	Urinary calcium to creatinine ratio
Circulating trophoblast	Insulin-like growth factor	Placental protein 13	Urinary kallikrein
Corticotrophin release hormone	Insulin-like growth factor binding protein	Plasminogen activator inhibitor-2	Vascular endothelial growth factor
Cytokines	Insulin resistance	Platelet activation	Visfatin
Dimethylarginine (ADMA)	Isoprostanes	Platelet count	Vitamin D

s-flt-1: PLGF

Placental growth factor (PLGF), a pro-angiogenic peptide produced by the placenta, is a potent vasodilator of resistance vessels mediated by nitric oxide (NO).(266, 267) Soluble fms-like tyrosine kinase-1 (s-flt-1), the soluble form of the PLGF receptor,(268) binds circulating PLGF, inhibiting its angiogenic and vasodilator effects.(269) In the second trimester of normal pregnancy, the PLGF concentrations are high and s-flt-1 concentrations low, creating a pro-angiogenic state. At 33 to 36 weeks the s-flt-1 levels increase corresponding to the late gestational decrease in free PLGF observed in normal pregnancy. In women with EOPE, s-flt-1 increases earlier in pregnancy, reaching a higher concentration when compared to normal pregnancy.(270)

Imbalance between pro- and anti-angiogenic factors is implicated in the pathogenesis of preeclampsia and accumulating evidence suggests that ratios of these markers can predict onset and clinical course of the disease.(259, 262, 263, 271) Soluble fms-like tyrosine 1 acts as an angiogenesis inhibitor by binding to and inactivating vascular endothelial growth factor (VEGF-A) and PLGF.(254, 265) Maynard *et al* reported in 2003 that s-flt-1 is up regulated and PLGF decreased in preeclampsia, indicating an overall anti-angiogenic state.(264) Endoglin is an anti-angiogenic coreceptor for transforming growth factor (TGF)- β 1 and β 3 and is highly expressed on cell membranes of vascular endothelium and syncytiotrophoblasts.(272, 273) Similarly to s-flt-1, placental endoglin and maternal serum endoglin are increased in preeclampsia.(273)

Early and late- onset preeclampsia are considered to be distinct disorders.(274) Verlohren *et al* reported that the s-flt-1: PLGF ratio permits identification of women with EOPE at risk of imminent delivery.(262) Similarly, Gómez-Arriaga *et al* evaluated the ratio of s-flt-1: PLGF (in combination with gestational age at onset and

uterine artery pulsatility-index) and demonstrated a significant association with perinatal complications and value in predicting timing of delivery in EOPE.(275) More recently, the landmark PROGNOSIS study was a prospective, multicentre, observational study, in which serum s-flt-1: PLGF ratios were measured in 1050 women with suspected preeclampsia between 24 + 0 and 36 + 6 weeks' gestation. The authors derived and validated a serum s-flt-1:PLGF ratio that predicts lack of progression to preeclampsia in the subsequent week.(261)

It is well established that the s-flt-1: PLGF ratio is increased in preeclampsia and may be useful for ruling out preeclampsia in suspected cases.(261, 276) The PLGF, s-flt-1 levels and their ratio has been shown to assist in identifying women at risk of EOPE from 20 weeks gestation.(277, 278) The ratio of s-flt-1: PLGF is a useful diagnostic tool to rule out of likelihood of progression of EOPE and this test is supported by a recent cost-benefit analysis.(279) The 2016 NICE guidelines concluded that “there is currently insufficient evidence to recommend (the) routine adoption of the s-flt-1: PLGF ratio for diagnosing preeclampsia” and recommended further research.(280)

Apelin 13

Apelin, is an adipocytokine, which is synthesised as a 77 amino acid prepropeptide and is cleaved into a mature 36 amino acid peptide. Apelin was first isolated from the bovine stomach in 1998.(281) Shorter more active isoforms such as Apelin 12, Apelin 13, Apelin 16, Apelin 17, Apelin 19 and a modified form of Apelin 13 with a pyroglutamate as the first element (Pyr¹ Apelin 13) have been identified.(282-284) However, Pyr¹ Apelin 13 has been reported as its' most potent form.(284) Apelin has many actions through its G-protein receptor (APJ receptor), and is found in the cardiovascular system and a variety of other human tissues.(285, 286)

Apelin is a potent positive inotrope, which mediates vasodilatation of resistance vessels and the venous system via Nitric Oxide (NO), a pro-angiogenic factor required for blood vessel growth and endothelial cell proliferation.(287) In addition, Apelin appears to have an opposing effect to the vasoconstrictive action of angiotensin II through both the NO dependent and independent pathways.(288, 289) It also been reported that the APJ G-protein receptor and Angiotensin II receptor display similarities in both transmembrane domain sequencing and tissue expression.(290) There have been numerous studies to date regarding the active form of Apelin 13 in relation to its role in the cardiovascular system.(291-296)

In normal pregnancy, maternal serum total Apelin increases until late in the third trimester when levels decline.(297, 298) In PE, Apelin levels are lower throughout the pregnancy due to decreased production from an ischaemic placenta.(299) This may contribute to the systolic heart failure seen in EOPE, and also to the increased vascular resistance and decreased stroke volume in normotensive pregnancies with FGR. Bortoff et al have demonstrated lower levels of serum Apelin 36 at time of PE diagnosis in comparison to normotensive controls. However, their findings were limited by unmatched normotensive controls (300) A recent study in 2016 by Yamaleyeva et al has reported the use of Pyr¹ Apelin 13 as a potential treatment for PE. In this study they treated the previously reported low total Apelin levels and this supplementation resulted in a reduction in MAP, proteinuria.(301) Down regulation of placental Apelin 13 has been linked with PE.(302) However, this has not yet been mapped prospectively in maternal serum in the first trimester.

Mean Platelet Volume (MPV)

Although the precise aetiology remains poorly characterised, preeclampsia is a pro-inflammatory state associated with platelet and coagulation activation. As a result of this known association of thrombocytopenia with PE much of the research to date has involved investigating platelet function and responsiveness in the setting of PE. A rise in mean platelet volume (MPV), which is associated with a reduction in overall platelet count is suggestive of active turnover of platelet production in bone marrow as a result of platelet consumption in PE.(303-305) MPV, is a sensitive indicator of platelet activation and consumption,(306) and is a routine parameter reported on all full blood counts (FBC). It is a simple and inexpensive test, which has been performed as an indicator of a variety of clinical conditions such as myocardial infarction, sepsis, hyperthyroid and acute exacerbation of chronic obstructive pulmonary disease.(307-310)

The MPV has gained recent interest as a potential marker and predictor for preeclampsia. However, an elevated MPV should be interpreted with caution as it has demonstrated to be influenced by both ethnicity, body mass index (BMI) and in the setting of gestational diabetes (GDM).(311, 312) In contrast, a reduction in MPV value has also been reported by Ulkumen et al in pregnancies complicated by pre-term labour.(313)

In the first trimester Myatt et al demonstrated a significant increase in MPV levels when comparing women with preeclampsia to unaffected controls.(311) In direct contrast Gezer et al have reported that women who subsequently developed preeclampsia had significantly lower levels of MPV than normotensive controls.(314) There is a growing body of evidence confirming the significant association of an elevated MPV and preeclampsia when assessed in the second and third trimesters.(315-322) Despite this evidence both Saleh et al and Yavuzcan et al were unable to

replicate these findings and in their studies they demonstrated that there was no significant difference in the MPV of preeclamptic women in comparison to normotensive controls.(323, 324) When assessing the role of MPV in distinguishing pregnancies complicated by gestational hypertension Karalis et al and Ohshige et al also reported no difference in MPV levels between women with gestational hypertension and normotensive controls.(325, 326) Longitudinal data extending to the postpartum is scarce and again conflicting results with the MPV parameter. Aune et al compared the postnatal blood parameters of women with preeclampsia to unaffected controls and whilst they demonstrated a significant increase in platelet counts there was no difference in the MPV values in the two groups.(317) A study by Jaremo et al in contrast demonstrated that the significant increase in MPV of women with preeclampsia, in comparison to unaffected controls, extended to the postpartum.(327)

Although preeclampsia is associated with significant increases in MPV, evidence regarding the ability of MPV to predict the disease remains conflicting. Altinbas et al and Dogan et al have confirmed that preeclampsia is significantly associated with increased MPV. However, this rise in MPV did not translate to an ability to discriminate which pregnancies will ultimately develop mild versus severe preeclampsia.(328, 329) Several authors have provided a “cut-off” value of MPV as a significant predictor for preeclampsia with values ranging from 8.65- 9.95 and dependant on gestational age with varying sensitivities and specificities.(320, 328, 330)

1.8 Previous research in predicting FGR

The most commonly employed method in predicting growth disturbances in pregnancy is the measurement of fetal biometry and approximation of fetal weight. In combination with maternal factors the addition of fetal biometry has been demonstrated to

predict between 61-71% of infants with a birthweight <3rd centile.(331-333) These predictive capabilities have been shown to perform better at later gestations e.g. 32 weeks' gestation vs. 19-24 weeks' gestation and up to 90% when performed within 2 weeks of delivery.(331, 334)

A recent meta-analysis in 2014 examined the use of first trimester uterine artery Doppler in assessing risk of an adverse pregnancy outcome. Within the meta-analysis the included studies were examined for their ability to predict FGR. Four studies involving over 26,000 women found that for the prediction of early onset FGR an abnormal Uterine artery Doppler flow wave volume (FWV) had a sensitivity of 39.2% (95% CI: 26.3- 53.8) and a specificity of 93.1% (95% CI: 90.6-95.0) The sensitivity and positivity of uterine Doppler in predicting FGR at any gestation were 15.4% (95% CI: 12.4- 18.9%) and 93.3% (95% CI: 90.9-95.1%) respectively for abnormal FWV. Notching in the uterine artery Doppler waveform had a sensitivity and specificity for predicting FGR of 58.5% (95% CI: 49.7- 66.7) and 56.1% (95% CI: 49.6- 62.5) respectively.(244) These findings regarding the predictive value of Uterine artery PI (UtPI) were further described at 30-34 weeks' gestation by Valino et al.(335) The addition of MAP and UtPI between 30-33 weeks' gestation has been reported to detect 90% FGR within 4 weeks of measurement.(336) However, the use UtPI and MAP at 35-37 weeks' gestation performs no better than maternal risk factors and biometry alone.(337)

An Italian study of 35 patients in 2004 examined maternal haemodynamics via maternal echocardiography in normotensive pregnancies complicated by SGA and FGR. They demonstrated a maladaptation of the maternal cardiovascular system correlated with FGR. They postulated that the absence of the compensatory mechanisms might be secondary to abnormal trophoblastic invasion, resulting in reduced perfusion and eventually FGR.

Cardiac output, SV, Maternal HR, MAP, total vascular resistance and end-diastolic volume were all haemodynamic factors, which have demonstrated statistical significance in predicting FGR. The importance of which is that these findings occurred prior to manifestation of clinical FGR and have not been replicated at later gestations.(213, 338, 339)

In addition there have been a variety of studies investigating multivariable analyses including a variety of biomarkers e.g. alpha fetoprotein (AFP), PAPP-A, placental growth factor (PLGF) and human chorionic gonadotrophin (hCG) in isolation and combination.(340-343) Within all studies there was a dose-response effect with best predictive value in severe FGR i.e. <3rd centile or requiring delivery <32 weeks' gestation.(344-347)

1.9 Why HANDLE?

More women are now aware of their reproductive health and delaying childbirth for a variety of reasons. A direct consequence of this practice is that the demographics of our obstetric population is changing. Several of these factors such as increasing maternal age, maternal BMI, co-existing medical conditions and use of assisted reproductive methods result in an increased risk of obstetric complications such as uteroplacental disease. Therefore there is a need to develop an effective screening process to aid earlier recognition and diagnosis of these conditions. The challenge now lies in evaluating the performance of non-invasive maternal Haemodynamic assessment in the prediction of PE and FGR within a low-risk obstetric cohort.

Hypothesis

My hypothesis is that the first trimester NICOM® obtained TPR in the setting of PE will be higher than the normal patient group by 200dyne.s.cm^{-5} and this this will have the ability to predict the emergence of PE.

Primary Study Objectives

- To assess serial haemodynamic changes in cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR) throughout pregnancy and postnatally.
- To compare maternal haemodynamic variables across the following four groups of patients:
 1. Pregnancies with PE (defined as maternal blood pressure of $\geq 140/90$ on 2 occasions and proteinuria $\geq 300\text{mg}/24$ hours).
 2. Pregnancies with GH (defined as maternal blood pressure of $\geq 140/90$ on 2 occasions in the absence of proteinuria or other signs of end organ damage).
 3. Pregnancies complicated by FGR (defined as an infant where the birth weight was less than the 10th centile when plotted on the WHO gender specific Neonatal and Infant close monitoring charts -Appendices 2.8 & 2.9).(348)
 4. Pregnancies uncomplicated by PE or FGR.
- To assess the ability of NICOM® to predict the evolution of PE and FGR.
- To evaluate the resolution or persistence of haemodynamic changes in the postnatal period.

Secondary study objectives

- To validate the use of non-invasive cardiac monitoring (NICOM®) using bioreactance in the obstetric setting.
- To investigate the correlation between first trimester biomarkers (soluble fms-like tyrosine 1 (s-flt-1), placental growth factor (PLGF), mean platelet volume (MPV) and Apelin) and maternal cardiac function and assess their ability to predict the evolution of uteroplacental disease.
- To investigate the impact of gestational hypertension on neonatal myocardial performance in the immediate postnatal period.

The thesis will introduce the HANDLE study methods in the next chapter and subsequently (i) explore the acceptability of NICOM® in the obstetric population,(221) (ii) describe the HANDLE patient population and declining preeclampsia rates in our local population, (iii) describe longitudinal changes in maternal haemodynamics in pregnancy, (iv) analyse the ability of NICOM® and biomarkers to predict the evolution of uteroplacental disease,(349) (v) assess the affect of gestational hypertension on the neonatal myocardium(350) and finally (vi) close with a discussion of the key findings of this thesis.

Chapter 2 MATERIALS AND METHODS

2.1 The HANDLE Study

The majority of the content of this thesis originates from data from the HANDLE study (**HAEMODYNAMIC** Assessment **iN** pregnancy **anD** neonatal **E**chocardiography assessment), which was conducted at the Rotunda Hospital, Dublin between May 2014 and October 2016. This study was designed to identify abnormal haemodynamic profiles in pregnancy as a predictor of adverse obstetric outcome and characterise the neonatal myocardial performance in infants born to mothers with an abnormal haemodynamic profile, in particular, those born to mothers with gestational hypertension.

This was a multidisciplinary project with input from the obstetric, neonatal and anaesthetic departments at the Rotunda Hospital. The study was funded by the HRB and via the Rotunda Foundation (formerly known as the Friends of the Rotunda). The Rotunda Foundation organisation was established in 1971 primarily to raise funds for Rotunda-based research and this project is an excellent example of such a collaborative research project, based at the hospital. This study was carried out solely in the Rotunda Hospital, a tertiary referral maternity centre with almost 9,000 deliveries per annum. The hospital also has a neonatal intensive care unit on site, which allowed for follow up of the postnatal paediatric outcomes.

This observational study enrolled eligible patients to serial antenatal assessments of maternal haemodynamics using bio-reactance technology with the Non-Invasive Cardiac Output Monitor (NICOM®) (Cheetah Medical, Maidenhead, Berkshire, United Kingdom). NICOM®-acquired haemodynamics, maternal variables, pregnancy outcomes, delivery outcomes and neonatal characteristics were recorded for all study participants and

maintained on a centralized database on the HANDLE study encrypted laptop and encrypted external hard drive for data management and analysis.

2.2 Study Design and Methods

2.2.1 Study Design

A pilot study evaluating the use of NICOM® in a high-risk cohort of patients identified distinct haemodynamic changes in both preeclampsia (PE) and fetal growth restriction (FGR) (208, 243). These aberrations were identified early in the course of pregnancy, prior to the emergence of clinical evidence of disease. The challenge now has been to evaluate this investigative tool in the low-risk population as a potential predictor of such obstetric complications. Non-invasive assessment of maternal haemodynamics represents a novel approach to the evaluation of maternal cardiovascular parameters during pregnancy. Profiling the changes that occur in these parameters across pregnancy provides the opportunity to identify a unique haemodynamic profile associated with underlying uteroplacental dysfunction. The ability to identify a cohort of women with an increased risk of developing PE and FGR could enable tailored antenatal care with these patients engaging in intensive antenatal surveillance of both maternal and fetal well being. There also exists the possibility that early, targeted intervention in this cohort may result in improved perinatal and maternal outcomes, prolong gestation and ultimately improve perinatal outcomes.

This was a prospective observational study enrolling 422 nulliparous women at their first antenatal appointment. Patients were referred to the study if they met the inclusion criteria, namely being a low risk nulliparous pregnancy. On recruitment to the

study, participants completed a written consent form, were assigned a study number, had first trimester serum bloods banked and underwent their first NICOM® assessment. Patients underwent two further antenatal interrogations of their haemodynamics timed to coincide with their routine antenatal care, as well as one additional assessment at least 6 weeks postpartum. The HANDLE study was observational and descriptive in nature, there were no pre-defined management or delivery criteria and all decisions were made by the lead clinician managing the case, who was blinded to the maternal haemodynamic profile.

The cohort was divided into four groups based on their final clinical diagnosis:

- PE
- gestational hypertension (GH)
- fetal growth restriction (FGR)
- uncomplicated pregnancy

The Institute of Obstetricians and Gynaecologists (IOG), Royal College of Physicians Ireland (RCPI) in collaboration with the Clinical Strategy and Programmes Directorate, Health Service Executive (HSE) and the International Society for the study of Hypertension in pregnancy (ISSHP) define PE as blood pressure of 140 mm Hg systolic or higher or 90 mm Hg diastolic or higher that occurs after 20 weeks of gestation in a woman with previously normal blood pressure and proteinuria, defined as urinary excretion of 0.3 g protein or higher in a 24-hour urine specimen. Gestational hypertension was similarly defined but in the absence of proteinuria or end organ dysfunction. These BP measurements were based on at least two measurements, taken using the same arm, several hours apart (13, 14). The IOG & RCPI guidelines currently define FGR as a fetus with estimated weight <10th centile for gestational age(120).

2.2.2 Clinical data and Outcome Measures

The following maternal details were collected at enrolment:

- Maternal age, weight and height
- Socioeconomic grouping
- Ethnicity
- Education
- Smoking, alcohol and drug use
- Medical history and Medication use
- Obstetric history
- Family history of preeclampsia or diabetes
- Booking ultrasound variables and gestational age

The primary study objectives were:

- To assess serial haemodynamic changes in cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR) throughout pregnancy and postnatally.
- Maternal haemodynamic variables were compared across the following four groups of patients:
 - Pregnancies with PE (defined as maternal blood pressure of $\geq 140/90$ on 2 occasions and proteinuria $\geq 300\text{mg}/24$ hours)
 - Pregnancies with GH (defined as maternal blood pressure of $\geq 140/90$ on 2 occasions in the absence of proteinuria or other signs of end organ damage)
 - Pregnancies complicated by FGR (defined as an infant where the birth weight was less than the 10th centile when plotted on the WHO gender specific Neonatal and Infant close monitoring charts -Appendices 2.8 & 2.9).(348)
 - Pregnancies uncomplicated by PE or FGR
- Assess the ability of NICOM® to predict the evolution of PE and FGR.
- Evaluate resolution or persistence of haemodynamic changes in the postnatal period

Secondary outcomes include:

- NICOM® validation in the obstetric setting
- The correlation between first trimester biomarkers (soluble fms-like tyrosine 1 (s-flt-1), placental growth factor (PLGF), mean platelet volume (MPV) and Apelin) and maternal cardiac function and their ability to predict the evolution of uteroplacental disease.
- The impact of gestational hypertension on neonatal myocardial performance in the immediate postnatal period

2.2.3 Eligibility Criteria and Recruitment

Ms. Lisa McSweeney and Dr. Cathy Monteith approached nulliparous patients attending the antenatal clinic for their booking visit for inclusion in the study. Only patients with adequate knowledge of the English language were invited to participate. All patients recruited to the study were provided with a patient information leaflet, assigned a study number and provided written informed consent prior to participation. Baseline demographics as detailed above were collected. Any medical problems and current medications, initial systolic and diastolic blood pressures and baseline dating ultrasound measurements were recorded. A routine comprehensive review of fetal anatomy was performed between 18 – 22 weeks' gestation. Participants were excluded in the event of a diagnosis of a fetal abnormality.

Inclusion criteria:

- Nulliparous patient
- Booking for antenatal care prior to 16 0/7 weeks' gestation

Exclusion Criteria:

- multiple gestation
- known fetal anomaly
- pre-existing medical condition such as hypertension, pre-existing renal disease or systemic lupus erythematosus
- hypertension at first booking visit

2.2.4 Period of Study

This prospective single centre observational cohort study was carried out at the Rotunda Hospital, Dublin, Ireland which is a stand alone tertiary referral maternity hospital with almost 9,000 deliveries annually. Approximately 40% of annual pregnancies occur in nulliparous women (up to 4,000 patients per year). It was conservatively expected that it would take 2 years to enrol 400 pregnancies for this observational study.

The recruitment phase of the study ran from May 2014 to January 2016 inclusive and 422 nulliparous women were recruited to the study. All patients completed the study protocol including a postnatal review by October 2016. Patients who consented to the study were monitored using NICOM® at the following time points:

- 14⁺⁰ - 16⁺⁰ weeks; (first booking visit)
- 20⁺⁰ - 22⁺⁰ weeks; (fetal anatomy scan)
- 28⁺⁰ – 30⁺⁰ weeks; (28 week Antenatal visit/ Glucose tolerance test)
- Postnatal (at least 6 weeks following delivery).

2.2.5 Sample Size and Power calculation

The primary outcome was the total peripheral resistance (TPR) in an uncomplicated pregnancy vs. pregnancies complicated by FGR or PE. In the pilot study, TPR within each subject group was normally distributed with standard deviation of 200dyne.s.cm⁻⁵. We have based our sample size calculation on the assumption that TPR at 20-22 weeks' gestation in the PE group would be higher than the normal patient group by 200dyne.s.cm⁻⁵. We planned a study of a continuous variable (TPR) from independent controls (normal pregnancy) and experimental subjects (PE group) with one control per experiment subject. Assuming 80% statistical power and an overall 5% level of significance (2.5% per comparison), the study would require 20 subjects per group (total 60 subjects). Given that the estimated PE rate in the Rotunda nulliparous population is 5% (351-356) we recruited 422 patients over a two-year period to obtain 20 patients with PE. Given a 10% rate of normotensive FGR in the Rotunda population, we anticipated acquiring 40 participants in the FGR group. Consequently, this would allow a clear delineation of the haemodynamic profiles between the three groups and assess the predictive value of NICOM®.

Dr. Patrick Dicker, a medical biostatistician at the RCSI Department of Obstetrics and Gynaecology, and Department of Epidemiology and Public Health, performed all data checks, statistical analyses and supported this project fully from a statistical point of view. Distribution normality was tested using the Shapiro-Wilk Test. Continuous data were presented as means (SD) or as medians [IQR] as appropriate. Categorical data was presented as absolute values and percentages. Three-group comparisons were conducted using the one-way ANOVA or Kruskal-Wallis one-way analysis of variance as appropriate. Two-

group comparisons were conducted using the independent t-test or Mann-Whitney U test. Proportions were compared using the Chi square test (or Fisher's exact test where appropriate).

Haemodynamic trends over time were displayed using line charts.

A Receiver Operating Characteristic (ROC) analysis was performed to determine the ability of TPR by 16 weeks' gestation to predict the evolution of later PE and FGR. SPSS version 23 (IBM Corporation, NY, USA) was used to conduct the analyses.

2.3 Overview of the Study Procedures

2.3.1 Measurement of the Maternal Haemodynamic profile

The assessment of the haemodynamic profile of participants was conducted using the NICOM® machine (Figure 2.1-Cheetah Medical, Maidenhead, Berkshire, United Kingdom). A research assistant (Lisa McSweeney) and Dr. Cathy Monteith were trained in the haemodynamic assessment. Further input and technical assistance was available from Cheetah Medical. Prior to the commencement of the study, a training day was set up and led by a representative from Cheetah Medical. This training day provided a step-by-step instruction on the application of the equipment, obtaining the measurements and archiving the data for later analysis.



Figure 2-1 NICOM machine and single use electrodes (Image courtesy of Cheetah Medical).

The NICOM® monitor was turned on to allow for haemodynamic monitoring. The following patient details were input into the NICOM® device: patient age, patient gender, patient height, current weight and HANDLE study number (used in place of patient name and hospital number to maintain patient anonymity). Haemodynamic monitoring was performed with the patient lying semi-recumbent, slightly tilted to the left, so as to avoid aorto-caval compression. Four double NICOM® electrodes were attached: 2 below the clavicle in the mid clavicular line and 2 at the costal margin in the mid-clavicular line (see Figure 2.2). A non-invasive blood pressure cuff was placed on the patients' upper arm to measure brachial artery pressure at 5-minute intervals. The NICOM® was allowed to calibrate and then haemodynamic monitoring continued for 15 minutes.

Given that the HANDLE study was observational and descriptive in nature, there was no pre-specified cut off limit for TPR and no prevention strategies were employed (e.g. commencement of low dose aspirin). NICOM® monitoring was repeated at the time points as described above. Prior to each episode of haemodynamic

monitoring patients were re-weighed using a hospital-calibrated scales. Their NICOM® profile was retrieved by HANDLE study number and was updated with their current weight.

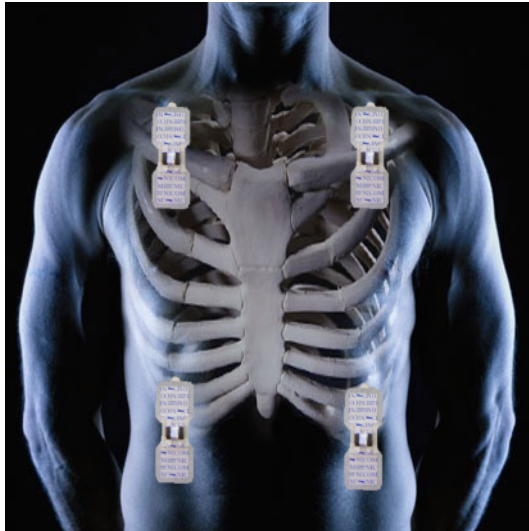


Figure 2-2 Application of NICOM electrodes (Image courtesy of Cheetah Medical).

2.3.2 Retrieval of data from NICOM®

The NICOM® device was powered on and connected to the study laptop via a USB cable. Scrolling down to the settings tab occurred, followed by tapping the menu icon and selecting the device mode. Once in device mode, monitoring mode was changed to device mode to allow for data transfer. On the study desktop computer, NICOM EMR (version 1.5.5) was selected, and login with user details was performed. Retrieve data icon was selected from the drop down menu and on-screen instructions to allow data import were followed.

The patient study number was entered in the search tab and the relevant date was selected as required for study episodes 1-4 and a report was generated by test. A copy of the 3-page pdf executive

report (which graphically summarised the episode of monitoring) as detailed in Figures 2.3-2.5 was saved to the study laptop. A report by patient was then selected which generated a copy of the excel report which was then saved to the relevant study number folder on the desktop of the encrypted study laptop, detailed in figure 2.6. A mean for each variable was calculated in excel using the Autosum average function and excluded the first two values to allow for calibration. The mean values were then inserted under the selected patient and time point in the study database.

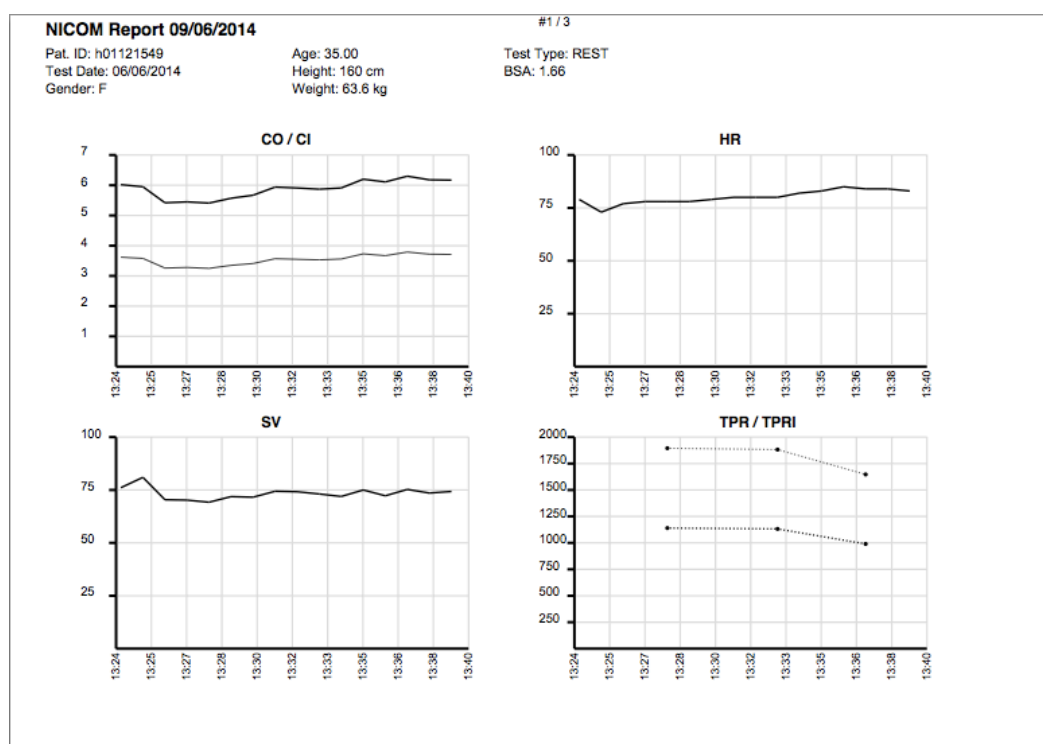


Figure 2-3 NICOM example report page 1

Abbreviations: F- female, BSA- Body surface area, CO- Cardiac Output, CI – Cardiac Index, HR Heart rate, SV- Stroke volume, TPR – total peripheral resistance and TPRI- indexed total peripheral resistance.

NICOM Report 09/06/2014

Pat. ID: h01121549
Test Date: 06/06/2014
Gender: F

Age: 35.00
Height: 160 cm
Weight: 63.6 kg

#2 / 3

Test Type: REST
BSA: 1.66

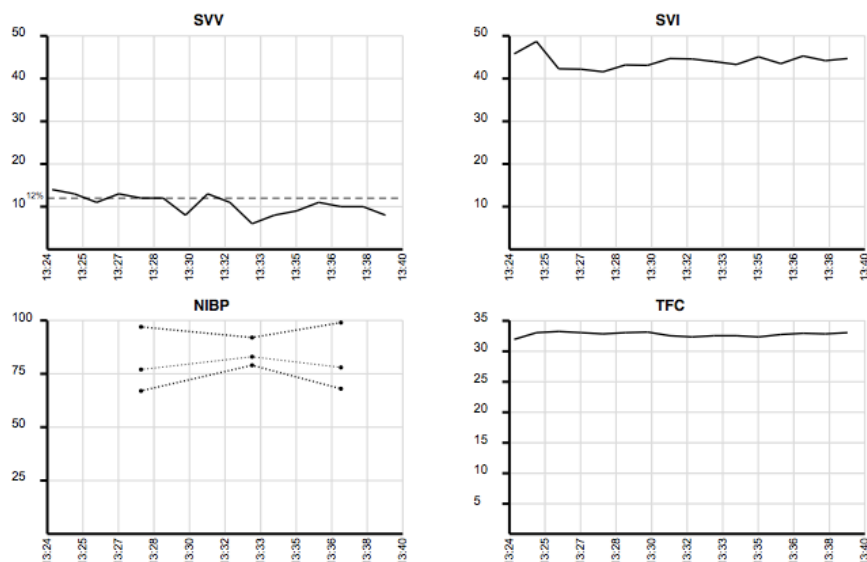


Figure 2-4 NICOM example report page 2

Abbreviations: F- female, BSA- body surface area, SVV- stroke volume variation, SVI- indexed stroke volume, NIBP- non-invasive blood pressure and TFC- thoracic fluid content

Report Date: 09/06/2014

#3 / 3

Pat. ID: h01121549

Test Date: 06/06/2014

Samples

Sample #	S.Time	CO	CI	HR	NIBP	MAP	TPR	TPRI	SV	SVI	SVV	SPO2	DO2I	TFC	EV
1	13:24:13	6	3.6	79	--/--	--	--	--	76.1	46	14%	--	--	31.9	--
2	13:25:13	5.9	3.6	73	--/--	--	--	--	81	49	13%	--	--	33	--
3	13:26:13	5.4	3.3	77	--/--	--	--	--	70.4	42	11%	--	--	33.2	--
4	13:27:13	5.4	3.3	78	--/--	--	--	--	70.2	42	13%	--	--	33	--
5	13:28:13	5.4	3.2	78	97 / 67	77	1140	1895	69.2	42	12%	--	--	32.8	--
6	13:29:13	5.6	3.3	78	--/--	--	--	--	71.9	43	12%	--	--	33	--
7	13:30:13	5.7	3.4	79	--/--	--	--	--	71.6	43	8%	--	--	33.1	--
8	13:31:13	5.9	3.6	80	--/--	--	--	--	74.4	45	13%	--	--	32.5	--
9	13:32:13	5.9	3.5	80	--/--	--	--	--	74.2	45	11%	--	--	32.3	--
10	13:33:13	5.9	3.5	80	92 / 79	83	1132	1882	73.1	44	6%	--	--	32.5	--
11	13:34:13	5.9	3.6	82	--/--	--	--	--	71.9	43	8%	--	--	32.5	--
12	13:35:13	6.2	3.7	83	--/--	--	--	--	75	45	9%	--	--	32.3	--
13	13:36:13	6.1	3.7	85	--/--	--	--	--	72.3	44	11%	--	--	32.7	--
14	13:37:13	6.3	3.8	84	99 / 68	78	990	1647	75.3	45	10%	--	--	32.9	--
15	13:38:13	6.2	3.7	84	--/--	--	--	--	73.6	44	10%	--	--	32.8	--
16	13:39:13	6.2	3.7	83	--/--	--	--	--	74.3	45	8%	--	--	33	--

Figure 2-5 NICOM example report page 3.

Abbreviations: CO- cardiac output, CI- cardiac index, HR- Heart rate, NIBP- non-invasive blood pressure, MAP – mean arterial pressure, TPR– total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, SVV- stroke volume variation and TFC- thoracic fluid content.

	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	'Study No'	'CO'	'CI'	HR	NIBP	'MAP'	'manMAP'	'TPR'	'TPRI'	'CP'	'CPI'	'SV'	'SVI'
2	1	6.02	3.62	79	∞/∞							76.09	46
3	2	5.95	3.58	73	∞/∞							80.97	49
4	3	5.42	3.26	77	∞/∞							70.39	42
5	4	5.45	3.28	78	∞/∞							70.21	42
6	5	5.41	3.25	78	97/67	77		1140	1895	0.9	0.6	69.22	42
7	6	5.57	3.35	78	∞/∞							71.87	43
8	7	5.67	3.41	79	∞/∞							71.6	43
9	8	5.94	3.57	80	∞/∞							74.4	45
10	9	5.91	3.55	80	∞/∞							74.18	45
11	10	5.87	3.53	80	92/79	83		1132	1882	1.1	0.6	73.12	44
12	11	5.91	3.56	82	∞/∞							71.94	43
13	12	6.2	3.73	83	∞/∞							75.01	45
14	13	6.11	3.67	85	∞/∞							72.26	44
15	14	6.3	3.79	84	99/68	78		990	1647	1.1	0.7	75.27	45
16	15	6.18	3.72	84	∞/∞							73.56	44
17	16	6.17	3.71	83	∞/∞							74.3	45

Figure 2-6 NICOM example excel report.

Abbreviations: CO- cardiac output, CI- cardiac index, HR- Heart rate, NIBP- non-invasive blood pressure, MAP – mean arterial pressure, TPR– total peripheral resistance, TPRI- indexed total peripheral, resistance, CP- cardiac power, CPI- cardiac power index, SV- stroke volume and SVI- indexed stroke volume.

2.3.3 Collection of first trimester maternal serum: blood draw, sample labelling and transport

Once written informed consent was obtained, 8ml of venous blood was drawn from the antecubital vein using a 19-gauge needle at the time of the routine booking visit phlebotomy and connected to a red 8mL VACUETTE® Z Serum Sep Clot Activator bottle. Samples were then labelled with patient initials, individual study number and date of birth. Samples were stored at room temperature and were transported from phlebotomy in a biohazard bag to the Rotunda Research Department laboratory for analysis. All samples were processed within 240 minutes from time of blood draw, to minimise potential platelet instability beyond these limits.

2.3.4 Initial sample processing and storage

Once in the Rotunda Research Department laboratory, I centrifuged blood samples at 3000rpm (Eppendorf 5804, Eppendorf AG, Barkhausenweg, Hamburg, Germany) for 5 minutes at room temperature. Following centrifuge, I removed the supernatant (approximately 3ml plasma in an 8ml serum blood sample) using a Pasteur transfer pipette with care taken to avoid contamination with red blood cells. Supernatant was then aliquoted in 1 ml samples into each 1ml tube (between 1-3 sample dependant). The remaining red cell sample and pasteur transfer pipettes were discarded into the clinical waste yellow sharps bin. Each 1ml supernatant sample was labelled with the corresponding study number and samples were placed in the local -80°C freezer (Haier Biomedical, DW 86L628, Qingdao, China) located in the main hospital until time of analysis. Once all pregnancy outcome data were available, samples eligible for analysis were thawed to room temperature on the day of analysis. All samples deemed ineligible for processing were disposed of as per hospital protocol.

2.4 Sample Assay processing

Serum samples selected for processing included all available disease states and a range of maternal BMI. The samples included 61 controls of varying BMI, five with pregnancy complicated by PE, 13 with pregnancy complicated by GH and 18 with pregnancy complicated by FGR. On the day of sample processing samples were identified and removed from the -80°C freezer. An independent dual review was performed by a laboratory technician and myself. One 1ml frozen aliquot from selected patients were placed on a rack and transferred to the Biochemistry Laboratory in the Rotunda Hospital, and all remaining samples were returned to the -80°C freezer. On arrival in the Biochemistry laboratory, frozen aliquots were placed on a roller (Spiramix roller, Thermo Scientific,

Hants, United Kingdom) and allowed to thaw to room temperature. Once thawed to room temperature, samples were transferred to Roche Diagnostic standard false bottom tubes (Roche Holding AG, Indiana, USA)) and labelled with a local laboratory barcoded sample ID as detailed in Appendix 2.1.

2.4.1 Soluble fms-like tyrosine kinase 1 (s-flt-1) assay

All samples were processed in the Rotunda Hospital, an INAB accredited (ISO 15189) laboratory, in a single analytical run and using standard quality control procedures. Serum s-flt-1 levels were determined in a commercially available Electro-chemiluminescence immunoassay –ECLIA (Elecsys® s-flt-1, Cobas, Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Cobas analyser (Cobas e601, Roche Diagnostics GmbH, Mannheim, Germany).

Quality control and calibration for the s-flt-1 immunoassay was performed on the day prior to sample analysis using PreciControl Multimarker (Cobas, Roche Diagnostics GmbH, Mannheim, Germany). The lyophilized s-flt-1 control serum was provided in two concentrations: approximately 100 pg/ml and approximately 1000 pg/ml. The lyophilized s-flt-1 control serums were reconstituted at room temperature by adding 2.0ml of deionized water and allowing to stand closed for 30 minutes. The reconstituted controls were then transferred into the supplied empty label snap-cap bottles (ControlSet vials) and the supplied bar-code label was applied. The reconstituted control serum was treated in the same way as a patient sample and data was read into the Cobas e601 analyser. QC was run prior to the samples being analysed and was within specification. Following sample thaw as described above, samples in the Roche false bottom tubes were placed in a paediatric rack and processed in the Cobas e601 automated immunoassay.

Once processing was complete samples were placed on ice prior to transfer to Dublin Institute of Technology for the Apelin ELISA assay. The analyser automatically calculates the analyte concentration of each sample in pg/ml. The measuring range is reported as 10-85,000 pg/ml with values below 10 pg/ml reported as <10 pg/ml and values greater than 85,000 pg/ml reported as >85,000 pg/ml. These automated results were printed as per HANDLE study number and entered into the database according to same.

2.4.2 Placental growth factor (PLGF) assay

All samples were processed in the Rotunda Hospital, an INAB accredited (ISO 15189) laboratory, in a single analytical run and using standard quality control procedures. Serum PLGF levels were determined in a commercially available Electro-chemiluminescence immunoassays –ECLIA (Elecsys® PLGF, Cobas, Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Cobas analyser (Cobas e601, Roche Diagnostics GmbH, Mannheim, Germany).

Quality control and calibration for the PLGF immunoassay was performed on the day prior to sample analysis using PreciControl Multimarker (Cobas, Roche Diagnostics GmbH, Mannheim, Germany). The lyophilized PLGF control serum was provided in two concentrations: approximately 100 pg/ml and approximately 1,000 pg/ml. The lyophilized PLGF control serums were reconstituted at room temperature by adding 2.0ml of deionized water and allowing to stand closed for 30 minutes. The reconstituted controls were then transferred into the supplied empty label snap-cap bottles (ControlSet vials) and the supplied bar-code label was attached. The reconstituted control serum was treated in the same way as a patient sample and data was read into the Cobas e601 analyser.

QC was run prior to the samples being analysed and was within specification. Following sample thaw as described above, samples in the Roche false bottom tubes were placed in a paediatric rack and processed in the Cobas e601 automated immunoassay. Once processing was complete, samples were placed on ice prior to transfer to Dublin Institute of Technology for the apelin ELISA assay. The analyser automatically calculates the analyte concentration of each sample in pg/ml. The measuring range is reported as 3 – 10,000 pg/ml with values below 3 pg/ml reported as <3 pg/ml and values greater than 10,000 pg/ml reported as >10,000 pg/ml. These automated results were printed as per HANDLE study number and entered into the database according to same.

2.4.3 Quantification of serum apelin levels

All samples were processed in Dublin Institute of Technology in a single analytical run and using standard quality control procedures. Serum apelin levels were determined using a commercially available Enzyme Linked Immunosorbent Assay (Nori® Human Apelin 13 ELISA Kit-2 Plates, Product number GR111133-2, Genorise Scientific, Inc. Glen Mills, PA, USA) The results were read using the Epoch Microplate spectrophotometer and Gen 5 image software (Epoch™, BioTek Instruments, Inc., VT, USA). I assisted my collaborator Dr. Greg Byrne during the Apelin ELISA preparation and analytical run.

Preparations of Assay reagents

All of the following reagents were brought to room temperature prior to use:

Phosphate buffered saline (PBS):

This was provided in two 30ml vials at 20x concentration. The 60ml concentrate was mixed with distilled water to a total volume of 1200ml.

Assay buffer:

This was provided in 10ml vials and prior to use 1 x Assay buffer was diluted with 1 x PBS.

Reagent Diluent: This was provided in two 21ml vials and prior to use 1 x Reagent diluent was diluted with 1 x PBS to give 84ml in total.

Human apelin 13 detection antibody:

The lyophilized detection antibody was placed in a centrifuge (Eppendorf Centrifuge 5417C, Eppendorf AG, Barkhausenweg, Hamburg, Germany) for 1 minute at 6,000xg to bring down material prior to opening the vial. Each vial contained sufficient detection antibody for one 96 well plate. Four vials of the detection antibody were then reconstituted by introducing 200µL of sterile phosphate buffered saline (PBS) into each and vortexed (Yellowline Test tube shaker (TTS), IKA®- WERKE GMBH & Co., Staufen, Germany) for 30 seconds. The entire 800µL of detection antibody was then mixed with 42ml of reagent diluent and the working dilution of the detection antibody was ready for use for four 96 well plates.

Human apelin 13 standard:

The lyophilized Human apelin 13 was placed in a centrifuge (Eppendorf Centrifuge 5417C, Eppendorf AG, Barkhausenweg, Hamburg, Germany) for 1 minute at 6,000xg to bring down material prior to opening the vial and each vial contained sufficient for generating a standard curve. Two vials of the human apelin 13 were then reconstituted by introducing 500µL of 1 x assay buffer to each standard vial to provide a concentration of 2,000pg/ml. This was vortexed for 30 seconds (Yellowline Test tube shaker (TTS), IKA®- WERKE GMBH & Co., Staufen, Germany) and allowed to rest for 5 minutes prior to use. A seven-point standard curve was generated using 2-fold serial dilutions with the assay buffer with a

30 second vortex for each step. A standard curve was generated for each set of samples assayed. Thorough mixing of the standards at each set of the dilutions was performed to ensure a normal standard curve.

Conjugate:

Each vial contained 106 μ L conjugate which was sufficient for two 96 well plates. Prior to opening the vial it was placed in the centrifuge (Eppendorf Centrifuge 5417C, Eppendorf AG, Barkhausenweg, Hamburg, Germany) for one minute at 6,000xg to bring down the material. To process four 96 well plates 212 μ L of conjugate was added to 42 ml of the Reagent diluent and mixed thoroughly to make a working dilution of conjugate at a 1:200 dilution.

Substrate solution:

This was provided as ready to use in a 21mL vial.

Stop solution:

This was provided as ready to use in an 11mL vial.

The thawed serum sample was diluted with an equal volume of 1 x Assay Buffer and vortexed for one minute prior to performance of the assay. All standards and samples were performed with triplicates on the same plate. See Appendix 2.2 for copy of the well plate maps.

Assay Procedure

The assay began by peeling back the plate cover from the top left corner of each commercially available antibody precoated Enzyme Linked Immunosorbent Assay -ELISA plates (Nori® Human Apelin 13 ELISA Kit-2 Plates, Product number GR111133-2, Genorise Scientific, Inc. Glen Mills, PA, USA) taking care to cover the wells that were not used. Samples were briefly vortexed prior to removal

for the assay. Samples were performed in triplicate with 100 μ L of serum sample or standards pipetted per well. The four 96 well plates were then covered and incubated for one hour at room temperature.

Following the first one-hour incubation the contents of each well were aspirated and washed with Assay buffer. The wash was performed using a multi-channel pipette (Eppendorf Research [®] plus, Eppendorf AG, Barkhausenweg, Hamburg, Germany) to fill each well with 300 μ L of Assay buffer and repeated for a total of 3 washes. To ensure good performance complete removal of liquid was performed following each wash. Following the third repeated wash, all assay buffer was removed by inverting the plates and blotting them against clean paper towels.

Following thorough wash all tips from the multichannel pipette were discarded and replaced with fresh tips. This allowed further use of the multichannel pipette to add 100 μ L of the working dilution of the detection antibody to each well in the four plates. The four plates were again covered and incubated for a second hour at room temperature. Following the second one-hour incubation the contents of each well were aspirated and washed with Assay buffer. The tips from the multichannel pipette were again discarded and replaced with fresh tips. The wash was performed using the multi-channel pipette to fill each well with 300 μ L of Assay buffer and repeated for a total of 3 washes. To ensure good performance complete removal of liquid was performed following each wash. Following the third repeated wash, all assay buffer was removed by inverting the plates and blotting them against clean paper towels.

Following the second incubation and wash, the tips from the multi-channel pipette were again discarded and replaced with fresh tips. The multi-channel pipette was used to add 100 μ L of the working dilution of conjugate to each well on all four plates. The plates

were covered, placed in the dark and incubated for the third time but for a period of 20 minutes at room temperature. Following the third incubation lasting 20 minutes the contents of each well were aspirated and washed with Assay buffer. The tips from the multichannel pipette were again discarded and replaced with fresh tips. The wash was performed using the multi-channel pipette to fill each well with 300 μ L of Assay buffer and repeated for a total of 3 washes. To ensure good performance, complete removal of liquid was performed following each wash. Following the third repeated wash, all assay buffer was removed by inverting the plates and blotting them against clean paper towels.

Following the third incubation and wash, the tips from the multi-channel pipette were again discarded and replaced with fresh tips. Using the multi-channel pipette 100 μ L of Substrate solution was added to each well in the four plates. The four plates were again covered, placed in the dark and incubated for 20 minutes at room temperature.

Following the fourth incubation and wash, the tips from the multi-channel pipette were again discarded and replaced with fresh tips. Using the multi-channel pipette 50 μ L of Stop solution was added to each well in the four plates. Following addition of the stop solution the plates were gently tapped to ensure thorough mixing.

The optical density of each well was read immediately using an automatic microplate reader set to 450nm (Epoch™, BioTek Instruments, Inc., VT, USA). The results were automatically read using the Gen 5 Microplate reader and imager software (Epoch™, BioTek Instruments, Inc., VT, USA). To allow for wavelength correction and improved accuracy the optical density of each well was repeated using the microplate reader set to 570nm. To correct for these optical imperfections in each plate, readings at 570nm were subtracted from the reference readings at 450nm.

Calculation of Results:

The mean was calculated from each set of triplicate readings for each standard, control and sample. Using Graphpad Prism (Horsham, PA, USA) a standard curve was generated and apelin serum levels were extrapolated from same.

Quantification of human apelin 13:

The detection range for this human apelin 13 ELISA assay is between 31 – 2,000 pg/mL with a sensitivity of 4pg/mL.

2.4.4 Mean platelet volume (MPV)

All pregnant women in our institution have a full blood count (FBC) performed from a venous sample at their first antenatal visit.

Venous blood was collected as part of routine booking bloods via a 22-gauge needle into 3ml vacutainers containing K3EDTA (VACUETTE® 3ml K3E, K3EDTA, Greiner Bio-One International, Austria). Samples were routinely analysed in the Rotunda Hospital accredited laboratory on the same day within an hour of sampling by a haematology analyser (Cell- Dyn Sapphire, Abbott Core Laboratory, IL, USA). Mean platelet volume (MPV) at this first antenatal visit was retrieved from an electronic laboratory database. Where a HANDLE study participant had subsequent antenatal venepuncture following their booking visit, this enabled retrieval of additional MPV measurements from the electronic laboratory database. The MPV was retrieved and timing of collection was assigned into six time points: first booking visit, 24 – 33 weeks gestational age, 34 – 40 weeks gestational age, days 2 – 4 postpartum, greater than day 5 postpartum and greater than day 28 postpartum where available.

2.5 Maternal Echocardiography.

NICOM® validity was further tested in the obstetric setting (via maternal transthoracic echocardiography; echo) in pregnant participants recruited to the study. Thirty participants in the HANDLE study were approached to participate in this aspect if they were scheduled for review assessment on the day of echo technician availability. Simultaneous NICOM® measurements of cardiac output and echo- measured cardiac output were obtained in participants in a rested state at either 18-22 weeks or 28-30 weeks' gestation for the assessment of agreement.

An additional thirty non-pregnant women were recruited to a cross-sectional study and underwent simultaneous NICOM and echocardiographic assessments. Recruitment posters were placed in the hospital canteen with contact details for the research team for participants to opt in. Women were deemed eligible for recruitment if they were similarly low risk with no pre-existing medical disease and with no requirement for regular medication.

Evaluations were performed using the Vivid S6 echocardiography system (GE Medical, Milwaukee, USA) with an adult cardiology 4MHz multi-frequency probe (M4S). Cine loops were obtained at end expiration in raw DICOM format and stored in an archiving system for later analysis (EchoPac, General Electric, version 112 revision 1.3). This echo machine is primarily dedicated to research and was made available for our study. An archiving system is available at the Rotunda Hospital and has enough storage capacity for 8 years with an activity of 500 - 700 scans per year.

Two senior clinical research investigators with extensive experience in performing and interpretation of echocardiographic studies performed the echocardiograms. All studies were performed in a quiet area in the Rotunda Day-care assessment

unit, with the woman placed in the left lateral semi-recumbent position. A two-dimensional (2D) echocardiogram was performed and the examiner was blinded to the simultaneous NICOM® assessment. All image acquisition and study analyses were conducted according to the recommendations of the American Society of Echocardiography (357). Offline analysis of all the scans was conducted at the end of the study by a single investigator (AEK) who was blinded to the NICOM values.

During the offline analysis stroke volume and cardiac output were calculated as follows. The aortic root diameter was obtained from the long axis parasternal view of the left ventricle (LV) and the resultant aortic cross sectional area (AoCSA) was calculated using this formula: $AoCSA = \pi \times (\text{aortic root radius})^2$. A pulsed wave Doppler of the LV outflow tract at the level of the aortic valve was obtained in the apical 5-chamber view, which enabled calculation of the velocity time index (VTI) see Figure 2.7. The ECG signal enabled calculation of heart rate (HR) from the R wave to R wave interval. Stroke Volume (SV) was calculated using the following formula: $SV = AoCSA \times VTI$. Cardiac Output was derived from Starling's Law: $CO = SV \times HR$. The Bioreactance (NICOM®) obtained CO and Bioreactance (NICOM®) obtained SV measurements were timed to the same minute of acquisition of the echocardiography obtained CO and echocardiography obtained SV. These NICOM® and echocardiography data sets were compared.

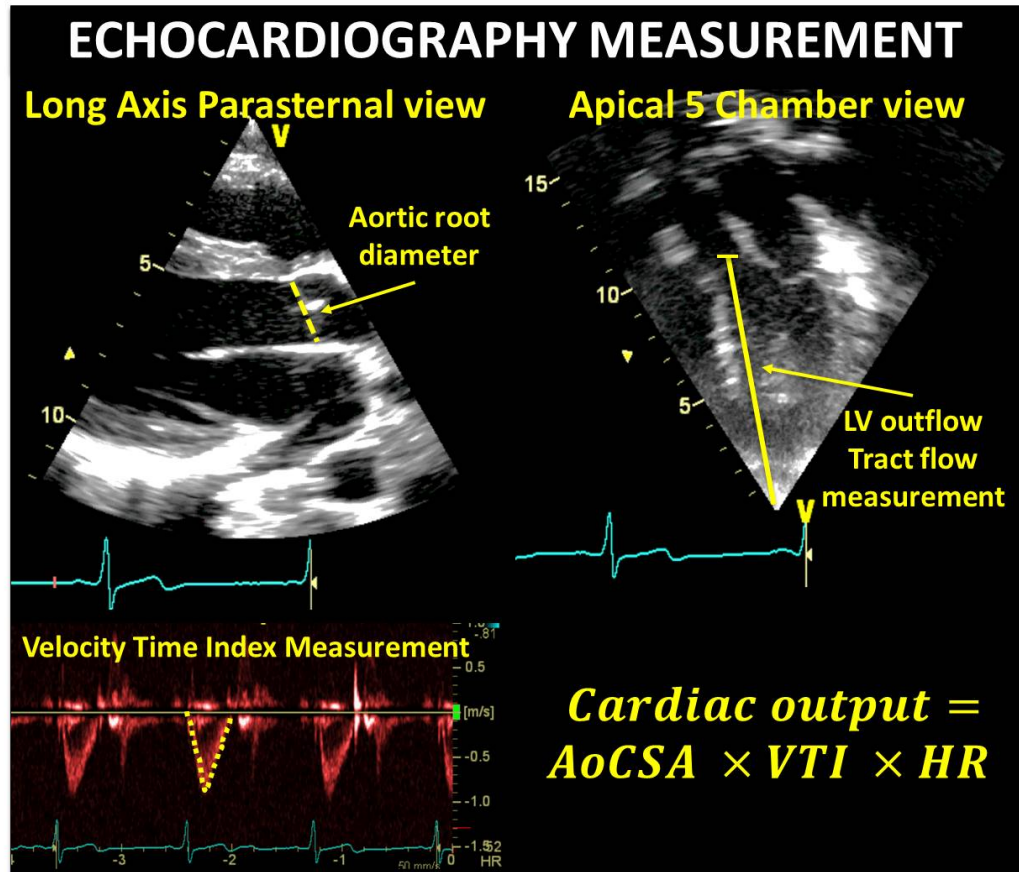


Figure 2-7 Echocardiographic measurement of cardiac output and stroke volume.

LV function was measured using tissue Doppler imaging (TDI) to derive mitral valve annular systolic velocity (s'), early (e') and late (a') diastolic velocities of the LV lateral wall. Diastolic e' and a' waves were expressed as a ratio ($e':a'$) to assess diastolic function. In addition, speckle-tracking echocardiography (STE) was used to derive LV strain. TDI values were obtained from the apical 4-chamber using a pulsed wave Doppler sample gate of 2 – 4 mm at the level of the mitral valve annulus. The cursor was aligned with the longitudinal plane of LV motion to maintain an angle of insonation $< 20^\circ$. LV s' was obtained from averaging three consecutive waves. For longitudinal strain analysis, grey-scale images were recorded from the apical four-chamber view at a

frame rate of 80 frames per second. Images were optimized to visualize the myocardial walls. To derive longitudinal LV strain, the endocardial border was manually traced at end-systole. The region of interest (ROI) was maintained within the myocardial wall.

The software divides each of the LV lateral wall and the septal wall into three segments (basal, mid and apical) and calculates the strain in each segment. An average strain for the entire LV in the 4-chamber plane calculated from the six segments is then provided. The analysis was accepted after visual inspection and when the software indicated adequate tracking. If tracking was suboptimal, the endocardial border was retraced. If satisfactory tracking was not accomplished within 5 min, the non-tracking segments were excluded from analysis. End-systolic strain values were measured at the time of aortic valve closure. The BRT-CO and BRT-SV measurements were timed to the same minute of acquisition of the echo-CO and echo-SV. These NICOM and echocardiography data sets were compared.

2.6 Sonographic Standards and References

All women attending the Rotunda Hospital undergo a formal fetal anatomy scan between 18-22 weeks' gestation. At this point mother's who had a fetal anomaly detected were excluded from the study. All ultrasound data were recorded in an ultrasound software system (Viewpoint; MDI Viewpoint, Jacksonville, FL)

2.6.1 Fetal Biometry and biophysical profiling

Antenatal suspicion of FGR was assessed via sonographic measurement of fetal biometry in the Rotunda Fetal Assessment Unit (FAU). Fetal biometry provides an EFW via calculation of the

fetal head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). The fetal head was examined in the transverse plane at the level of the thalami and cavum septum pellucidum. The fetal head circumference was measured by tracing a track ball in this plane and the biparietal diameter by leading edge to leading edge (outer to inner). The abdominal circumference was measured in a transverse plane of the abdomen at the level of the stomach and umbilical vein and included the outer perimeter of the fetal tissues. The femur length was measured in the longitudinal plane and included the proximal and distal femoral diaphysis excluding the proximal and distal epiphysis(358). The EFW was calculated using the Hadlock 4 equation(359):

$$\text{Log}_{10}\text{EFW} = 1.3596 - 0.00386(\text{AC} \times \text{FL}) + 0.0064(\text{HC}) + 0.00061(\text{BPD} \times \text{AC}) + 0.0425(\text{AC}) + 0.174(\text{FL}).$$

The Biophysical Profile (BPP) is an ultrasound assessment used to evaluate fetal wellbeing. The fetus is scored 8/8 if it meets all ultrasound parameter tests or 10/10 with the addition of a normal fetal non-stress test (CTG). There are 4 sonographic parameters assessed for the BPP each of which can score a maximum 2/2. Fetal breathing movements scores 2/2 when rate is >60; fetal movements scores 2/2 when there are at least 3 gross movements in 30 minutes; fetal tone scores 2/2 when there are one or more episodes of active flexion and extension; amniotic fluid index scores 2/2 when there is a single deepest pool of amniotic fluid that measures greater than 2cm.

2.6.2 Doppler Sonography standards

All Doppler assessments of the umbilical artery and middle cerebral artery were performed in the Rotunda Fetal Assessment Unit.

Umbilical artery (UA) Doppler assessments were performed by identifying a free cord loop and placing the sample gate over the umbilical artery. The waveform was then measured either using the automatic function or manually over 3-5 waveforms. For the purpose of this study we have recorded end-diastolic blood flow and the pulsatility index (PI) when a UA Doppler was performed (360).

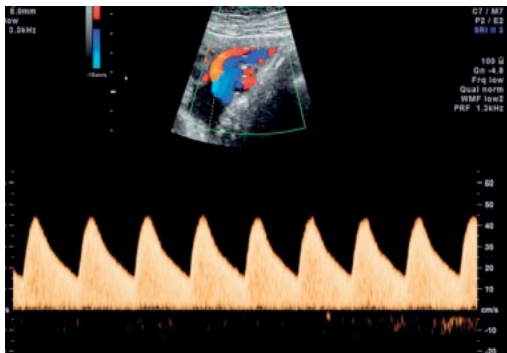


Figure 2-8 A normal umbilical artery waveform with continuing forward flow during diastole.(360).

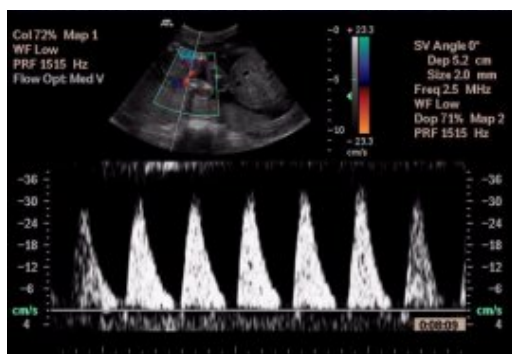


Figure 2-9 An abnormal umbilical artery waveform with absent flow during diastole.

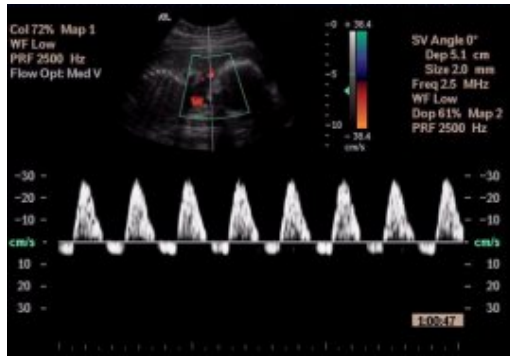


Figure 2-10 An abnormal umbilical artery waveform with reversed flow during diastole.

The middle cerebral artery (MCA) was performed in an axial section of the brain including the thalami and sphenoid bone wings. Colour mapping was used to identify the Circle of Willis and the proximal MCA. Once identified the pulsed wave sample gate was placed over the proximal third of vessel with the angle of insonation kept as close to 0°. Between 3 and 10 recordings were performed and the highest point of the waveform was considered as the peak systolic velocity (PSV).

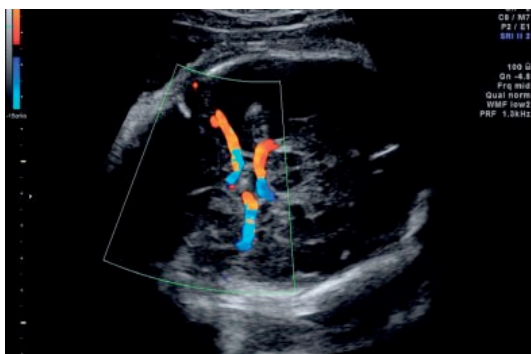


Figure 2-11 Demonstrates colour flow mapping the Circle of Willis.

(360).

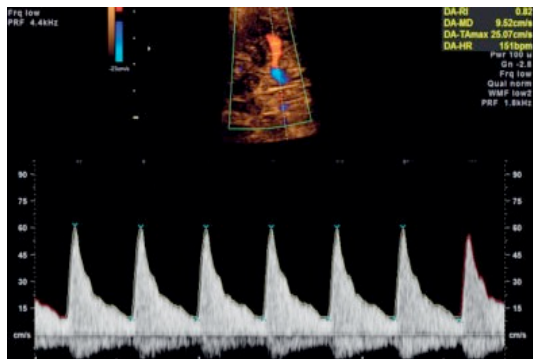


Figure 2-12 An acceptable Middle Cerebral artery Doppler shift waveform with an angle of insonation near 0°.(360)

2.7 Neonatal Echocardiography

A number of senior neonatal clinical research collaborators performed all neonatal evaluations within the first 48 hours of life. A cross section of babies from recruited pregnancies were included across 2 groups: 1- normal healthy appropriately-grown infants at term (defined as birth >37 weeks' gestation) born to mothers without significant maternal illness (diabetes, PE, hypertension, clinical chorioamnionitis, abnormal umbilical artery Doppler at any point of pregnancy); 2- infants where the pregnancy was complicated by hypertensive disease in the absence of proteinuria (GH). Infants in the uncomplicated group were recruited pragmatically at time of neonatal researcher availability. Parental informed consent was obtained prior to echocardiography. Neonatal data collection included birth details, neonatal characteristics including gestational age, timing of echocardiogram, birth weight, and Apgar score at 5 minutes of life. Evaluations were performed using the Vivid S6 echocardiography system (GE Medical, Milwaukee, USA) with a neonatal 7MHz multi-frequency probe. This echo machine is primarily dedicated to research and was available for our study.

A senior neonatal clinical research fellow who has extensive experience in performing and interpretation of echocardiographic

studies performed the echocardiograms. All studies were conducted using a standardized functional protocol adapted from recently published guidelines (361). The scans were recorded as raw data on the machine's internal hard drive and then transferred to the EchoPAC (Version 112, revision 1.3; GE Vingmed) archiving system for offline measurements and validation. Offline analysis of the parameters was performed at a later date and the assessor was blinded to the NICOM® obtained haemodynamic profile of the mother.

A comprehensive echocardiographic assessment to measure biventricular function was performed (111-113, 362). Ejection fraction (EF) was measured using the Simpson's Biplane method, and LV dimensions including length in diastole, mitral valve (MV) annular diameter and LV end diastolic diameter (LVEDD). Deformation imaging [LV and RV systolic longitudinal strain and systolic strain rate] was measured using a validated image acquisition and analysis protocols (363-366) and a frame rate to heart rate ratio (FR/HR) between 0.7 – 0.9 was utilised during imaging for optimal tracking and event timing (365). LV rotational mechanics [apical rotation, basal rotation, twist, twist rate and untwist rate] were obtained. We recorded clockwise rotation as negative, and anticlockwise rotation as positive. The value for twist was the net effect of the apical and basal rotation using the following formula: $\text{Twist (}^\circ\text{)} = \text{apical rotation (}^\circ\text{)} - \text{Basal rotation (}^\circ\text{)}$. Torsion is the value assigned when LV twist is indexed to LV length in end diastole (in cm) using the following formula: $\text{Torsion (}^\circ\text{/cm)} = \text{LV Twist (}^\circ\text{)} \div \text{LV length (cm)}$. Left ventricular twist rate (LVTR) is the speed at which the LV twists ($^\circ\text{/s}$) in systole and LV untwist rate (LVUTR) is the speed at which the LV untwists ($^\circ\text{/s}$) in early diastole.

The RV annulus, mid-cavity diameters, as well as RV length were obtained from an RV-focused four-chamber view at end diastole.

Tricuspid annular plane systolic excursion (TAPSE) was measured from the apical four-chamber view using M-mode (367). Fractional area change (FAC) was measured from the RV four-chamber view (368).

2.8 Pregnancy and Paediatric follow-up

All women were recruited to the study at their first booking visit prior to 16 weeks' gestation. Patient activity was monitored using the Rotunda Hospital iPIMS software and appointments were made with the researchers for the same day as their subsequent visits for their anatomy scan (18-22 weeks) and 28-week antenatal visit (alternatively at time of glucose tolerance testing - GTT, antenatal classes or routine administration of prophylactic Anti-D). The iPIMS system was also used to monitor the date of participants' delivery. Once patients had delivered they were offered to attend the Rotunda after they had reached 6 weeks postpartum. At this visit postnatal variables were collected, participants had a repeat haemodynamic assessment and all infants were offered a paediatric review. As this was an observational study of a low risk population, additional review following the 6-week postnatal review was not deemed necessary.

2.9 Postnatal review.

After a minimum of 6 weeks postpartum all women were invited to attend for their final NICOM® assessment. At the time of this assessment I was present to perform the NICOM® assessments and to provide an obstetric review answering any questions in relation to the course of their pregnancy or the HANDLE study. In addition a neonatal collaborator was also present to perform a 6-week baby check and to address any concerns the first time mothers had in relation to their infants. At this appointment a sub-cohort of women were also invited to participate in a two page

questionnaire to assess patient experience and acceptability of NICOM® assessment (see Appendix 2.3).

2.10 Data Collection and compilation

All data were collected as a single encrypted database on a password-protected study laptop with a back-up copy on the study-encrypted external hard-drive. Patient details were recorded under a specific study number, rather than a patient name and access to the database was password-protected to ensure data protection. The maternal and neonatal data collection was completed at the time of six-week postnatal review. The variables collected included maternal demographics as detailed earlier, antenatal course of each pregnancy to include antenatal complications, medications and admissions; details on delivery outcome were recorded including gestational age at delivery, indication for induction, mode of delivery, indication for mode of delivery, birth weight, gender and maternal complications during birth.

Neonatal characteristics included gestational age at delivery, birth weight, if small for gestation, arterial and venous cord blood pH (where available), Apgar scores, colour of amniotic fluid, evidence of prolonged rupture of membranes, other risk factors for sepsis, admission to the neonatal intensive care, course of neonatal care, length of neonatal stay and adverse perinatal outcome. For the purpose of the HANDLE analysis, adverse perinatal outcome was defined as a composite outcome of intraventricular haemorrhage (IVH -all grades), periventricular leucomalacia (PVL), hypoxic ischaemic encephalopathy (HIE - all grades), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), sepsis or death. Given that the study site is a member of the Vermont Oxford Network, definitions for IVH, PVL, HIE, NEC, BPD and sepsis were standardized as derived from the Vermont Oxford Network Manual(369).

2.11 Equipment, Resources and Quality assurance

With Health Research Board (HRB) funding the study team purchased two NICOM® monitors (Cheetah, Medical, Maidenhead, Berkshire, United Kingdom) and 1,600 sets of NICOM® electrode stickers (400 patients x 4 time points) to perform haemodynamic assessments on all recruits. In addition, a laptop and an external hard drive were purchased for sole use in this study. The HRB grant also funded a research assistant post to help with recruitment and co-ordinate haemodynamic assessments at participant's subsequent reviews. (Rachel Cushion, Lucy Shirren, Lisa McSweeney)

The following resources were available to the study group:

- Use of the Rotunda adult outpatient department for both the recruitment of patients and performance of the haemodynamic assessments.
- Use of the Rotunda day-care assessment unit for the performance of all Maternal Echoes and haemodynamic assessments.
- Use of the Rotunda paediatric outpatient department for the postnatal reviews of all recruited mothers and babies.
- Administrative support from the Rotunda Hospital staff in accessing all maternal and neonatal charts.
- Use of the Vivid echocardiography system (GE Medical, Milwaukee) courtesy of the Neonatal Research Department
- Use of the adult cardiology multi-frequency probe (courtesy of the Department of Anaesthesia).

Quality assurance-

This study was approved under the supervision of the Perinatal Ireland Research Group and received a charitable grant from the Rotunda Foundation and the Health Research Board. Professor Fergal Malone assumed overall responsibility and control for project management and coordinated the contributions of the rest

of the investigators involved. He controlled, with the aid of the project manager (Dr. Elizabeth Tully), the financial and administrative aspects of the project. The steering group comprised of Professor F. Malone, Dr. E. Tully, Dr. C. Monteith, Prof. A. EL-Khuffash, Dr. P. Thorton, Dr. C. Breathnach, Dr. A. Doherty, Dr. E. Kent and L. McSweeney. The steering group formally met 15 times since the study commenced and have submitted annual progress reports to the Heath Research Board in June 2014 and June 2015. The findings of this study are presented in keeping with the STROBE checklist for observational studies (Appendix 2.4).

2.12 Feasibility of the study

I have actively participated and overseen the day-to-day management of the recruitment of patients and reported regularly to the team on activities and progress to ensure the project proceeded in a timely manner. I estimated that over the two-year period, we would be able to identify close to 8,000 nulliparous patients who were booking for antenatal care in the Rotunda Hospital and estimated that 80% of these would meet the inclusion criteria for our study. Given their low risk demographic background, many of these women would be suitable for midwifery-led care in Rotunda satellite clinics. Therefore over the full twenty-four months of the study, the required numbers for recruitment (400) were achieved; taking into account time for lead-in and study closeout. The existing RCSI infrastructure and governance strategies allowed the proposed study to be effectively implemented without difficulty. Perinatal Ireland and the RCSI Department of Obstetrics and Gynaecology have run three major national studies (ESPRiT, PORTO and Genesis) in recent years and to this effect, have demonstrated an excellent track record in clinical project management.

2.13 Ethics and Consent

Institutional ethical approval was granted by the Rotunda Research Ethics Committee (reference REC-2013-017 Appendix 2.4).

Prior to commencing the study, patients were provided with an information leaflet (Appendix 2.6) and a copy of the maternal consent form (Appendix 2.7). All recruits completed a written informed consent prior to commencement. This included full disclosure of the nature, risks and benefits of participation in the study.

2.14 Funding

This project was supported by the following research grants:

Medical Research Charities Group/ Health Research Board of Ireland; Grant file reference MCRG/2013/9 (€188,125.70)

Applicants: Professor Fergal Malone, Prof. Afif EL-Khuffash, Dr. Etaoin Kent, Dr. Niamh Hayes, Dr. Anne Doherty

Rotunda Foundation (formerly known as Friends of the Rotunda) Research Grant (Reference: FoR/EQUIPMENT/101572)

Purchase of the Vivid echocardiography system

Applicants: Prof. Afif EL-Khuffash

Chapter 3 VALIDATION

My hypothesis is that NICOM® is a valid alternative to echocardiography in the haemodynamic assessment of pregnant women.

3.1- The validity of NICOM® as a method of haemodynamic assessment.

To allow for validation of NICOM® in the obstetric population a small cross sectional study of 35 women from the overall patient cohort of 366 were selected to undergo simultaneous echocardiographic and NICOM® assessments. I aimed to compare simultaneous cardiac output readings obtained using NICOM by means of bioimpedance and echocardiography in a group of healthy nulliparous women, and assess the relationship between maternal characteristics, cardiac output and myocardial performance.

Continuous variables were tested for normality using the Shapiro-Wilk test and presented as means and standard deviations (SD) or medians (inter-quartile range IQR) as appropriate. Paired data were compared using a paired t-test or a Wilcoxon signed-rank test as appropriate. Independent data were compared using an independent student t-test or a Mann Whitney U test as appropriate. Correlations between variables of interest were tested using Pearson Correlation Coefficient. Agreement between ECHO and bioimpedance measured stroke volume (SV) and cardiac output (CO) was tested using Bland Altman analysis (to derive bias and limits of agreement (LOA) between the two methods) and the intra-class correlation coefficient (ICC version 2.1).

The percentage error between the SV and CO measurements obtained from bioimpedance and ECHO was calculated using the following formula (370):

$$\text{Mean percentage error} = \frac{(100 \times 1.96 \times \text{SD of bias between the two methods})}{\text{Mean CO between the two methods}}$$

A mean percentage error is considered acceptable if the value is less than 30%.(229) In addition, I calculated the precision of bioreactance CO measurements in each subject by calculating the coefficient of error (CE) of 13 bioreactance- CO measurements obtained one minute apart from subjects in a resting state when no changes in CO were expected.

The following formula for coefficient of error was used, where n represents the number of repeated measurements:

$$CE = \frac{\text{Coefficient of variation (CV)}}{\sqrt{n}}$$

CV was calculated as follows:

(SD of absolute differences between repeated measurements ÷ mean of all repeated measurements) × 100%

SPSS version 23 (IBM Corporation, NY, USA) was used to conduct the analyses and a p-value <0.05 was deemed as statistically significant.

The 35 women had a mean (±SD) age and booking weight of 29.6 (±5.5) years and 67.8 (±14.7) kg respectively and underwent paired haemodynamic assessment to coincide with either the 20 week anomaly scan visit or their 28 week routine antenatal visit. Of the 35 women undergoing the paired assessments there were 2 (5.7%) cases complicated by preeclampsia and a further 3 (8.6%) cases complicated by fetal growth restriction. The demographics of participants in this cross sectional study are further detailed in Table 3.1.

Table 3-1 Maternal Demographics and Fetal characteristics (n=35).

Characteristic	N (%) / mean +/- SD
Age, years	29.6 (±5.5)
Ethnicity (White European)	31 (88.6)
Spontaneous Conception	34 (97.1)
Maternal height, cm	164.7 ±5.1
Maternal weight at booking, kg	67.8 ±14.7
BMI, kg/m ²	25.0 ± 5.0
Smokers	9 (25.7)
FGR	3 (8.6)
PE	2 (5.7)
GA at delivery, weeks	39.3 ± 2.2
Birthweight, g	3258 ± 604

BMI- body mass index, FGR- fetal growth restriction, GH- gestational hypertension, PE- preeclampsia, GA- Gestational age

Of those undergoing paired haemodynamic assessment 11 (31.4%) were performed to coincide with the anomaly scan with 24 (68.6%) coinciding with the 28-week visit. This resulted in a median [IQR] gestational age of assessment of 28 [21 – 28] weeks. Table 3.2 illustrates the stroke volume (SV), cardiac output (CO) and heart rate (HR) using the two techniques in the overall cohort. There was no overall difference in the echo obtained CO (BRT-CO) and the NICOM® CO (NICOM-CO) or between the BRT-HR and NICOM-HR. NICOM-SV was lower than BRT-SV, however, the difference was small.

Table 3-2 Observed differences between NICOM® and echocardiography in the cohort (n=35).

	NICOM®	ECHO	p-value
Heart Rate bpm	88 (13)	87 (14)	0.6
Cardiac Output (L/min)	6.1 (1.1)	6.4 (1.1)	0.1
Stroke Volume (ml)	69 (15)	75 (18)	0.01

Values are presented as means \pm SD and compared via a paired t-test.

When assessing independently the second and third trimester values there was no difference between the SV, CO and HR values obtained via the two techniques at 20 weeks' gestational age. During assessment at 28 weeks' gestational age there was no difference in values obtained for HR or CO. While the differences in SV measurements were small at 6ml, they reached statistical significance ($p=0.02$). Overall, the NICOM® obtained values continued to be acceptable independent of gestational age, detailed further in Tables 3.3 & 3.4.

Table 3-3 Difference between NICOM® and ECHO when assessed at 20 weeks' gestational age.

	NICOM®	ECHO	p-value
Heart Rate	81 (10)	82 (12)	0.94
Cardiac Output	6.2 (1.1)	6.0 (1.0)	0.48
Stroke Volume	78 (16)	73 (13)	0.22

Values are presented as means \pm SD and compared via a paired t-test.

Table 3-4 Difference between NICOM® and ECHO when assessed at 28 weeks' gestational age.

	NICOM®	ECHO	p-value
Heart Rate	91 (15)	91 (12)	0.59
Cardiac Output	6.4 (1.1)	6.2 (1.2)	0.14
Stroke Volume	73 (19)	67 (17)	0.02

Values are presented as means +/- SD and compared via a paired t-test.

There was good agreement between the stroke volumes ascertained by ECHO and bioreactance with a mean bias of 6ml (LOA -18 – 19) and an Intraclass Correlation Coefficient (ICC) of 0.8 (95% CI 0.6- 0.9; $p < 0.001$). Similarly there was good agreement between the measurement of CO from the two methods with a mean bias of 0.2L (LOA -1.3 – 1.7) and an ICC of 0.8 (95% CI 0.7 – 0.9; $p < 0.001$) – see Table 3.5. The mean percentage error between the two methodologies was $\pm 26\%$. There was a strong correlation between both methodologies measuring SV and CO, as further detailed in Figure 3.1. There was no difference in the mean bias of CO (0.21 vs. 0.24L), or SV (5 vs. 6mL) between subjects assessed during the second vs. third trimesters (all $p > 0.5$). Similarly, there was no difference in the mean bias of CO or SV between the lowest and highest age quartiles (all $p > 0.4$). The coefficient of error (CE) representing the precision of BRT-CO was 3.4% (Table 3.5).

Table 3-5 Agreement between Echocardiography and NICOM® measurements.

Measurement and Type of Analysis	Result
Stroke volume	
<i>Bias and Limits of Agreement*</i>	6mL (-18 – 29)
<i>ICC (95% Confidence interval)</i>	0.8 (0.6 – 0.9)
<i>Correlation (r, p value)</i>	0.8, p<0.001
<i>Mean Percentage Error</i>	±29%
<i>NICOM Coefficient of Error (Precision)</i>	3.9%
Cardiac output	
<i>Bias and Limits of Agreement*</i>	0.2L (-1.3 – 1.7)
<i>ICC (95% Confidence interval)</i>	0.8 (0.7 – 0.9)
<i>Correlation (r, p value)</i>	0.7, <0.001
<i>Mean Percentage Error</i>	±26%
<i>NICOM Coefficient of Error (Precision)</i>	3.4%

*Bland Altman Analysis; ICC: Intraclass correlation coefficient

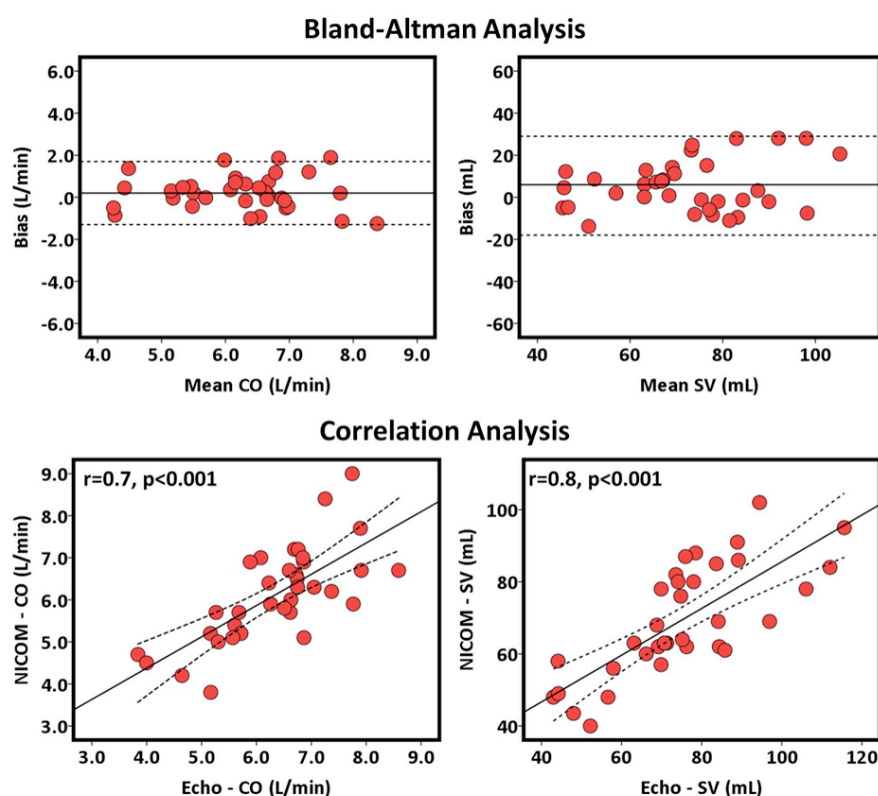


Figure 3-1 Bland Altman and correlation analyses between NICOM and echocardiography measured cardiac output and stroke volume.

3.2 Patient acceptability of NICOM® haemodynamic assessment.

Integrated into the HANDLE study was a questionnaire study designed to understand participants' experience and acceptability of NICOM® haemodynamic assessment. To date there have been no studies evaluating if patients have found this novel method to be an acceptable alternative for assessing their haemodynamic status. Additional ethical approval for this element was requested May 2016 and granted in June 2016. This allowed for patient participation via a questionnaire (Appendix 2.3) at the postnatal visits between July 2016 and October 2016. Ninety-five women who attended a postnatal review between July 2016 and October 2016 were invited to participate. Patient uptake of the questionnaire was excellent at 83.2%. Non-participation was a result of providing care for their infant or other commitments requiring prompt exit from the clinic. In addition, 10 women who self-selected to withdraw from the study were approached and asked to complete the questionnaire as their experience could prove invaluable during conceptualisation of any future study methodologies. Descriptive statistics were provided via SPSS version 23 (IBM Corporation, NY, USA).

The questionnaire was completed and recorded anonymously by a total of 89 women, representing 24.3% of the overall study population. Of the 89 women a total of 74 (83.1%) completed the study protocol and attended all four episodes of patient contact. Of 11 women surveyed who specified a missed appointment the most frequent missed study interaction in six (54.5%) women coincided with the fetal anatomy scan. The reasons for participants missing a haemodynamic assessment are detailed further in Table 3.6.

Table 3-6 Reasons participants were unable to complete haemodynamic assessment (n=14).

Reason	N (%)
Change of coinciding hospital appointment	4 (28.6)
Did not like sensation of BP cuff	1 (7.1)
Attending external satellite clinic	2 (14.3)
Work commitments	5 (35.7)
Family Bereavement	1 (7.1)
Monitoring took too long	1 (7.1)

Overall participant responses to the study were positive with 97% of those surveyed reporting that they would choose to partake in a similar study in a future pregnancy. This increased to 100% if patient-suggested improvements such as providing a patient copy of their individualised report or the addition of a designated research area in the outpatient department were included. The maximum number of haemodynamic assessments, which was felt to be acceptable, was calculated as a median [IQR] of 4 [3-4]. Patient experience in relation to the NICOM® monitor was similarly positive and is detailed further in the histograms in Figures 3.2 & 3.3.

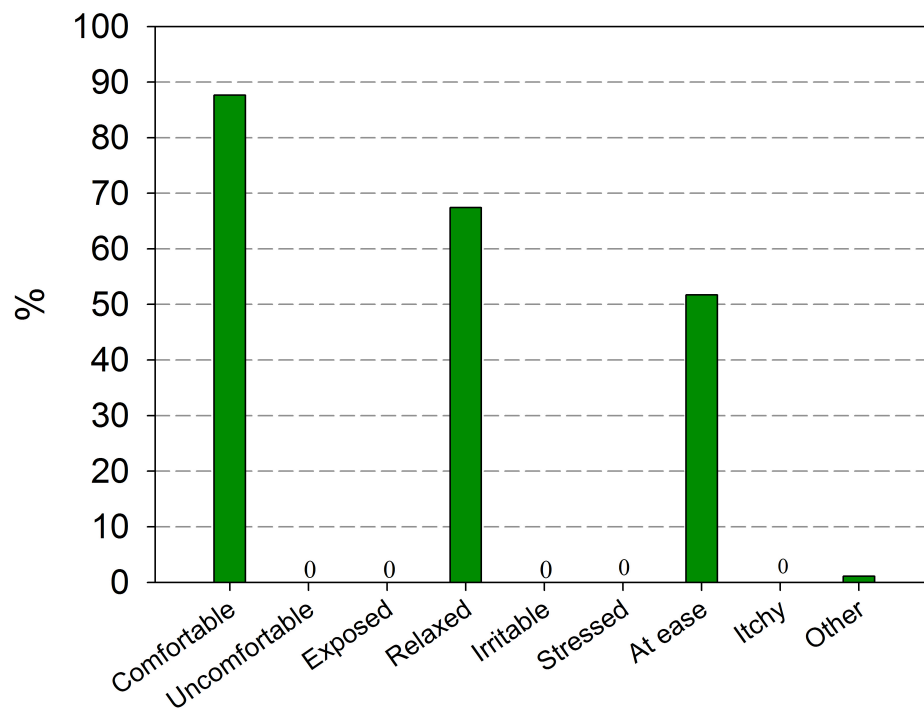


Figure 3-2 During the NICOM assessment participants felt.

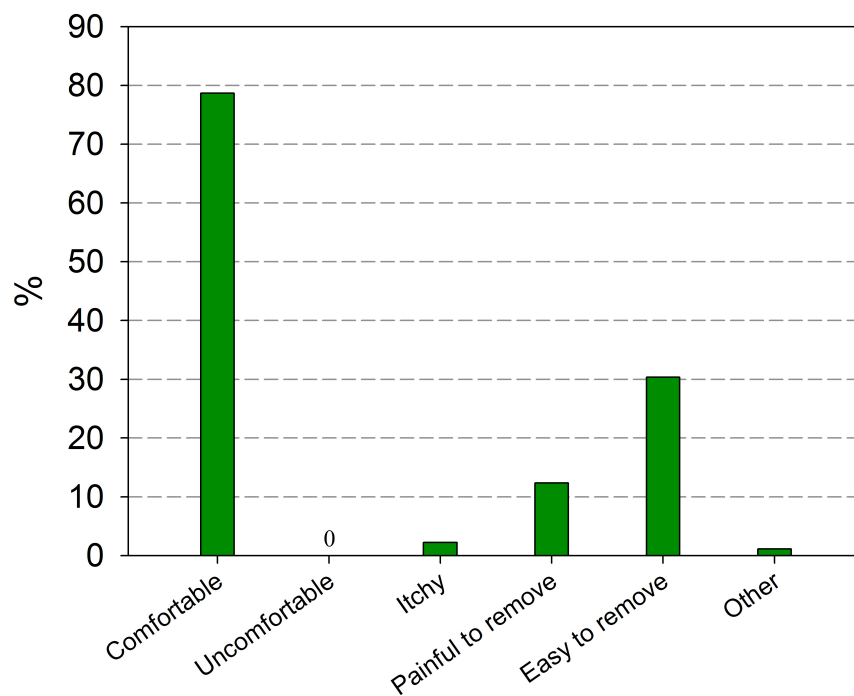


Figure 3-3 Participants' perception of the NICOM sensors.

Chapter 4 RESULTS -DEMOGRAPHICS

4.1 HANDLE patient population results and demographics.

Recruitment to the HANDLE study commenced in May 2014 and concluded in January 2016. During this time 15,298 women booked to the Rotunda Hospital of whom, 6,324 (41.3%) were nulliparous. Of all nulliparous women booking to the hospital during the study period a resultant 422 women from the public clinic were recruited to the study. For feasibility of patient follow-up this study was limited to patients attending the public clinic. Women booked through public clinic are provided with state-funded care. This is team led by a group of Consultant Obstetricians and Non-Consultant Hospital Doctors. Whereas women booked to the private clinic access services in a separate building of the hospital. Their care is provided through self-funded health insurance packages and additional premiums for individual consultant led care.

Of the 422 low risk nulliparous women recruited to the study 19 were excluded from the analysis: two (0.4%) due to chromosomal abnormality (Trisomy 18 and di George), eight (1.9%) miscarriages, two (0.4%) intrauterine deaths at 27 weeks' gestation (one unexplained, one placental abruption in a normotensive patient), three (0.7%) cases of multiple pregnancy, one (0.2%) patient was recruited to another research study and three women were recruited to the study in error (one using low molecular weight heparin, one taking aspirin and one with pre-existing hypertension). In addition to those excluded, 19 (4.5%) women did not attend subsequent NICOM® assessments and 18 (4.3%) delivered at an alternative obstetric unit and were lost to follow-up. This resulted in 366 patients completing the study protocol. Figure 4.1 & Table 4.1 further detail recruitment and reasons for not completing the study protocol.

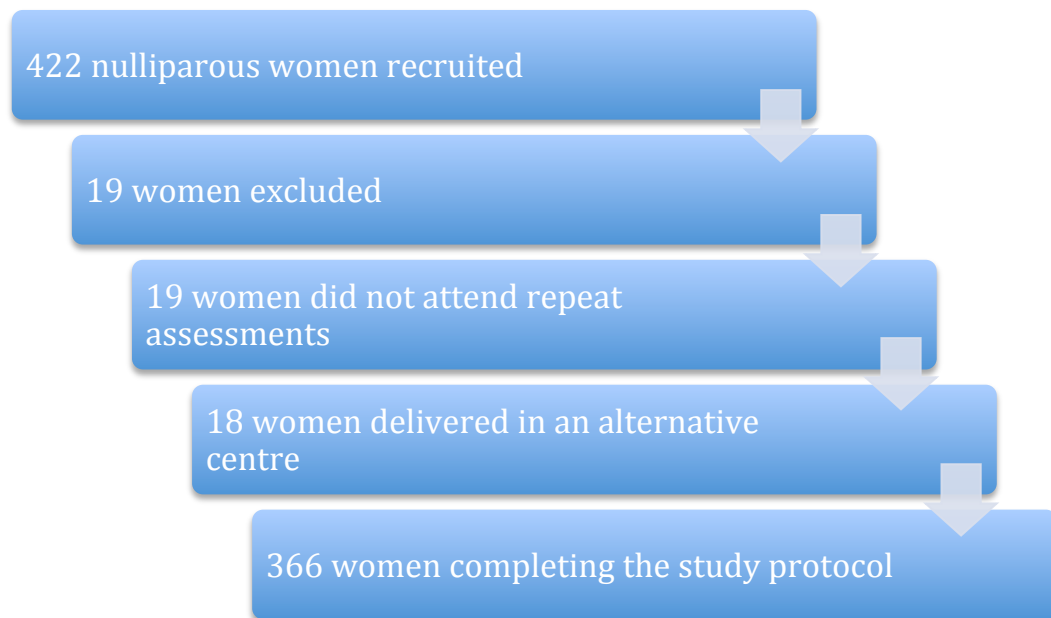


Figure 4-1 HANDLE recruitment and overall outcomes.

Table 4-1 Reasons for patient not completing the study protocol (n=56).

Reason for withdrawal	N=
Did not attend repeat assessments	19
Excluded from analysis	Total =19
Genetic anomaly	2
Intrauterine death	2
Miscarriage	8
Multiple pregnancy	3
Simultaneous recruitment to another study	1
Recruited in error	3
Delivered in another unit	Total =18
Delivered in Ireland	6
Delivered abroad	8
Unknown	4

As part of the study protocol all 366 women who completed the antenatal element were offered a postnatal review, neonatal check and a repeat NICOM assessment. Maternal outcomes were assigned at the time of the postnatal visit and Table 4.2 details patient outcomes for the 366 women who completed the study.

Table 4-2 Patient outcome.

Patient outcome	Number	Percentage
PE	6	1.6%
GH	18	4.9%
FGR	24	6.6%
Normal controls	318	86.9%
Total	366	

Abbreviations: PE- preeclampsia; GH- gestational hypertension; FGR- fetal growth restriction.

4.2 Patient Demographics:

There were 366 women recruited who completed the study protocol. The mean (\pm SD) gestational age at time of enrolment was 13.2 (\pm 1.4). The median [interquartile range- IQR] age of participants at time of recruitment was 29.0 years [22-36 years]. Table 4.3 details the maternal demographics and fetal characteristics of the cohort. The median weight and height of participants was 64.0kg [46.7 – 81.3kg] and 166.0cm [158 -174cm] respectively. This resulted in a mean body mass index (BMI) of 23.3kg/m² [17.7 – 28.8kg/m²] for the cohort. There were fewer than anticipated pregnancies complicated by preeclampsia, with an incidence of 1.6% in this cohort. This is in contrast to the 5-7% preeclampsia rate in nulliparous women that is quoted in the literature (26, 33, 371, 372).

Table 4-3 Maternal Demographics and Fetal characteristics (n=366).

Characteristic	Overall cohort N=366	Control N=318	PE N=6	GH N=18	FGR N=24
Age, years	29.0 [22-36]	29.0 [22-36]	33 [21-45]	30.5 [20.5 - 40.5]	28.5 [19.8 - 37.3]
Ethnicity					
-White European	316 (86.3)	276(86.8)	6 (100)	15(83.3)	19 (79.1)
-African	6 (1.6)	6 (1.9)	0 (0)	0 (0)	0 (0)
-Asian	11 (3.0)	7 (2.2)	0 (0)	2 (11.1)	2 (8.3)
Single	206 (56.3)	180 (56.6)	4 (66.7)	10 (55.6)	12 (50)
Tertiary education	205 (56.0)	177 (55.7)	2 (33.3)	12 (66.7)	14 (58.3)
Spontaneous Conception	351 (95.9)	307 (96.5)	6 (100)	16 (88.9)	22 (91.7)
Maternal height, cm	166 [158- 174]	166 [158- 174]	164.5 [151.5- 177.5]	167 [157- 177]	163.0 [152.2 - 173.8]
Maternal weight at booking, kg	64 [46.7-81.3]	64.2 [47.2 – 81.2]	66.2 [49.6- 82.8]	64.8 [43.9 – 85.7]	60.7 [46.7 - 74.6]*
BMI, kg/m ²	23.2 [17.7 -28.8]	23.4 [17.4 - 29.4]	24.8 [16.9 – 32.7]	24.4 [15.2- 33.6]	22.2 [17.7 - 26.7]
MAP, mmHg	88.2 ±7.4	87.9 ±7.1	91.5 ±5.0	96.5 ±4.6*	84.6 ±7.8
Smokers	66 (18.0)	56 (17.6)	1 (16.7)	2 (11.1)	7 (29.2)
FHx HTN	89 (24.3)	77 (24.2)	1 (16.7)	6 (33.3)	5 (20.8)
FHx DM	34 (9.3)	32 (10.1)	0 (0)	1 (5.6)	1 (4.2)
FHx both	43 (11.7)	35 (11.0)	1 (16.7)	4 (22.2)	3 (12.5)
GA at enrolment, weeks	13.2 ± 1.4	13.2 ± 1.5	14.0 ± 2.7	13.8 ± 1.7	12.8 ± 1.7
GA at delivery, weeks	39.8 ± 1.8	39.9 ± 1.8	36.0 ± 2.8 **	39.7 ± 1.4	39.6 ± 1.5
Birthweight, g	3399 ± 529	3475 ± 480	2478 ± 773**	3456 ± 477	2728 ± 332 **
Apgar at 5 minutes	10 [10-10]	10 [10-10]	10 [10-10]	10 [10-10]	10 [10-10]
Arterial Cord pH <7.1	6 (1.6)	5 (1.6)	0 (0)	0 (0)	1 (4.2)
NICU admission	38 (10.4)	32 (10.0)	2 (33.3)	1 (5.6)	3 (12.5)
Adverse perinatal outcome	2 (0.5)	2 (0.6)	0 (0)	0 (0)	0 (0)
Neonatal Deaths	1 (0.3)	1 (0.3)	0(0)	0(0)	0(0)

BMI- Body mass index, GH- Gestational hypertension, PE- preeclampsia, MAP- mean arterial pressure, FHx – Family History, HTN-hypertension, DM- Diabetes Mellitus, GA- Gestational age, FGR- fetal growth restriction and NICU- Neonatal intensive care. Figures presented as N (%), median [IQR] or mean ± SD. * p-value <0.05 ** p-value <0.01

A reduction in the incidence of preeclampsia in nulliparous women has been observed in the overall Rotunda population. During the study period only 3.3%, 2.4% and 2.8% of nulliparous women in 2014, 2015 and 2016 respectively had a pregnancy complicated by preeclampsia. Figure 4.2 further details the significant ($p < 0.001$) falling incidence of preeclampsia in the Rotunda nulliparous population between 2004 and 2016. During the 13 year period a total of 107,673 women were delivered of whom 3,117 (2.9%) had a pregnancy complicated by PE. During the 13 years the mean PE rate in nulliparous women was 4.3% and in multiparous women 1.8%.

There was an overall reduction in nulliparous women with PE in this time period from a peak of 5.3% in 2005 to a trough of 2.4% in 2015 ($p < 0.001$). Similar reductions, detailed in Table 4.4, were observed in the rates of eclampsia ($p = 0.02$), induction of labour for hypertension ($p = 0.03$), elective caesarean section indicated by PE (0.03) and high dependency unit admissions for hypertension ($p = 0.03$). There was no difference in the overall number of pregnancies delivering post-term (> 41 weeks' gestation) detailed in Figure 4.3. To assess for selection bias, patient demographics were compared to the Rotunda Annual Report data for 2014 and 2015 where available (373, 374), see Figures 4.4 – 4.7.

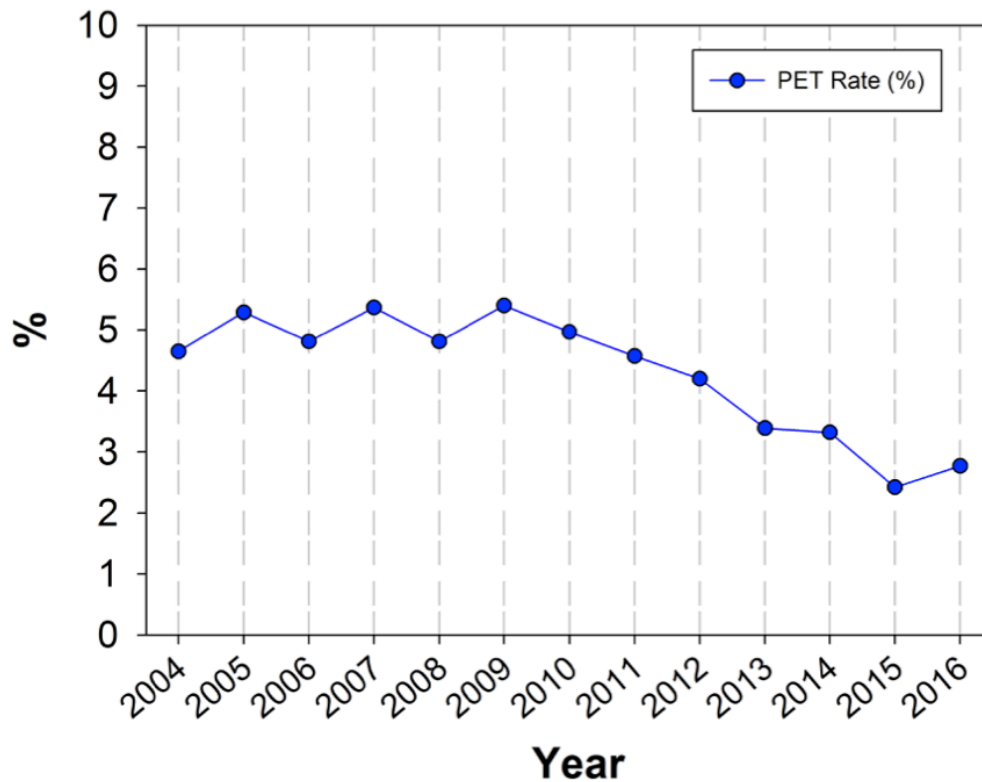


Figure 4-2 Incidence of preeclampsia in the Rotunda Hospital between 2004 -2016.

There was a significant reduction in preeclampsia $p<0.001$ after 2009.

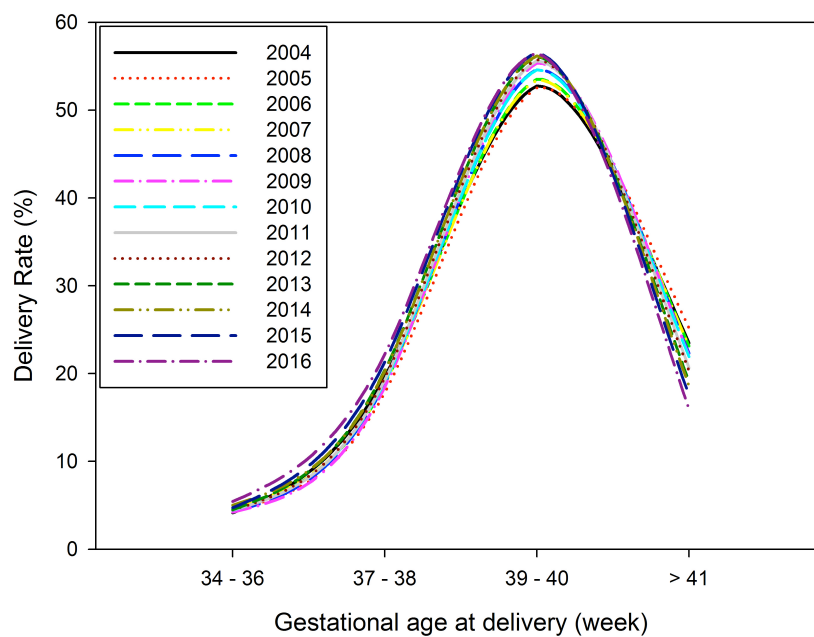


Figure 4-3 Trends in gestational age at time of delivery between 2004 - 2016.

Table 4-4 Changing demographics and preeclampsia prevalence.

Outcome	Years 2004-2009	Years 2010 - 2016	P-value*
Live births	7816 ± 945	8682 ± 262	0.015
PE rate (%)	3.5 ± 0.3	2.4 ± 0.7	<0.001
Nulliparous births	3568 ± 577	3778 ± 245	0.249
Nullip eclampsia (total)	10	1	0.024
Nullip PE rate (%)	5.1 ± 0.3	3.7 ± 0.9	<0.001
IoL	1577 ± 298	2497 ± 104	<0.001
HTN rate (%) in IoL	12 ± 2	10 ± 2	0.031
LSCS	2073 ± 306	2662 ± 160	<0.001
Nullip LSCS	1298 ± 196	1581 ± 98	<0.001
PE rate (%) in Nullip LSCS	2.2 ± 0.4	1.5 ± 0.4	0.106
GH rate (%) in Nullip LSCS	1.6 ± 0.7	1.3 ± 0.5	0.089
Multip LSCS	773 ± 118	1077 ± 88	<0.001
PE rate (%) in Multip LSCS	1.2 ± 0.3	0.8 ± 0.3	0.036
GH rate (%) in Multip LSCS	1.1 ± 0.3	0.8 ± 0.3	0.028
HDU	133 ± 20	201 ± 30	<0.001
HTN rate (%) in HDU	37 ± 7	25 ± 6	0.032
PE rate (%) < 36 weeks	4.79 ± 5.06	3.79 ± - 2.07	0.955
PE rate (%) 26-20 weeks	0.12 ± 0.05	0.15 ± 0.07	0.702
PE rate (%) 30-33 weeks	0.14 ± 0.02	0.14 ± 0.05	0.543
PE rate (%) 34-36 weeks	0.09 ± 0.02	0.10 ± 0.04	0.845
PE rate (%) 37-38 weeks	0.04 ± 0.01	0.05 ± 0.02	0.244
PE rate (%) 39-40 weeks	0.02 ± 0.01	0.02 ± 0.01	0.053
PE rate (%) > 41 weeks	0.01 ± 0.00	0.01 ± 0.01	0.063

Abbreviations: PE- preeclampsia; IoL- induction of labour; HTN- hypertension; LSCS- lower segment caesarean section; Nullip- nulliparous; Multip- multiparous; GH- gestational hypertension; HDU- high dependency unit.

Figure 4.4 and Figure 4.5 detail the spread of age and BMI respectively across the cohort in comparison to the total Rotunda nulliparous population. Almost 20% (n=63) of the study cohort were current smokers at time of their first antenatal visit. Of the women who were actively smoking, 25% admitted to moderate/heavy smoking (>10 cigarettes per day). The majority of the patient population were White European 86% (n=316). Figure 4.6 details further the ethnic diversity across the cohort.

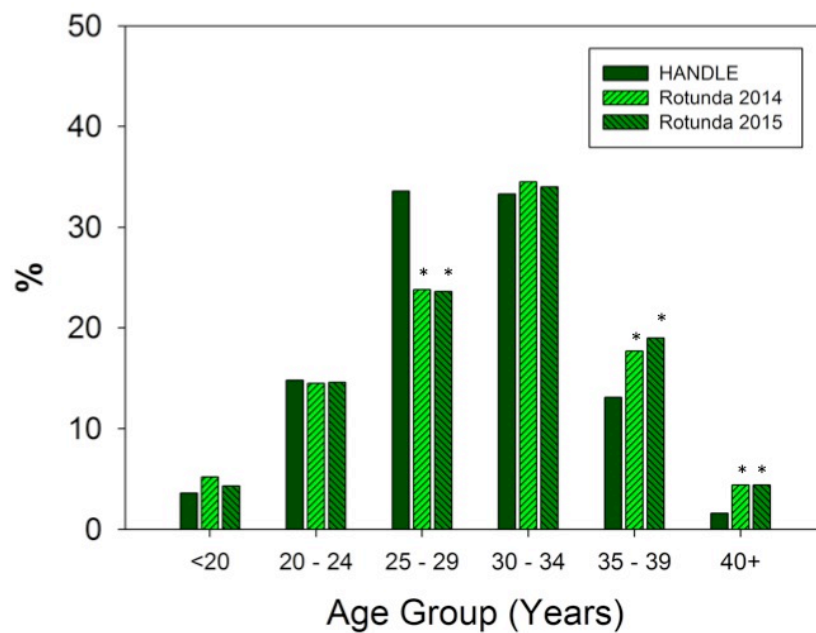


Figure 4-4 A comparison of age between the HANDLE cohort and the Rotunda Annual report nulliparous data.

* denotes a p-value <0.05.

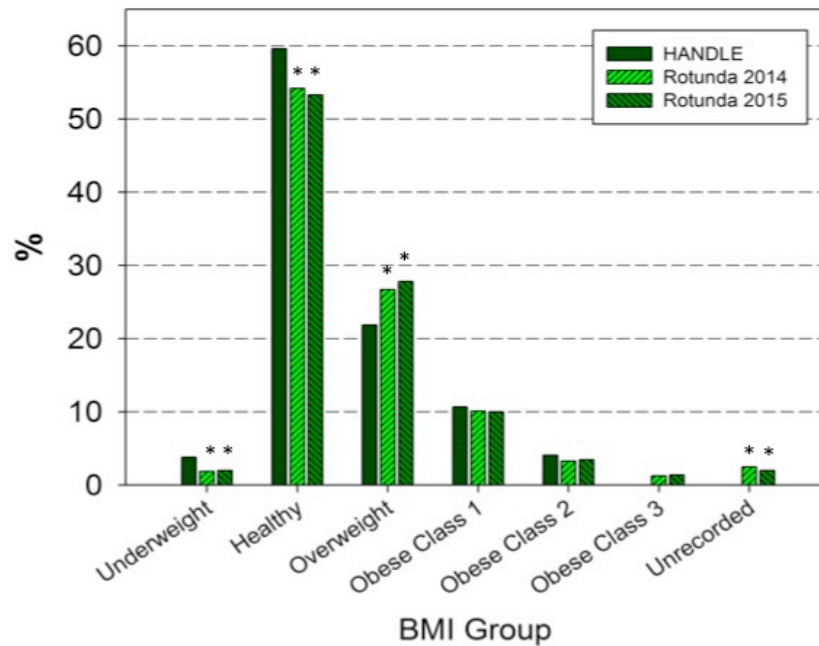


Figure 4-5 A comparison of BMI between the HANDLE cohort and the Rotunda Annual Report data (nulliparous & multiparous).

* denotes a p-value <0.05.

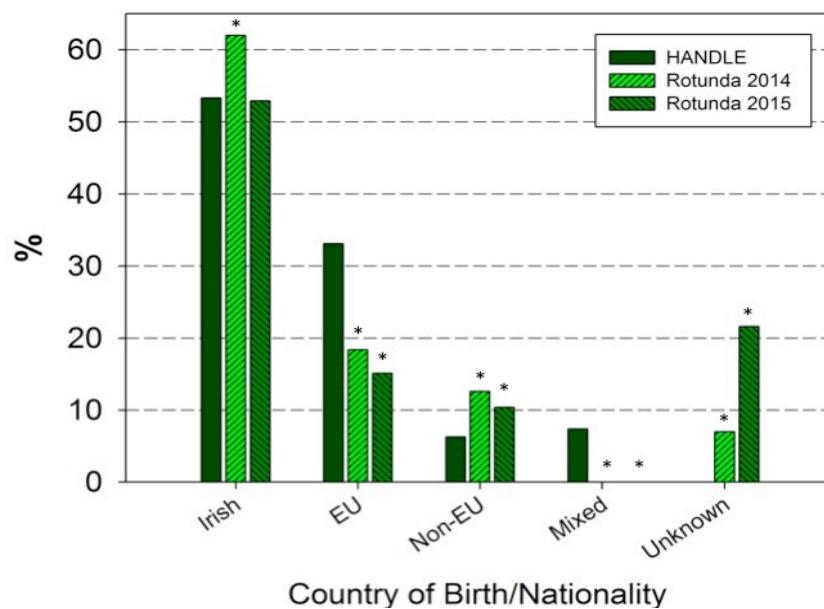


Figure 4-6 Country of birth & Nationality at time of delivery. Comparison between HANDLE cohort and Rotunda population (nulliparous & multiparous).

* denotes a p-value <0.05.

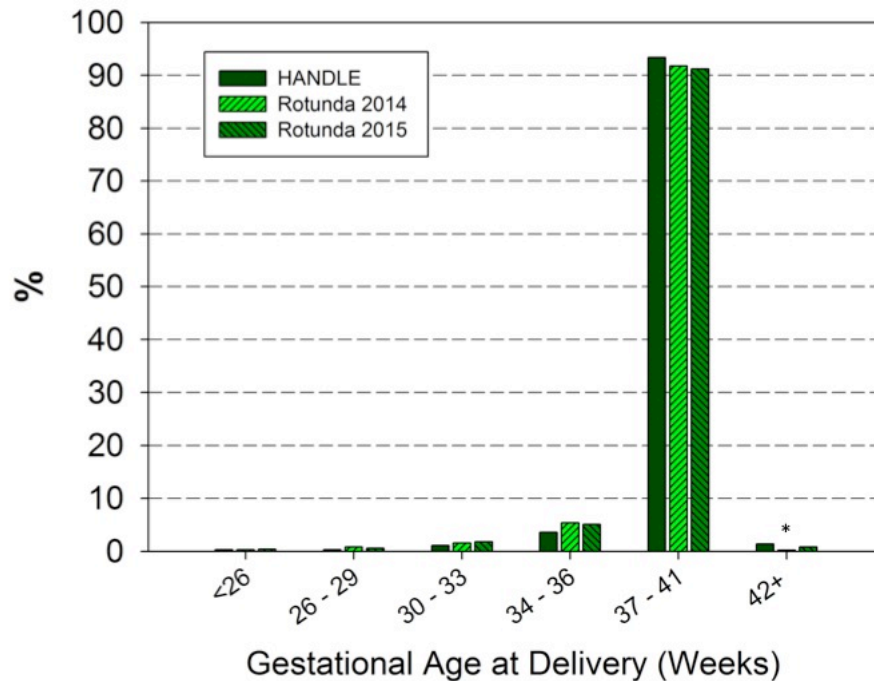


Figure 4-7 Comparison of the HANDLE cohort and Rotunda Nulliparous population and gestational age at time of delivery.

A formal repeat ultrasound was performed in the fetal assessment unit in 42.9% (n=157) of the cohort. A repeat departmental ultrasound was performed once in 95.5% (n=150), twice in 1.3% (n=2), three times in 1.9% (n=3) and four times in 1.4% (n=2). The most common indication for repeat imaging was placenta location in 15.3% (n=24) Table 4.5 details the indications for repeat departmental imaging. Sixteen women underwent a repeat departmental ultrasound the clinical indications for which included: suspected FGR (n=9), incomplete cardiac views (n=1), routine third trimester growth for GDM (n=2), placenta location (n=1), query rupture of membranes (n=1) and reduced fetal movements (n=2). Irrespective of clinical indication for repeat ultrasound only 4 (25%) pregnancies where an infant was delivered <10th centile did the corresponding antenatal imaging suggest a diagnosis of FGR.

Table 4-5 Indication for repeat department sonography in nulliparous patient.

Clinical indication	Frequency - n (%)
Placental location	24 (15.3)
Decreased fetal movements	23 (14.6)
Inpatient	23 (14.6)
Query FGR	21 (13.4)
GDM routine 3 rd trimester growth	21 (13.4)
Incomplete cardiac view	19 (12.1)
Multiple indications	14 (8.9)
Query oligohydramnios	3 (1.9)
Query polyhydramnios	2 (1.3)
Query rupture of membranes	2 (1.3)
Known 2 vessel cord	2 (1.3)
Query maternal cholestasis	1 (0.6)
Query macrosomia	1 (0.6)
External US query anomaly	1 (0.6)

Abbreviations: FGR- fetal growth restriction, GDM- Gestational Diabetes, US- ultrasound.

Of the 366 pregnancies completing the study protocol 6.6% (n=24) had a pre-labour caesarean section with the indication in over 50% (n=11) being a breech presentation. When a trial of labour was considered appropriate, induction of labour occurred in 36.6% (n=134), regional analgesia (epidural or combined spinal epidural) was used in 64.5% (n=236) and overall a vaginal delivery was achieved in 76.0% (n=278). These findings are detailed further in Figures 4.8 and 4.9.

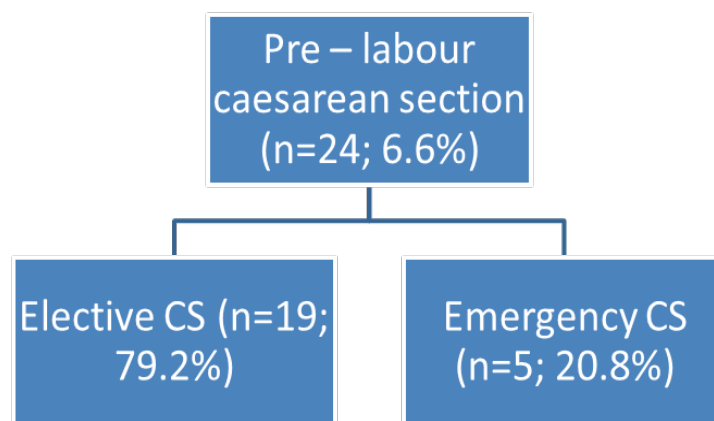


Figure 4-8 Pre-labour caesarean delivery in the overall HANDLE cohort (n=366).

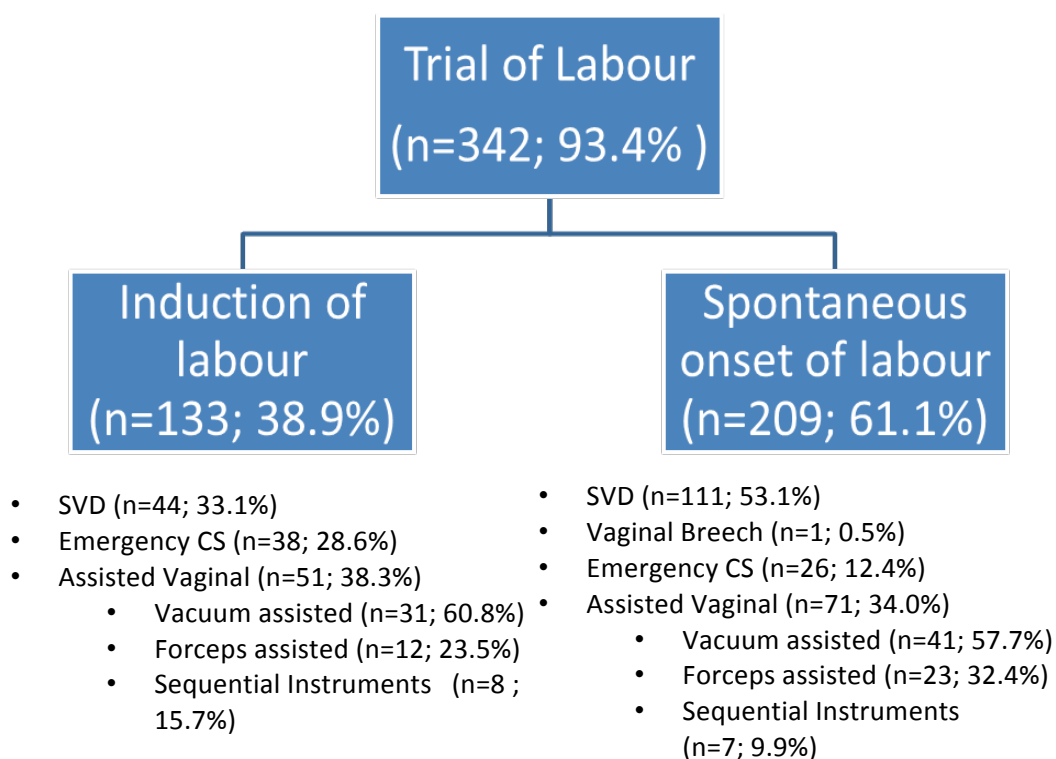


Figure 4-9 Mode of delivery in the overall HANDLE cohort where a vaginal delivery was anticipated (n=342).

Of six preeclamptic pregnancies, no patient presented in spontaneous labour. The mean gestational age at delivery was 35⁺⁶ with a mean birthweight of 2478g (± 773 g). Overall 66.7% (n=4) required emergency caesarean section delivery and 33.3% (n=2) achieved vaginal delivery. The four women who were deemed suitable for attempted vaginal delivery underwent an induction of labour with 50% (n=2) achieving vaginal delivery. The two remaining patients in the induced subgroup were delivered by caesarean section, which was due to failure to advance in labour.

In the 18 gestational hypertension affected pregnancies, the mean gestational age at delivery was 39⁺⁵ with a mean birthweight 3456g (± 477 g). Overall 27.8% (n=5) required caesarean section delivery and 72.2% (n=13) achieved vaginal delivery. Five women (27.8%) presented in spontaneous labour and all five successfully achieved a vaginal delivery. There were 16 women who were deemed suitable for attempted vaginal delivery of whom, 68.8% (n=11) underwent an induction of labour with 72.7% (n=8) achieving vaginal delivery. The remaining three women, who were induced, were delivered by caesarean section and this was due to failure to advance in labour.

There were 24 pregnancies complicated by FGR (defined as an infant where the birth weight was less than the 10th centile when plotted on the WHO gender specific Neonatal and Infant close monitoring charts; Appendices 2.8 & 2.9). Of these only one third (n=8) of infants diagnosed as FGR were suspected antenatally, none of whom had abnormal umbilical artery Doppler assessments. The mean gestational age at delivery was 39⁺⁴ with a mean birthweight 2728g (± 332 g). Of the 24 cases of FGR, eight were a gestation and gender corrected birthweight <3rd centile. Five of these eight infants went on to deliver at a gestational age of greater than 40 weeks. Overall 29.2% (n=7) required caesarean

delivery and 70.8% (n=17) achieved vaginal delivery. There were 10 women with fetal growth restriction who presented in spontaneous labour and all 10 achieved a successful vaginal delivery. There were three women delivered by elective caesarean section, with the indications being breech presentation (n=2), anhydramnios (n=1) and non-reassuring fetal cardiotocograph (n=1). Of the resultant 20 women who were deemed suitable for attempted vaginal delivery 50% (n=10) underwent an induction of labour with 70% (n=7) achieving vaginal delivery. The remaining three women, who were induced, were delivered by caesarean section, with the indication being non-reassuring fetal cardiotocograph.

Throughout the study period there were 12 maternal admissions to the High Dependency Unit (HDU). The indications for admission included: postpartum haemorrhage (PPH) n=5, maternal hypertension n=2, maternal sepsis n=3 and one case where the patient suffered a concurrent PPH and sepsis.

Completion of the postnatal component of the HANDLE protocol was successful with 291 (79.5%) undergoing a final NICOM assessment and completing the study protocol. The postnatal review was undertaken at a mean 52.9 days (± 9.5). At the time of postnatal review maternal weight and BP were again assessed. When compared to first trimester values, participants demonstrated a postnatal mean weight gain and fluctuation of systolic and diastolic blood pressure of +3.7kg (± 5.2), -1.6mmHg (± 15.1) and +10.25mmHg (± 10.0) respectively. Among participants it was noted that the incidence of breastfeeding was in excess of national initiation figures (57%) with an initiation rate of 77%(375). Breastfeeding at time of discharge was 73% again in excess of the national 46.3% reported(376). Breastfeeding rate at time of postnatal review was 61% which was almost double the national reported 34%(377). The postnatal patient attendance has been

successful with the assistance of my collaborators AEK, AJ and CRB who have provided a 6 week neonatal assessment at the time of the maternal NICOM assessment.

Table 4-6 Maternal and Fetal Morbidity.

Characteristic	N (%) / mean \pm SD/ median[IQR]
GH/PE	24 (6.6)
Eclampsia	0 (0)
Suspected FGR	9 (37.5)
Undetected FGR	15 (62.5)
PPH >2000ml	3(0.8)
Emergency Hysterectomy	0 (0)
Maternal HDU Admission	12 (3.3)
HDU LOS, days	1 [1 - 2.75]
Uterine Rupture	0 (0)
Pulmonary Embolus	0 (0)
NICU admission	38 (10.4)
NICU LOS, days	2 [1 – 5.5]
Adverse perinatal outcome composite of IVH, PVL, HIE, NEC, BPD, Sepsis & death	2 (0.6)
Apgar score < 7 at 5 mins	2 (0.6)
Cord Gas <7.1	5 (1.6)
Neonatal Deaths	1 (0.3)

Abbreviations: GH - Gestational hypertension, PET - Preeclampsia, FGR - Fetal growth restriction, PPH - Postpartum haemorrhage, HDU - High dependency unit, LOS - Length of stay, NICU - Neonatal intensive care, IVH - Intraventricular haemorrhage, PVL - Periventricular leukomalacia, HIE - Hypoxic ischaemic encephalopathy, NEC - Necrotizing enterocolitis, BPD – Bronchopulmonary dysplasia.

Of the 366 non-anomalous singleton pregnancies completing the study protocol, the mean (\pm SD) gestational age and birthweight were 39.8 weeks (\pm 1.8) and 3399g (\pm 529) respectively. The median [IQR] length of hospital stay for the cohort was 2 [2 -3] days. The reported neonatal condition in the cohort was good with a median [IQR] Apgar at 1 minute and 5 minutes of 10 [10-10] and 10 [10-10] respectively. There were two infants who had an Apgar score of less than seven at five minutes. Paired cord pH sampling was performed in 189 (51.9%) with an insufficient arterial sample in nine cases. The mean (\pm SD) arterial and venous pH values were 7.23 (\pm 0.7) and 7.30 (\pm 0.6) respectively. There were five cases of neonatal metabolic acidosis with an arterial pH less than 7.1.

There were five (1.4%) women who received prelabour magnesium sulphate for seizure prophylaxis, and 18 (4.9%) women who completed antenatal dexamethasone. Eighty three (22.7%) women had a risk factor for neonatal sepsis. These included 56 (15.3%) women reporting rupture of membranes for greater than 18 hours, 26 (7.1%) women who had a peripartum pyrexia and one woman who was culture positive for group B streptococcus. Overall 38 (10.4%) infants required admission to the neonatal intensive care unit (NICU) with one infant being admitted to the Coombe Women & Infants University Hospital following an in-utero transfer following an antepartum haemorrhage, as no NICU beds were available at the Rotunda Hospital. The median NICU length of stay was 2 [1 – 5.5]. Of infants requiring NICU admission there was one (0.3%) infant ventilated for a total of 20 days, 4 infants requiring CPAP for a median 4.5 [1.5 – 8.25] days and a mean airway pressure (MAP) of 9.8 [7.95 – 15] cmH₂O. Eight infants required oxygen supplementation for a median 4.0 [2.25 – 7.5] days.

There were two (0.6%) infants affected by adverse perinatal outcome, which was defined as either composite morbidity outcome (intraventricular haemorrhage, periventricular leucomalacia,

necrotising enterocolitis, hypoxic-ischaemic encephalopathy, sepsis) or mortality. The adverse outcomes reported were one case of grade two IVH and one case of grade three IVH. The case of grade three IVH also comprised the only mortality. This comprised a late neonatal death at 20 days of life secondary to the sequelae of prematurity and sepsis. This infant was delivered at a gestational age of 25⁺³ by an assisted vaginal breech delivery weighing 740g following a preterm prelabour rupture of membranes one day prior to delivery. The mother completed a course of antenatal steroids 12 hours prior to delivery and completed 24 hours of magnesium sulphate infusion for neuroprotection. However, the infant was concomitantly affected by bilateral grade three intraventricular haemorrhage, pulmonary haemorrhage, ruptured necrotising enterocolitis and candida sepsis refractory to treatment. In this case neonatal intensive care was withdrawn after 20 days due to multi-organ failure. A post-mortem was not performed in this case.

Chapter 5 RESULTS- HAEMODYNAMICS & BLOODS

My hypothesis is that first trimester NICOM® obtained TPR in the setting of PE will be higher than the normal patient group by 200 dynes.s.cm⁻⁵. Continuous data was presented as means (standard deviation) or as medians [inter-quartile ranges] as appropriate. Categorical data was presented as absolute values and percentages. Three group comparisons were conducted using the one-way ANOVA or Kruskal-Wallis one-way analysis of variance as appropriate. Two group comparisons were conducted using the independent t-test or Mann-Whitney U test. Proportions were compared using the Chi square test (or Fisher's exact test where appropriate). Haemodynamic trends over time were displayed using line charts. SPSS version 23 (IBM Corporation, NY, USA) was used to conduct the analysis

5.1 Non- Pregnant Profile.

Due to the nature of the HANDLE study it was not feasible to perform pre-pregnancy NICOM &/ or echocardiographic assessments as women were recruited in the first trimester of pregnancy. To allow for comparison of pre-pregnancy haemodynamic variables to those in pregnancy and the postpartum, thirty women were recruited to a cross-sectional study and underwent simultaneous NICOM and echocardiographic assessments. Recruitment posters were placed in the hospital canteen with contact details for the research team for participants to opt in. Women were deemed eligible for recruitment if they were similarly low risk with no pre-existing medical disease and with no requirement for regular medication. There were no differences in age, weight or body mass index between pregnant and non-pregnant women.

Table 5-1 Haemodynamic profile of Non-pregnant women.

Cardiac variables	Non-Pregnant
CO (L/min)	5.9 (1.0)
COI (L/min/m ²)	3.5 (0.6)
SV (mL)	76 (12)
SVI (mL/ m ²)	45 (8)
TPR (dynes.sec)	1179 (301)
TPRI (dynes.sec.cm ⁻⁵)	1972 (390)
HR (bpm)	79 (14)
SBP (mmHg)	116 (8)
DBP (mmHg)	79 (6)

Abbreviations: CO –cardiac output, COI –indexed cardiac output, TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, SBP- systolic blood pressure, DBP- diastolic blood pressure and HR- heart rate. Values are presented as means (Standard deviation).

Table 5-2 Pregnant and Non-pregnant demographics.

Characteristic	Overall HANDLE cohort N=366	Non-pregnant Control N=30
Age, years	29.0 [22.0- 36.0]	28.9 [25.0- 32.8]
Ethnicity		
-White European	316 (86.3)	22 (73.3)
-African	6 (1.6)	0 (0)
-Asian	11 (3.0)	(0)
Single	206 (56.3)	14 (46.7)
Tertiary education	205 (56.0)	17 (56.7)
Maternal height, cm	166.0 [158.0 -174.0]	167.0 [158.0 -176]
Maternal weight at first assessment, kg	64.0 [46.7 – 81.3]	64.6 [53.5 -75.7]
BMI, kg/m ²	23.3 [17.7 - 28.9]	22.6 [18.1 – 27.1]

Abbreviations: BMI- Body mass index, Figures presented as N (%) or median [interquartile range]

Table 5-3 Comparison of non-pregnant and postnatal preeclampsia cardiac variables.

Cardiac variables	Non-Pregnant N=30	PE N=4	p-value
CO (L/min)	5.9 (1.0)	5.7 (1.2)	0.59
COI (L/min/m ²)	3.5 (0.6)	3.2 (0.7)	0.41
SV (mL)	76 (12)	71 (20)	0.46
SVI (mL/ m ²)	45 (8)	41 (12)	0.33
TPR (dynes.sec)	1179 (301)	1422 (327)	0.24
TPRI (dynes.sec.cm ⁻⁵)	1972 (390)	2519 (733)	0.13
HR (bpm)	79 (14)	81 (8)	0.80
SBP (mmHg)	116 (8)	128 (7)	0.04
DBP (mmHg)	79 (6)	82 (4)	0.29

Abbreviations: PE-preeclampsia, CO –cardiac output, COI –indexed cardiac output, TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, SBP- systolic blood pressure, DBP- diastolic blood pressure and HR- heart rate. Values are presented as means (Standard deviation). Comparison via independent t-test and a p-value of <0.05 deemed statistically significant.

Table 5-4 Comparison of non-pregnant and postnatal gestational hypertension variables.

Cardiac variables	Non-Pregnant N=30	GH N= 17	p-value
CO (L/min)	5.9 (1.0)	6.0 (1.3)	0.71
COI (L/min/m ²)	3.5 (0.6)	3.3 (0.6)	0.34
SV (mL)	76 (12)	71 (21)	0.34
SVI (mL/ m ²)	45 (8)	39 (10)	0.03
TPR (dynes.sec)	1179 (301)	1456 (383)	0.11
TPRI (dynes.sec.cm ⁻⁵)	1972 (390)	2591 (657)	0.01
HR (bpm)	79 (14)	87 (10)	0.04
SBP (mmHg)	116 (8)	125 (10)	0.06
DBP (mmHg)	79 (6)	88 (7)	0.01

Abbreviations: GH-gestational hypertension, CO –cardiac output, COI –indexed cardiac output, TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, SBP- systolic blood pressure, DBP- diastolic blood pressure and HR- heart rate. Values are presented as means (Standard deviation). Comparison via independent t-test and a p-value of <0.05 deemed statistically significant.

Table 5-5 Comparison of non-pregnant and postnatal fetal growth restriction cardiac variables.

Cardiac variables	Non-Pregnant N=30	FGR N=18	p-value
CO (L/min)	5.9 (1.0)	5.5 (1.4)	0.21
COI (L/min/m ²)	3.5 (0.6)	3.2 (0.7)	0.16
SV (mL)	76 (12)	67 (19)	0.08
SVI (mL/ m ²)	45 (8)	40 (11)	0.07
TPR (dynes.sec)	1179 (301)	1393 (423)	0.24
TPRI (dynes.sec.cm ⁻⁵)	1972 (390)	2326 (660)	0.12
HR (bpm)	79 (14)	82 (11)	0.44
SBP (mmHg)	116 (8)	112 (12)	0.44
DBP (mmHg)	79 (6)	75 (11)	0.43

Abbreviations: FGR- fetal growth restriction, CO –cardiac output, COI –indexed cardiac output, TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, SBP- systolic blood pressure, DBP- diastolic blood pressure and HR- heart rate. Values are presented as means (Standard deviation). Comparison via independent t-test and a p-value of <0.05 deemed statistically significant.

5.2 Normal Pregnant Profile

The serial haemodynamic profile of 318 women with uncomplicated pregnancies was obtained. These changes are detailed in Table 5.6. Statistically significant results were defined as p<0.05 and marked within figures as *.

Table 5-6 Serial haemodynamic profile changes of normal pregnancy during the study period.

	14 weeks	20 weeks	28 weeks	Postnatal	ANOVA p
CO (L/min)	6.3 (1.3)	6.4 (1.3)	6.6 (1.4)*	5.6 (1.2)*	<0.001
SV (mL)	75 (17)	72 (17)*	71 (15)*	69(16)*	<0.001
TPR (dynes.sec)	1180 (272)	1131 (258)*	1102 (237)*	1350 (301)*	<0.001
HR (bpm)	85 (10)	90 (11)*	95 (11)*	83 (10)	<0.001

Abbreviations: CO –cardiac output, TPR- total peripheral resistance, SV- stroke volume and HR- heart rate. Values are presented as means (Standard deviations). One-way ANOVA with repeated measures was used to assess change over time. * Indicates p values <0.05 compared with baseline assessment at 14 weeks (Bonferroni adjustment).

5.3 Profile of Pregnancy complicated by Gestational Hypertension

The serial haemodynamic profile of 18 women with pregnancies complicated by gestational hypertension was obtained. These changes are detailed in Table 5.7 and comparison to uncomplicated pregnancy values is detailed in Figures 5.1 – 5.9. Overall pregnancies complicated with gestational hypertension had relatively unchanged cardiac output, an expected elevated blood pressure and increased heart rate. There were trends for increased total peripheral resistance combined with a reduction in stroke volume in comparison to unaffected controls. Two-way ANOVA with repeated measures was used to assess change over time. Two group comparisons were conducted using the independent t-test. Statistically significant results were defined as $p < 0.05$ and marked within figures as *.

Table 5-7 Serial Haemodynamic profile changes of gestational hypertension during the study period.

	14 weeks	20 weeks	28 weeks	Postnatal	ANOVA p
CO (L/min)	6.2 (1.3)	6.5 (0.8)	6.6 (1.2)	6.1 (1.3)	0.39
SV (mL)	73 (18)	71 (11)	66 (16)	71 (21)	0.20
TPR (dynes.sec)	1262 (204) †	1217 (204)	1243 (217)	1454 (396)*	0.02
HR (bpm)	87 (12)	92 (10)	101 (11)*	87 (10)	<0.001

Abbreviations: CO –cardiac output, TPR- total peripheral resistance, SV- stroke volume, HR- heart rate, SBP- systolic blood pressure, DBP diastolic blood pressure and Blood pressure units: mmHg. Values are presented as means (Standard deviations). One-way ANOVA with repeated measures was used to assess change over time. * indicates p values <0.05 compared with baseline assessment at 14 weeks (Bonferroni adjustment). † = p value < 0.05 compared with 14 week Normal cohort value.

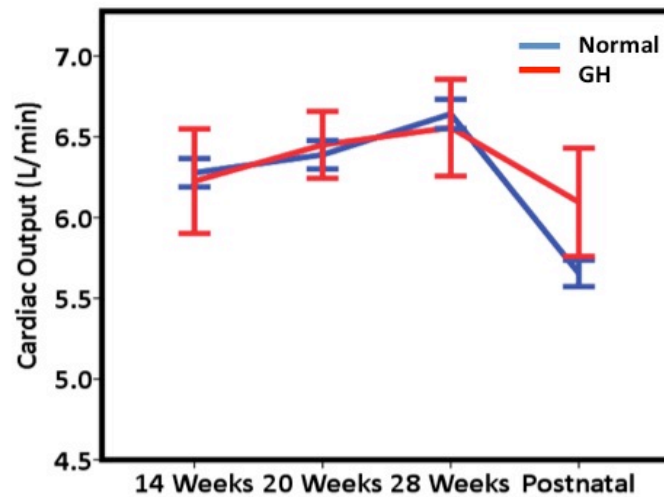


Figure 5-1 The serial changes in cardiac output in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

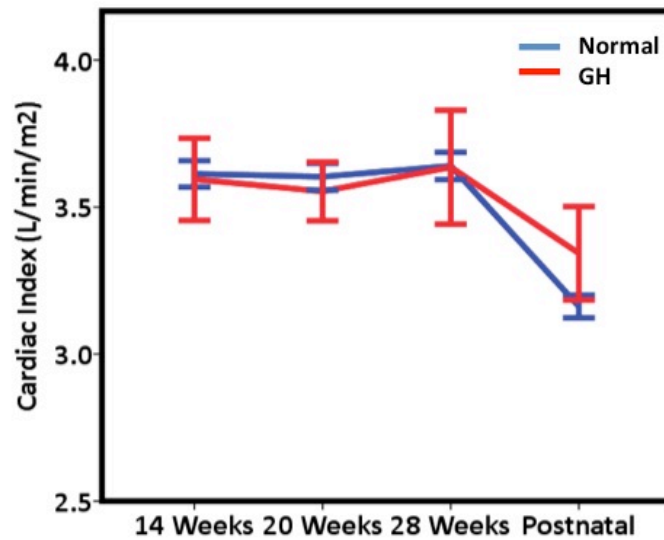


Figure 5-2 The serial changes in cardiac index in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

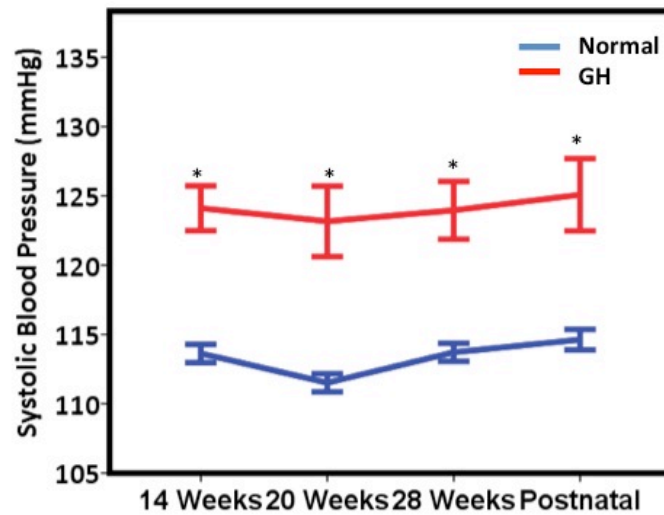


Figure 5-3 The serial changes in systolic blood pressure in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

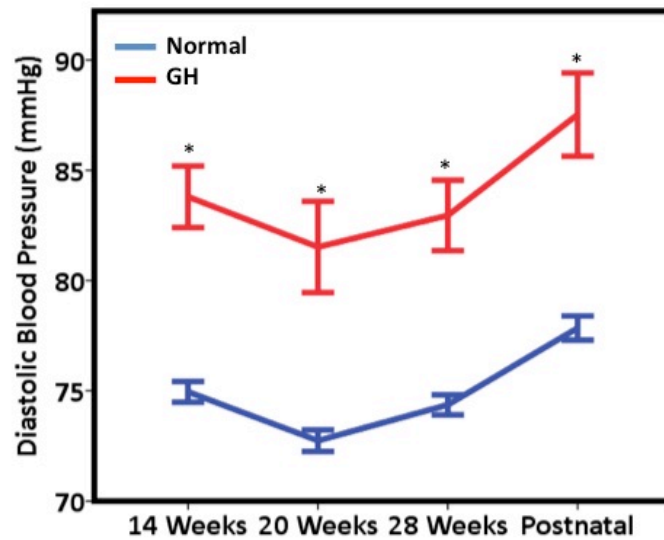


Figure 5-4 The serial changes in diastolic blood pressure in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

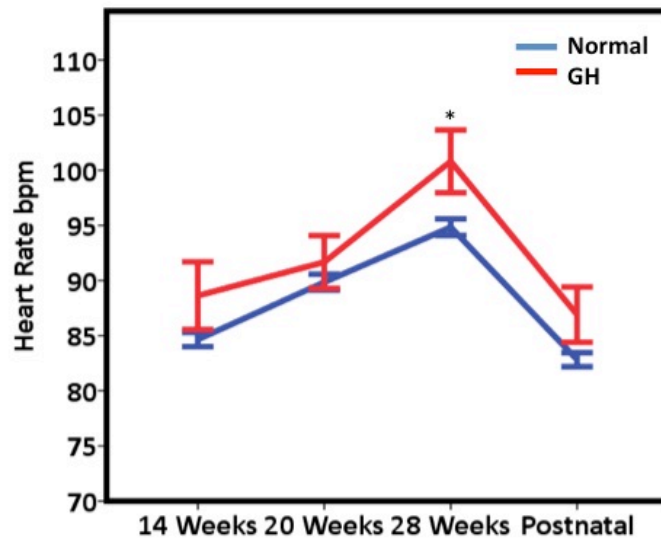


Figure 5-5 The serial changes in heart rate in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

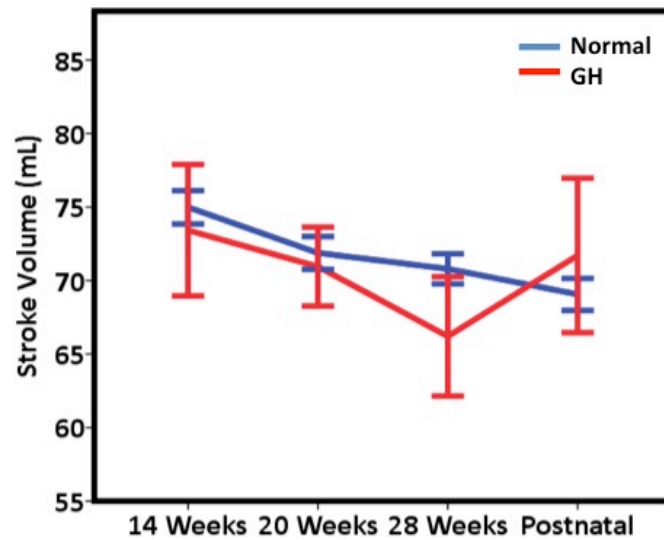


Figure 5-6 The serial changes in stroke volume in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

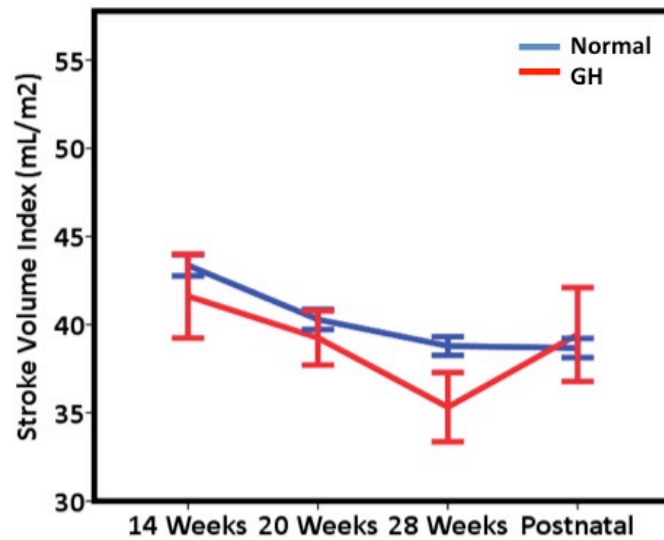


Figure 5-7 The serial changes in stroke volume index in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

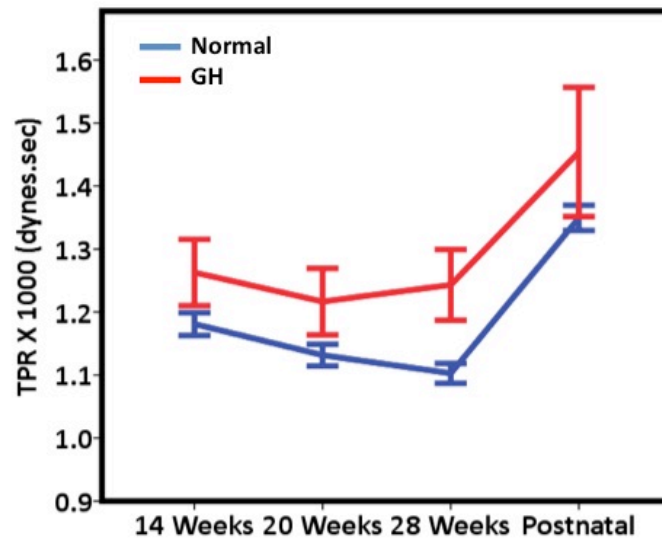


Figure 5-8 The serial changes in total peripheral resistance in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

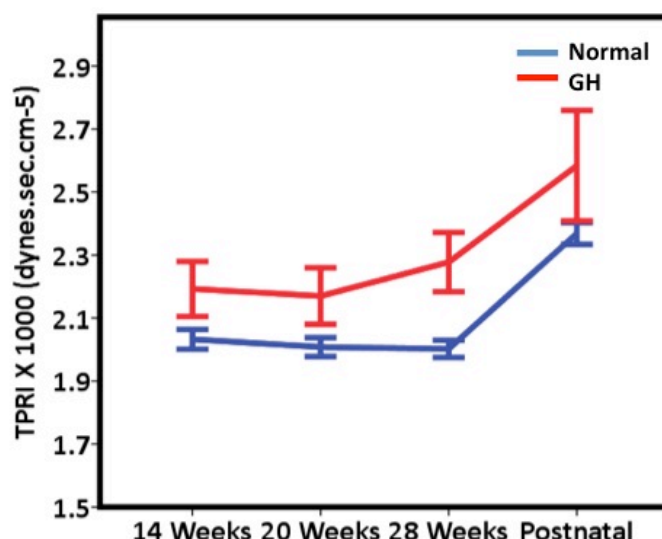


Figure 5-9 The serial changes in indexed total peripheral resistance in gestational hypertension and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

5.4 Profile of Pregnancy complicated by Preeclampsia

As detailed in Chapter 4, a reduction in the incidence of preeclampsia in nulliparous women has been observed in the overall Rotunda population. During the study period only 3.3%, 2.4% and 2.8% of nulliparous women in 2014, 2015 and 2016 respectively had a pregnancy complicated by preeclampsia. Figure 4.2 further details the significant ($p<0.001$) falling incidence of preeclampsia in the Rotunda nulliparous population between 2004 and 2016.

The serial haemodynamic profile of six women with pregnancies complicated by preeclampsia was obtained. These changes are detailed in Table 5.8 and comparison to uncomplicated pregnancy

values is detailed in Figures 5.10 – 5.18. Overall pregnancies complicated with preeclampsia had relatively unchanged cardiac output, an expected elevated blood pressure. There were trends for increased stroke volume and increased total peripheral resistance combined with a reduction in heart rate in comparison to unaffected controls. The differences between preeclampsia and gestational hypertension are further detailed in figures 5.19 - 5.27. Two-way ANOVA with repeated measures was used to assess change over time. Two group comparisons were conducted using the independent t-test. Statistically significant results were defined as $p < 0.05$ and marked within figures as *.

Table 5-8 Serial haemodynamic profile changes of preeclampsia during the study period.

	14 weeks	20 weeks	28 weeks	Postnatal	ANOVA p
CO(L/min)	5.7 (1.0)	6.3 (0.8)	6.4 (1.5)	5.7 (1.2)	0.62
SV (mL)	72 (20)	77 (9)	71 (12)	71 (20)	0.92
TPR (dynes.sec)	1302 (305)	1181 (194)	1309 (542)	1421 (327)	0.76
HR (bpm)	83 (10)	83 (9)	91 (14)	81 (8)	0.15

Abbreviations: CO –cardiac output, TPR- total peripheral resistance, SV- stroke volume and HR- heart rate. Values are presented as means (Standard deviations). One-way ANOVA with repeated measures was used to assess change over time. * indicates p values < 0.05 compared with baseline assessment at 14 weeks (Bonferroni adjustment).

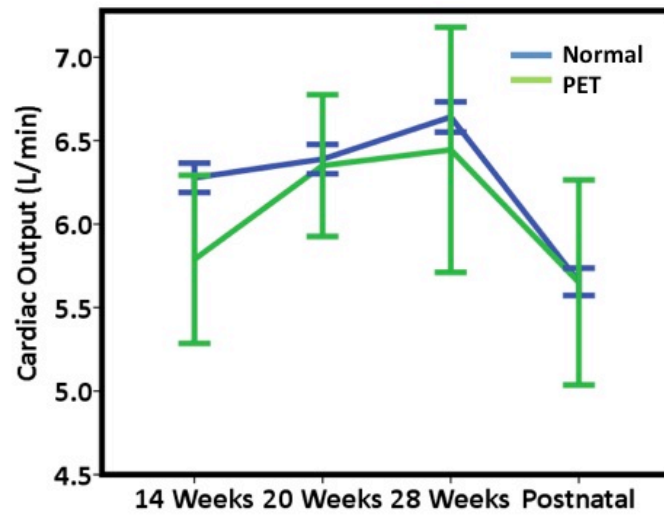


Figure 5-10 The serial changes in cardiac output in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

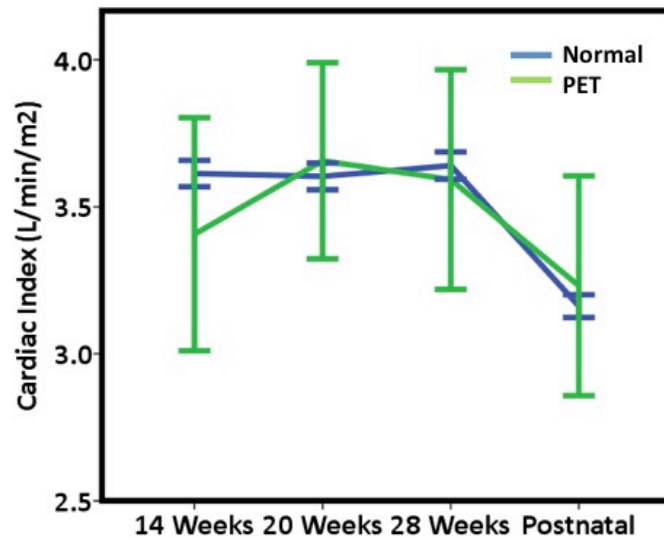


Figure 5-11 The serial changes in cardiac index in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

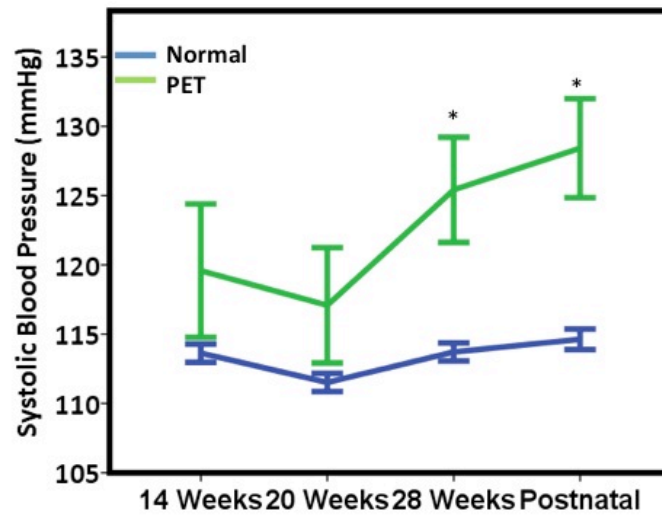


Figure 5-12 The serial changes in systolic blood pressure in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

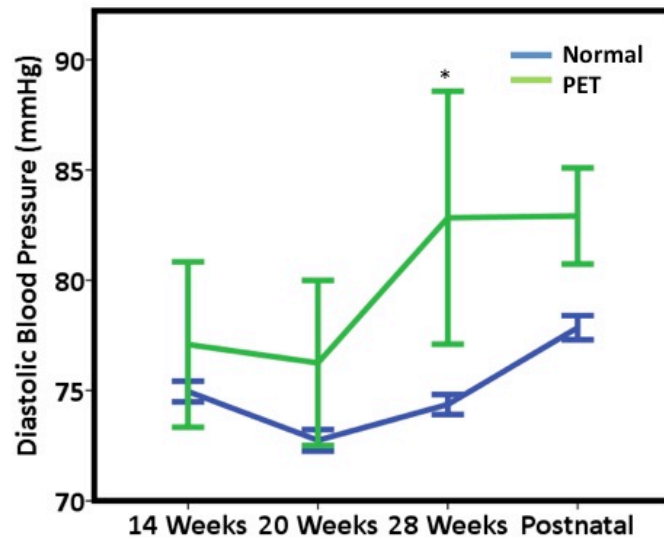


Figure 5-13 The serial changes in diastolic blood pressure in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

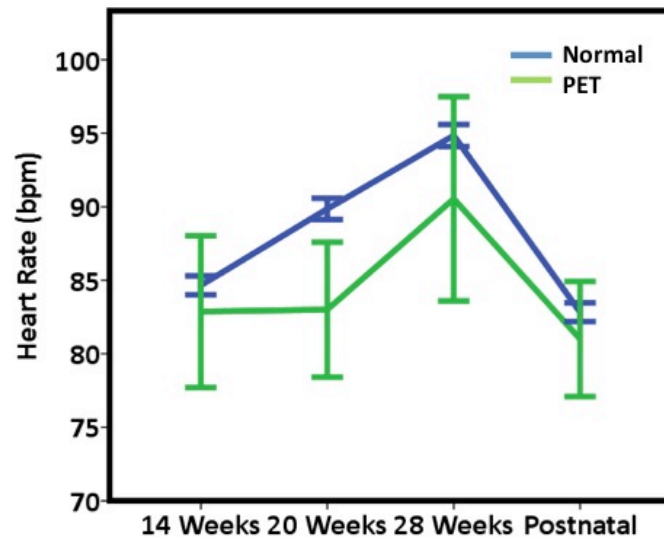


Figure 5-14 The serial changes in heart rate in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

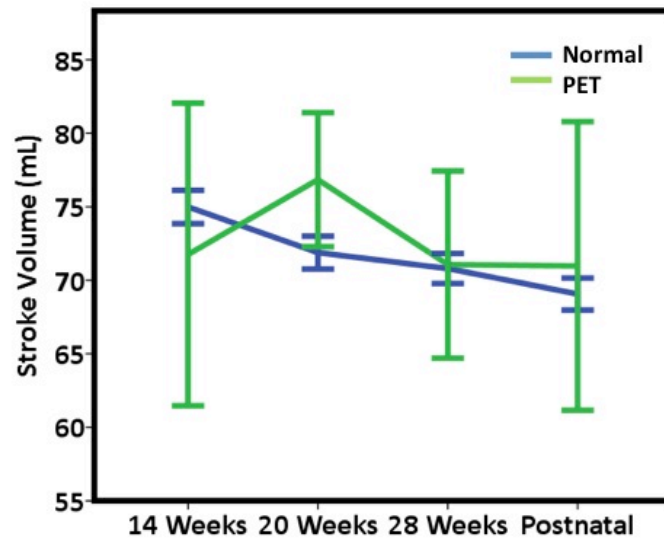


Figure 5-15 The serial changes in stroke volume in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

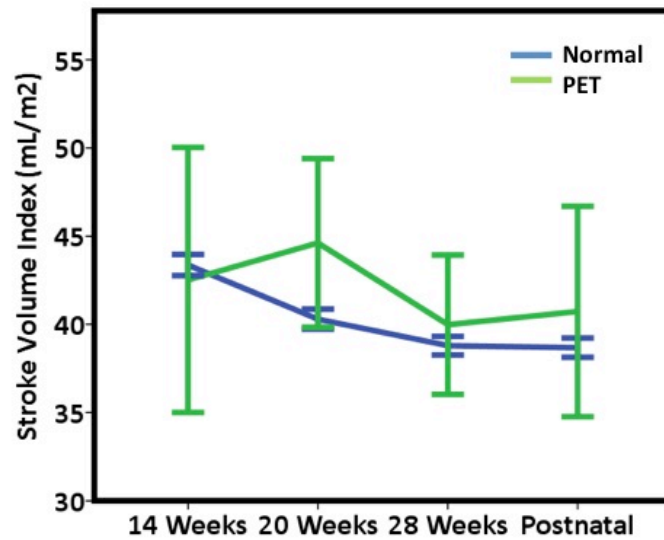


Figure 5-16 The serial changes in stroke volume index in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

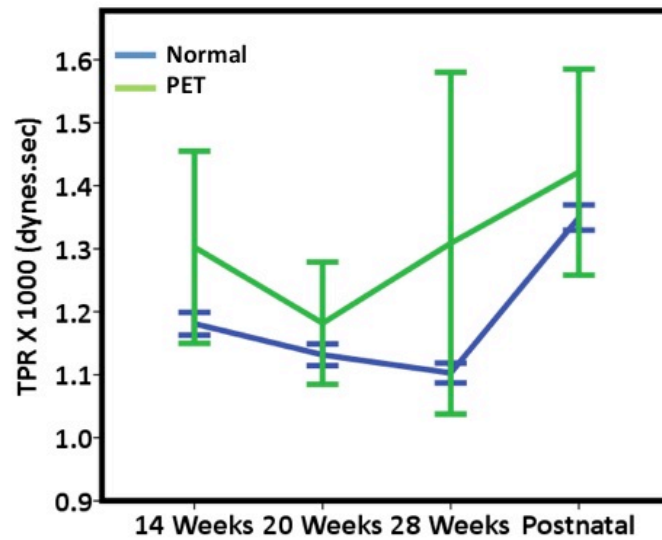


Figure 5-17 The serial changes in total peripheral resistance in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

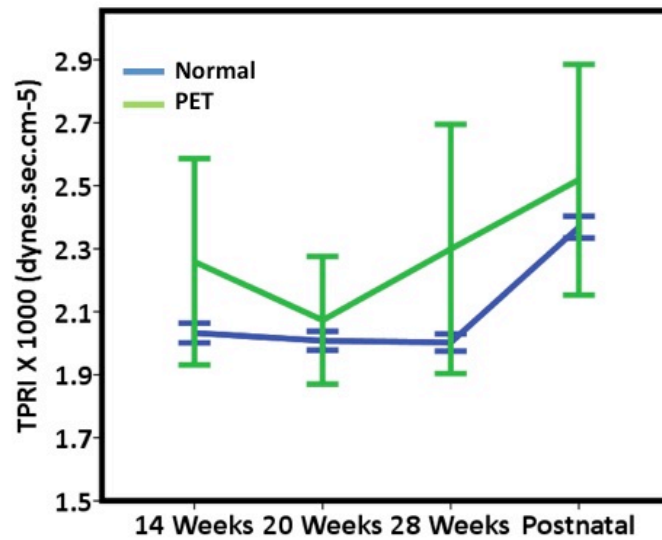


Figure 5-18 The serial changes in indexed total peripheral resistance in preeclampsia and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

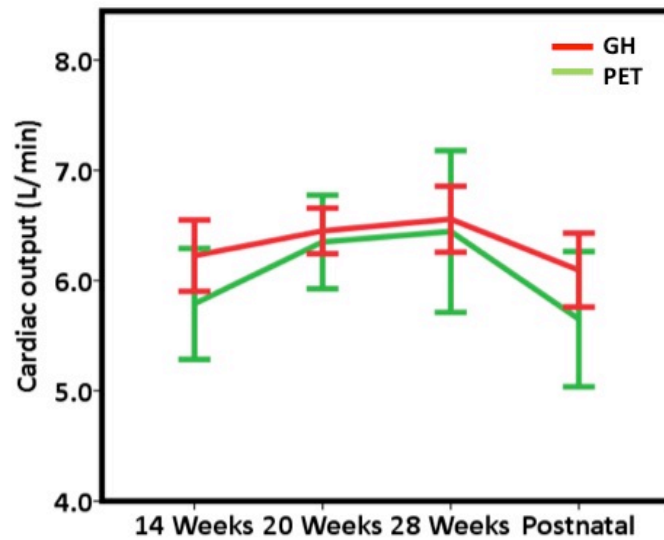


Figure 5-19 The serial changes in cardiac output in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

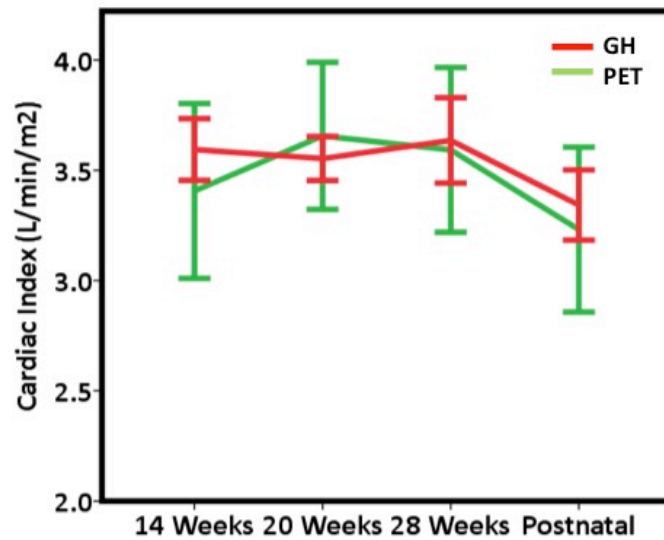


Figure 5-20 The serial changes in cardiac index in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

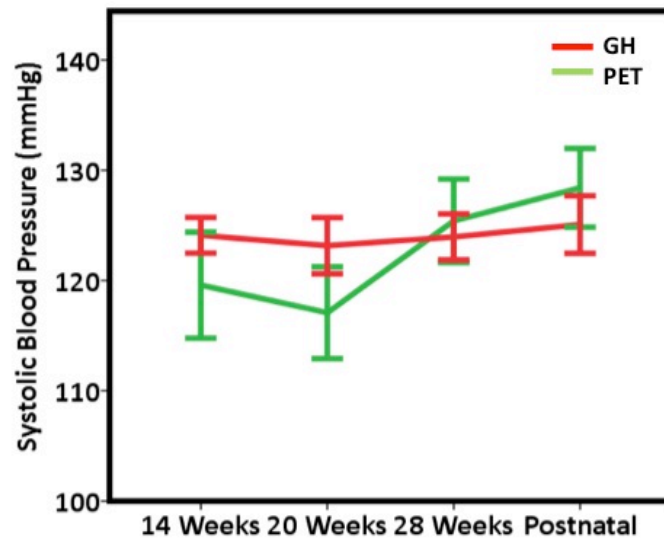


Figure 5-21 The serial changes in systolic blood pressure in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

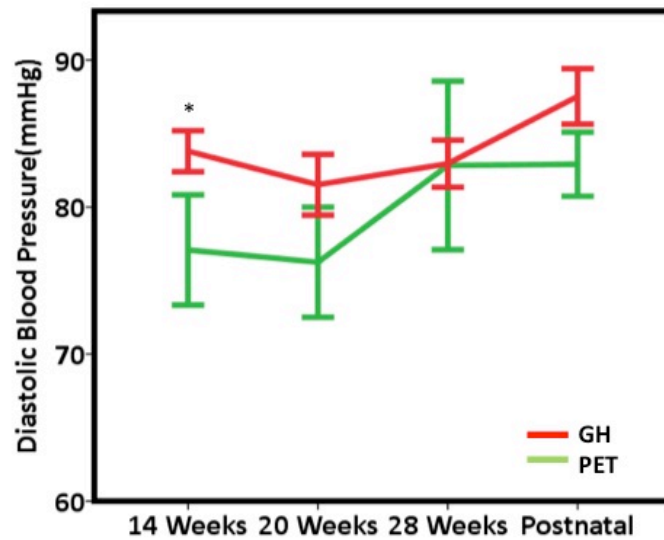


Figure 5-22 The serial changes in diastolic blood pressure in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

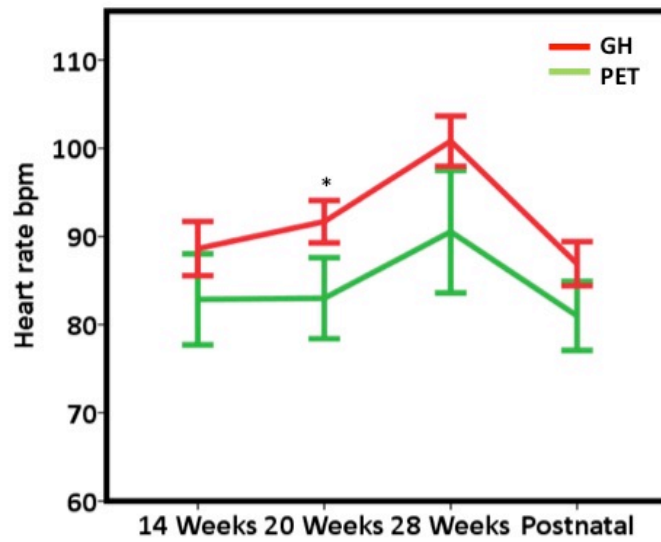


Figure 5-23 The serial changes in heart rate in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

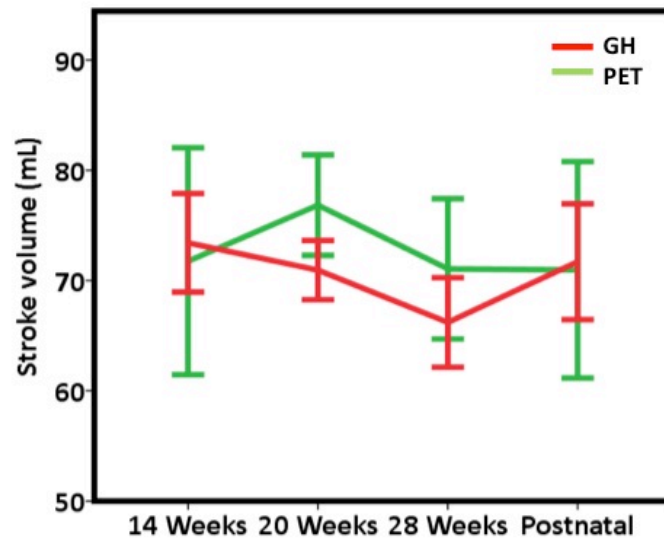


Figure 5-24 The serial changes in stroke volume in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

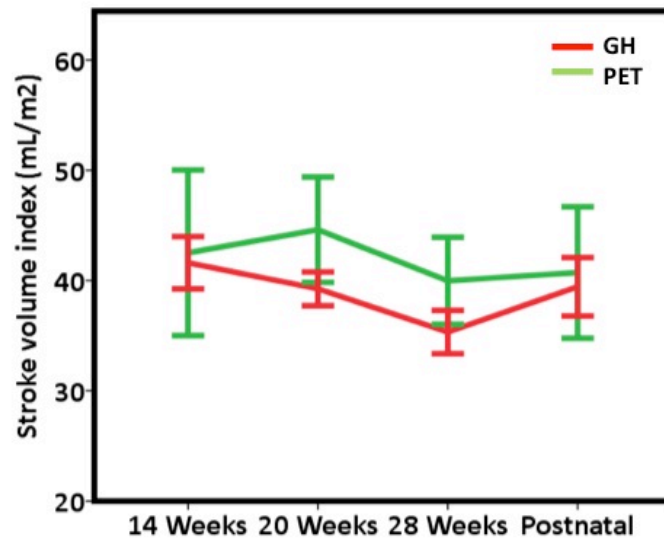


Figure 5-25 The serial changes in stroke volume index in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

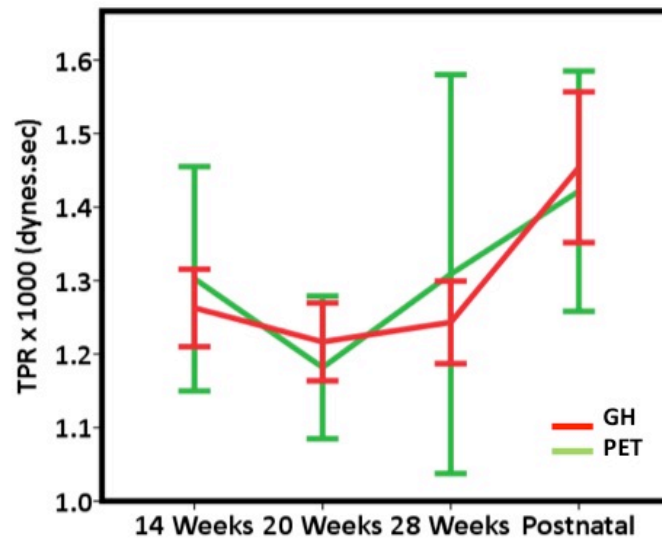


Figure 5-26 The serial changes in total peripheral resistance in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

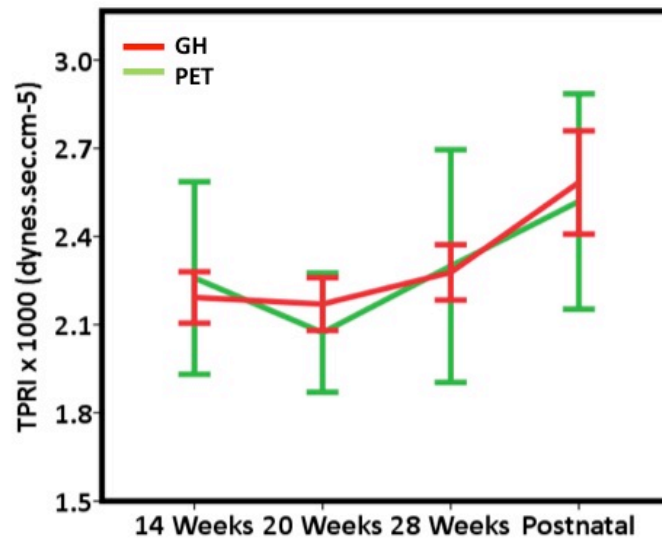


Figure 5-27 The serial changes in indexed total peripheral resistance in gestational hypertension and preeclampsia.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

5.5 Profile of Pregnancy complicated by fetal growth restriction

The serial haemodynamic profile of 24 women with pregnancies complicated by fetal growth restriction was obtained. These changes are detailed in Table 5.9 and comparison to uncomplicated pregnancy values is detailed in Figures 5.27 – 5.36. Overall pregnancies complicated with fetal growth restriction had lower blood pressure, reduced cardiac output, and lower stroke volume but an increase in total peripheral resistance in comparison to unaffected controls but not to the same extent as in hypertensive disease. Two-way ANOVA with repeated measures was used to assess change over time. Two group comparisons were conducted

using the independent t-test. Statistically significant results were defined as $p < 0.05$ and marked within figures as *.

Table 5-9 Serial haemodynamic profile changes of fetal growth restriction during the study period.

	14 weeks	20 weeks	28 weeks	Postnatal	ANOVA p
CO(L/min)	5.8 (1.1)	5.9 (1.1)	6.0 (1.4)	5.6 (1.3)	0.48
SV (mL)	72 (17)	69 (15)	65 (18)	69 (18)	0.27
TPR (dynes.sec)	1209 (284)	1183 (300)	1208 (291)	1350 (393)	0.06
HR (bpm)	83 (10)	87 (10)	94 (11)*	83 (11)	0.003

Abbreviations: CO –cardiac output, TPR- total peripheral resistance, SV- stroke volume, HR- heart rate, SBP- systolic blood pressure, DBP diastolic blood pressure and Blood pressure units: mmHg. Values are presented as means (Standard deviations). One-way ANOVA with repeated measures was used to assess change over time. * indicates p values < 0.05 compared with baseline assessment at 14 weeks (Bonferroni adjustment).

Table 5-10 Serial haemodynamic changes of fetal growth restriction <3rd centile in comparison to <10th centile at 14 weeks' gestation.

Variable	FGR <3 rd n=8	FGR <10 th n=16	p-value
CO	5.5 (1.1)	5.7 (1.1)	0.70
COI	3.5 (0.7)	3.5 (0.5)	0.89
SBP	110 (9)	109 (10)	0.79
DBP	72 (8)	72 (8)	0.94
HR	82 (12)	85 (9)	0.51
SV	69 (18)	70 (15)	0.85
SVI	44 (12)	42 (9)	0.60
TPR	1289 (321)	1184 (209)	0.35
TPRI	2012 (483)	1997 (361)	0.93

Abbreviations: FGR- Fetal growth restriction, CO –cardiac output, COI – indexed cardiac output TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, HR- heart rate, SBP- systolic blood pressure, DBP diastolic blood pressure and Blood pressure units: mmHg. Values are presented as means (Standard deviations). Means of two groups compared by independent t-test. P<0.05 deemed statistically significant.

Table 5-11 Serial haemodynamic changes of fetal growth restriction <3rd centile in comparison to <10th centile at 20 weeks' gestation.

Variable	FGR <3 rd n=8	FGR <10 th n=16	p-value
CO	5.3 (0.9)	6.1 (1.2)	0.11
COI	3.3 (0.6)	3.5 (0.6)	0.36
SBP	106 (7)	110 (8)	0.28
DBP	68 (6)	69 (9)	0.89
HR	88 (8)	89 (11)	0.82
SV	61 (13)	70 (15)	0.21
SVI	38 (8)	40 (8)	0.52
TPR	1300 (381)	1142 (221)	0.22
TPRI	2091 (590)	1956 (302)	0.47

Abbreviations: FGR- Fetal growth restriction, CO –cardiac output, COI – indexed cardiac output TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, HR- heart rate, SBP- systolic blood pressure, DBP diastolic blood pressure and Blood pressure units: mmHg. Values are presented as means (Standard deviations). Means of two groups compared by independent t-test. P<0.05 deemed statistically significant.

Table 5-12 Serial haemodynamic changes of fetal growth restriction <3rd centile in comparison to <10th centile at 28 weeks' gestation.

Variable	FGR <3 rd n=8	FGR <10 th n=16	p-value
CO	5.9 (0.9)	5.9 (1.6)	0.95
COI	3.6 (0.5)	3.3 (0.8)	0.32
SBP	115 (5)	110 (8)	0.097
DBP	77 (6)	73 (5)	0.078
HR	94 (10)	94 (10)	0.91
SV	63 (12)	63 (18)	0.98
SVI	39 (7)	35 (10)	0.42
TPR	1242 (221)	1245 (344)	0.98
TPRI	2041 (357)	2196 (521)	0.46

Abbreviations: FGR- Fetal growth restriction, CO –cardiac output, COI – indexed cardiac output TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, SV- stroke volume, SVI- indexed stroke volume, HR- heart rate, SBP- systolic blood pressure, DBP diastolic blood pressure and Blood pressure units: mmHg. Values are presented as means (Standard deviations). Means of two groups compared by independent t-test. P<0.05 deemed statistically significant.

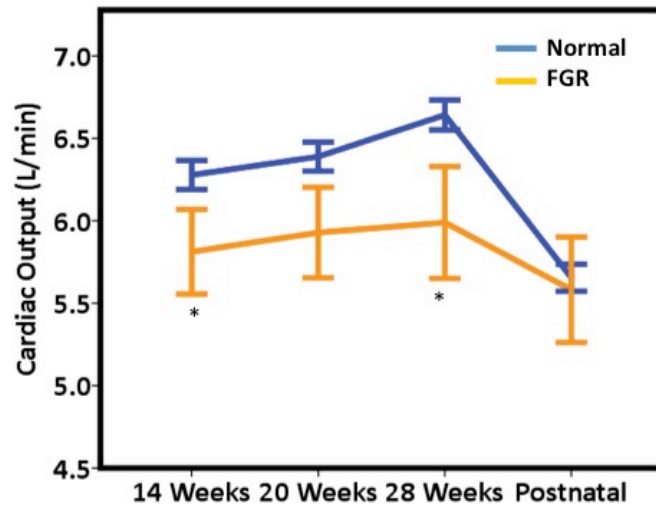


Figure 5-28 The serial changes in cardiac output in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

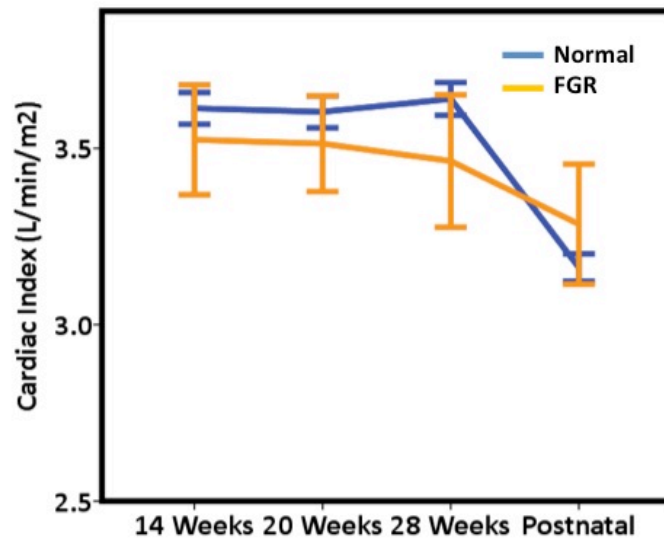


Figure 5-29 The serial changes in cardiac index in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

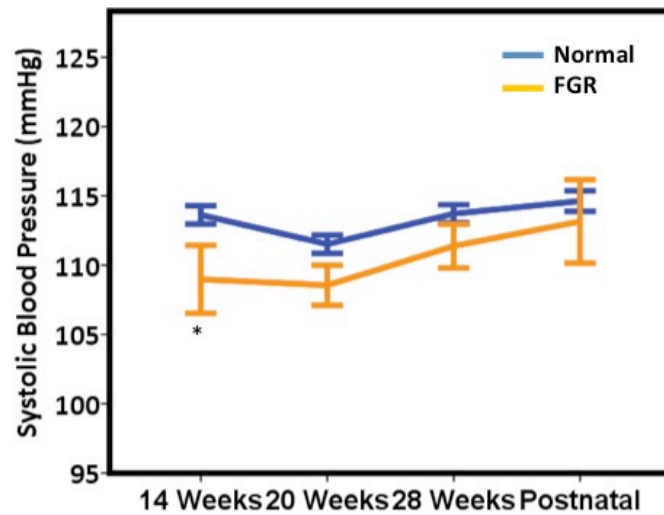


Figure 5-30 The serial changes in systolic blood pressure in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

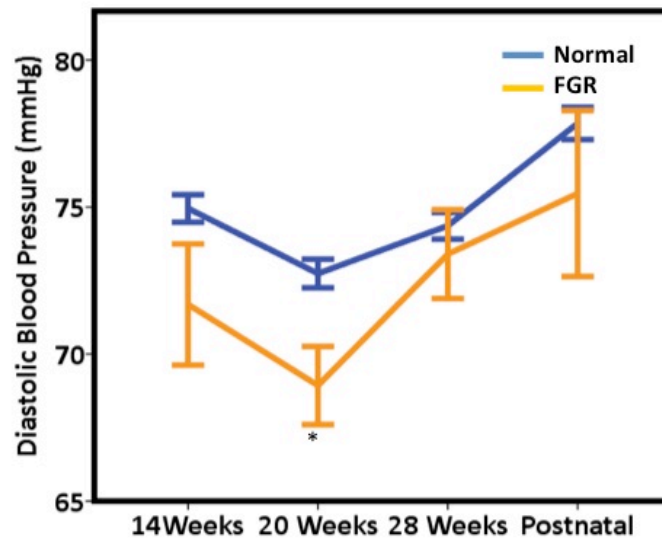


Figure 5-31 The serial changes in diastolic blood pressure in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

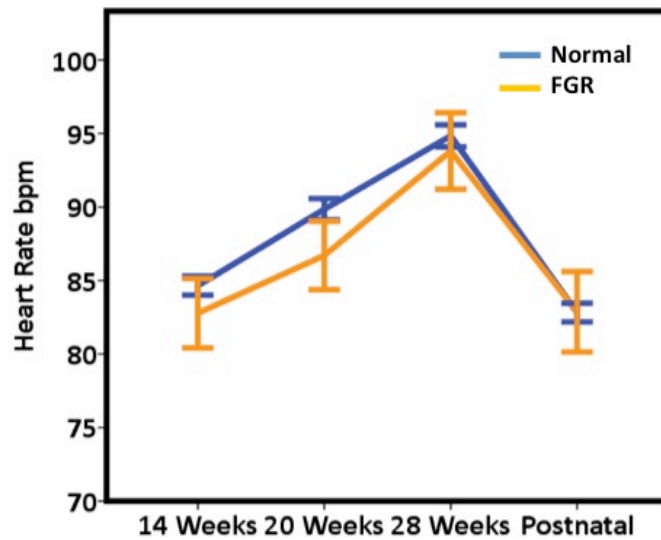


Figure 5-32 The serial changes in heart rate in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

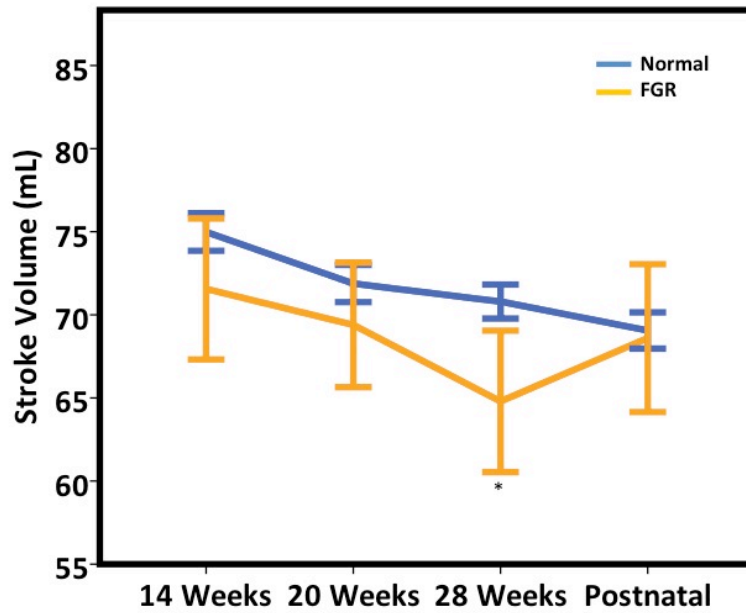


Figure 5-33 The serial changes in stroke volume in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

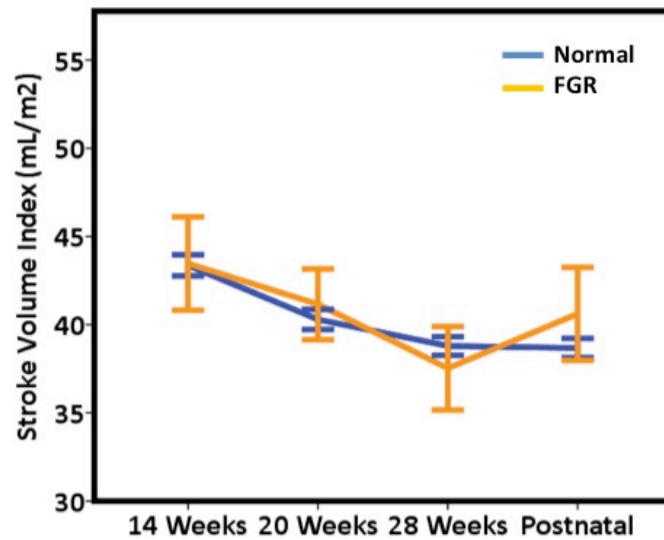


Figure 5-34 The serial changes in stroke volume index in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

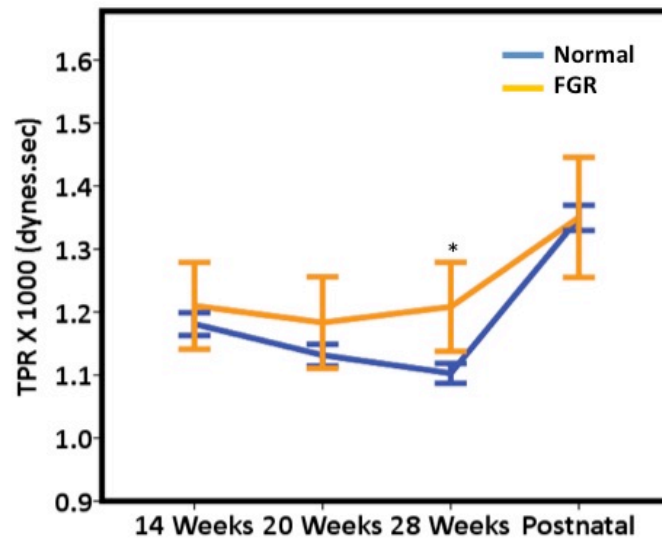


Figure 5-35 The serial changes in total peripheral resistance in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

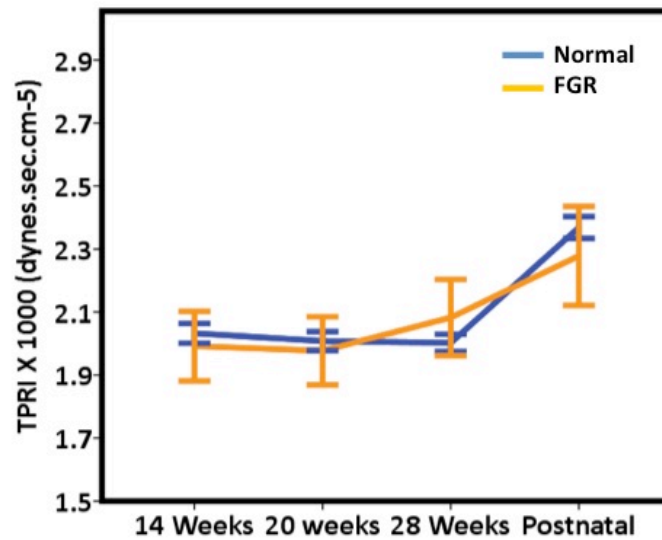


Figure 5-36 The serial changes in indexed total peripheral resistance in fetal growth restriction and unaffected pregnancies.

Bars represent 1 standard error around the mean. Variables were compared by independent t-test and significant p-value of <0.05 are marked as *.

The analysis of banked serum blood was included in the study protocol for participants #71-422 following procurement of additional funding to perform soluble fms-like tyrosine 1 (s-flt-1), placental growth factor (PLGF) and Apelin 13 assays. There were a total of 91 samples processed at a mean (SD) gestational age of 12⁺⁵ (11days). In addition, the mean platelet volume (MPV) was retrieved from their booking visit full blood count sample.

5.6 MPV

All pregnant women in our institution have a full blood count (FBC) performed from a venous sample at their first antenatal visit. Mean platelet volume (MPV) at this first antenatal visit was retrieved from an electronic laboratory database. Study participants had routine venepuncture for the assessment of their disease state, which included a repeat FBC this allowed for retrieval of the MPV from the electronic laboratory database. Comparison for each group was made to normal controls by one-way ANOVA with bonferroni adjustment. There was no significant difference in MPV between disease states or BMI class. Figures 5.37, Figures 5.38, Tables 5.13 and 5.14 further detail MPV by uteroplacental disease and BMI.

Table 5-13 First trimester MPV by disease state.

	n	MPV Mean (SD)	P value
Normal Control	61	8.5 (1.5)	NA
Gestational Hypertension	13	8.9 (2.2)	1.00
Preeclampsia	5	7.3 (0.4)	0.685
Fetal Growth restriction	18	8.2 (1.5)	1.00

Abbreviations: MPV- mean platelet volume; SD- standard deviation. Values are presented as means (Standard deviations). Comparison for each group was made to normal controls and p values were calculated by one-way ANOVA with bonferroni adjustment. A p value <0.05 is considered statistically significant

Table 5-14 First trimester MPV by BMI.

	N=	MPV Mean (SD)	P value
BMI <20.0	27	8.1 (1.2)	0.408
BMI ≥20.0 & <33.0	51	8.6 (1.9)	NA
BMI ≥33.0	19	8.5 (1.1)	1.00

Abbreviations: MPV- mean platelet volume; SD- standard deviation; BMI- body mass index. Values are presented as means (Standard deviations). Comparison for each group was made to normal controls (BMI ≥20.0 & <33.0) and p values were calculated by one-way ANOVA with Bonferroni adjustment. A p value <0.05 is considered statistically significant.

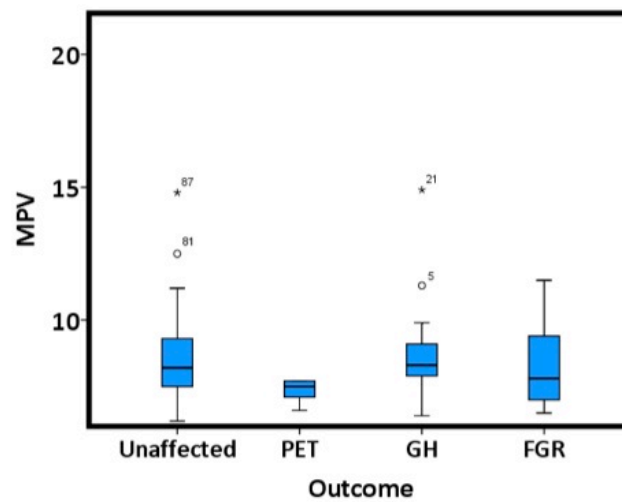


Figure 5-37 MPV spread of values in normal pregnancy and those complicated by uteroplacental disease.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: MPV- mean platelet volume, PET- preeclampsia, GH- gestational hypertension, FGR- fetal growth restriction, *- outlier values.

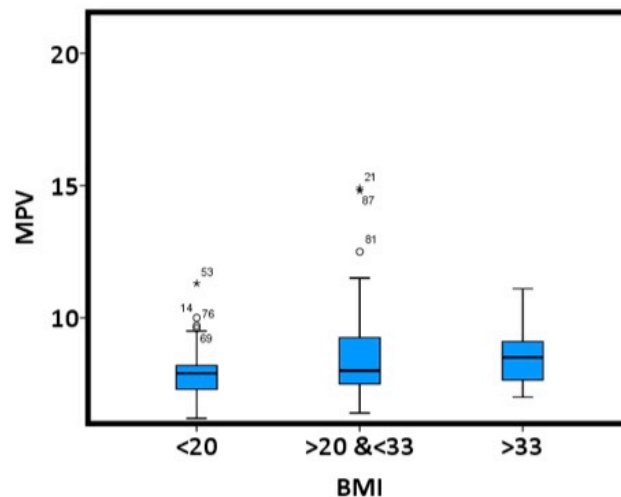


Figure 5-38 MPV values categorised by BMI.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: MPV- mean platelet value, BMI- body mass index, *- outlier values.

5.7 Soluble fms-like tyrosine-1

A soluble fms-like tyrosine-1 (s-flt-1) assay was performed on serum samples of 99 participants; two were excluded due to mislabelling. The samples included 61 controls of varying BMI, five with pregnancy complicated by PE, 13 with pregnancy complicated by GH and 18 with pregnancy complicated by FGR. Comparison for each group was made to normal controls by one-way ANOVA with bonferroni adjustment. The overall distribution of serum s-flt-1 values in this population ranged from a minimum 514.9 pg/ml to a maximum value of 3982 pg/ml. There were no significant differences between disease states. There was a difference between s-flt-1 values and extremes of BMI ($p=0.03$). The distribution is further described in Figures 5.39 & 5.40.

Table 5-15 The mean s-flt-1 values by control and disease state.

	n	s-flt-1 Mean (SD)	P value
Normal Control	61	1612.9 (662.4)	NA
Gestational Hypertension	13	1391.2 (534.2)	1.00
Preeclampsia	5	1171.0 (99.4)	0.699
Fetal Growth restriction	18	1329.9 (463.1)	0.49

Abbreviations: s-flt-1 –soluble fms-like tyrosine-1; SD- standard deviation. Values are presented as means (Standard deviations). Comparison for each group was made to normal controls and p values were calculated by one-way ANOVA with Bonferroni adjustment. A p value <0.05 is considered statistically significant

Table 5-16 The mean s-flt-1 values in the control population by BMI.

	N=	s-flt-1 Mean (SD)	P value
BMI <20.0	27	1730.8 (646.1)*	0.35
BMI ≥20.0 & <33.0	51	1484.8 (627.6)	NA
BMI ≥33.0	19	1253.1	0.44

Abbreviations: s-flt-1 –soluble fms-like tyrosine-1; SD- standard deviation; BMI- body mass index. Values are presented as means (Standard deviations). Comparison for each group was made to normal controls (BMI ≥20.0 & <33.0) and p values were calculated by one-way ANOVA with Bonferroni adjustment. A p value <0.05 is considered statistically significant. * P<0.05 when compared to BMI≥33.0.

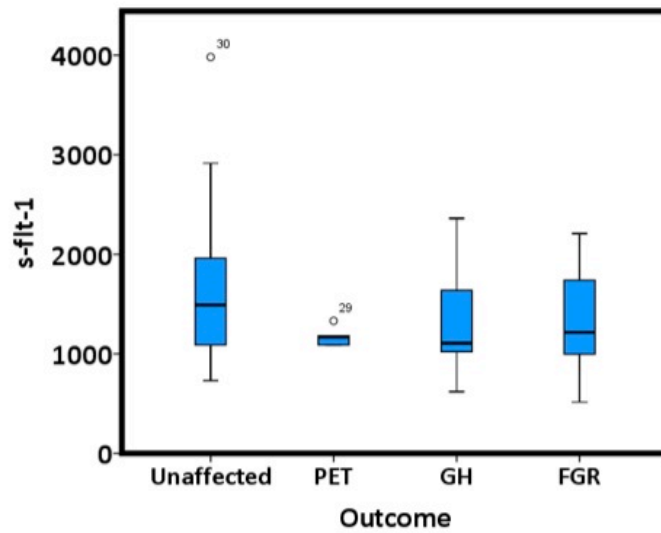


Figure 5-39 The distribution of s-flt-1 values in uteroplacental disease and unaffected pregnancies.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: s-flt-1 – soluble fms-like tyrosine 1, PET- preeclampsia, GH- gestational hypertension, FGR- fetal growth restriction, *- outlier values.

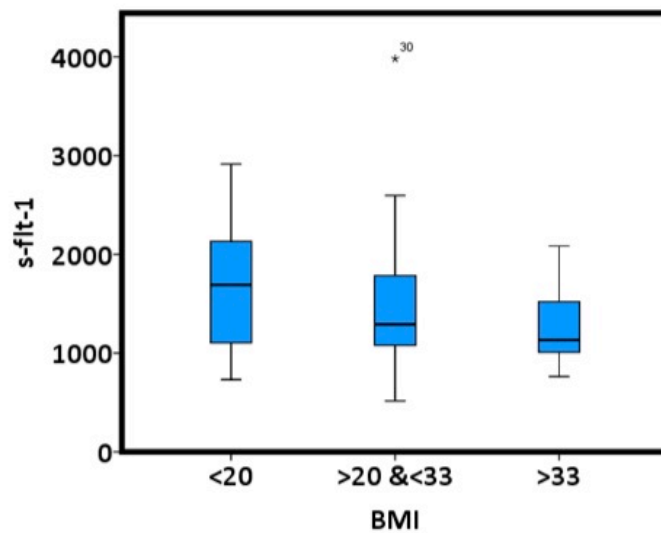


Figure 5-40 The distribution of s-flt-1 values categorised by BMI.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: s-flt-1 – soluble fms-like tyrosine 1, BMI- body mass index, *- outlier values.

5.8 Placental growth factor

A placental growth factor (PLGF) assay was performed on serum samples of 99 participants; two were excluded due to mislabelling. The samples included 61 controls of varying BMI, five with pregnancy complicated by PE, 13 with pregnancy complicated by GH and 18 with pregnancy complicated by FGR. Comparison for each group was made to normal controls by Mann Whitney U test. The overall distribution of serum PLGF values in this population ranged from a minimum 15.6 pg/ml to a maximum value of 137.7 pg/ml. There was no significant difference in PLGF between disease states or BMI class. The distribution is further described in Figures 5.41 & 5.42.

Table 5-17 The median PLGF values by control and disease state.

	N=	PLGF Median [IQR]	P value
Normal Control	61	44.7 [31.6 - 60.4]	NA
Gestational Hypertension	13	46.1 [32 - 58.9]	0.96
Preeclampsia	5	41.2 [19 - 81]	0.521
Fetal Growth restriction	18	42.2 [29.0 - 73.3]	0.82

Abbreviations: PLGF- placental growth factor; IQR- Inter-quartile range. Values are presented as medians [Inter-quartile range]. Comparison for each group was made to normal controls and p values were calculated by Mann Whitney U test. A p value <0.05 is considered statistically significant.

Table 5-18 The median PLGF values in the control population by BMI.

	N=	PLGF Median [IQR]	P value
BMI <20.0	27	48.1 [33.2 – 62.5]	0.862
BMI ≥20.0 & <33.0	51	43.8 [29.9 – 62.6]	NA
BMI ≥33.0	19	41.2 [31.7 – 50.5]	0.530

Abbreviations: PLGF- placental growth factor; IQR- Inter-quartile range. Values are presented as means medians [Inter-quartile range]. Comparison for each group was made to normal controls (BMI ≥20.0 & <33.0) and p values were calculated by Mann Whitney U test. A p value <0.05 is considered statistically significant.

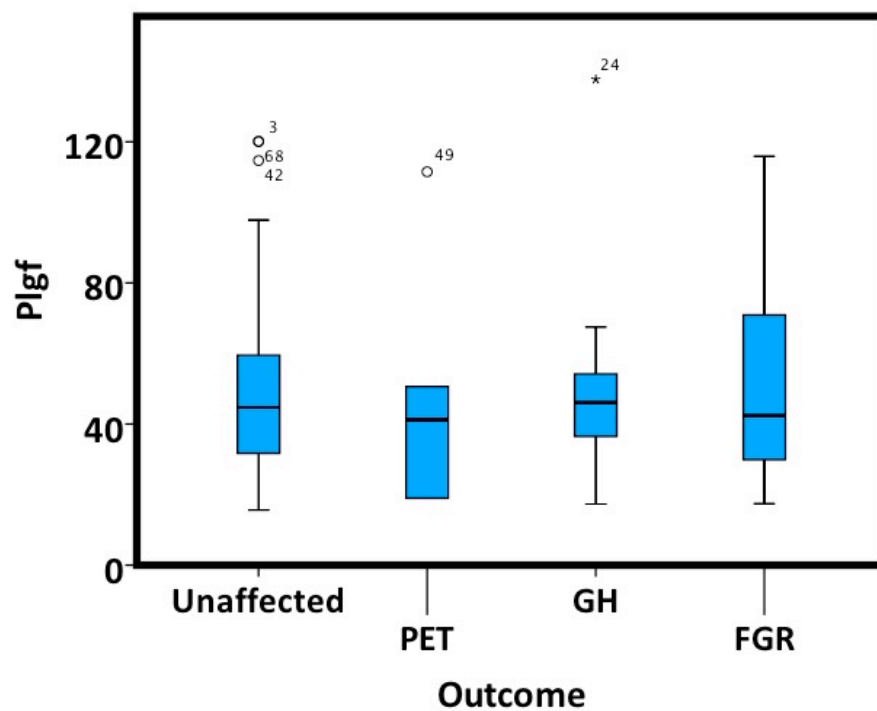


Figure 5-41 The distribution of PLGF values in uteroplacental disease and unaffected pregnancies.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: PLGF- placental growth factor, PET- preeclampsia, GH- gestational hypertension, FGR- fetal growth restriction, *- outlier values.

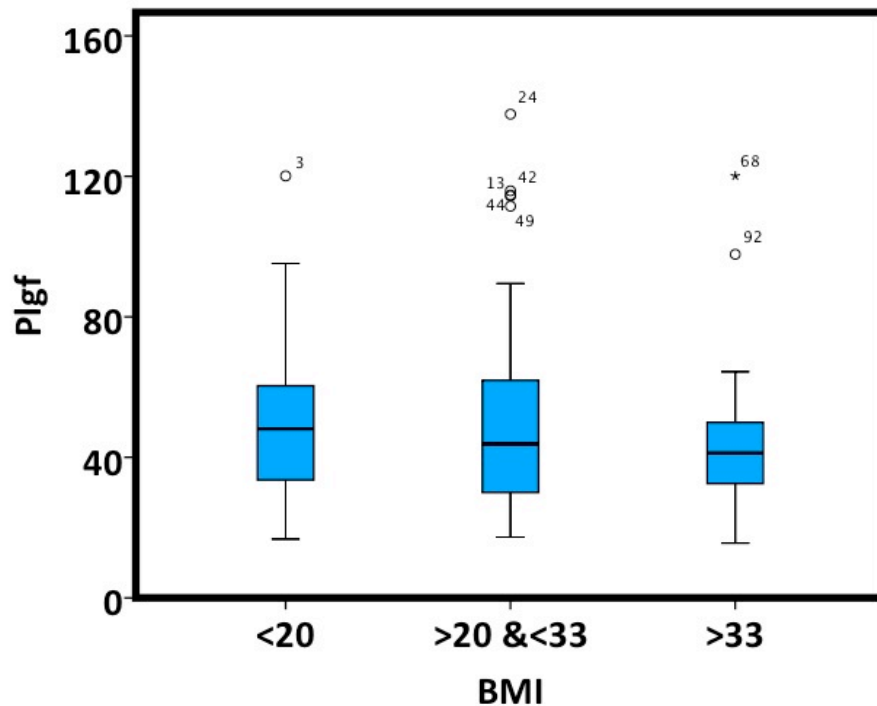


Figure 5-42 The distribution of PLGF values categorised by BMI.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: PLGF- placental growth factor, BMI- body mass index, *- outlier values.

5.9 Soluble fms like tyrosine 1: Placental growth factor ratio

A placental growth factor (PLGF) and soluble-fms like tyrosine-1 (s-flt-1) assay was performed on serum samples of 99 participants; two were excluded due to mislabelling. The ratio was calculated by dividing the serum s-flt-1/ PLGF. The samples included 61 controls of varying BMI, five with pregnancy complicated by PE, 13 with pregnancy complicated by GH and 18 with pregnancy complicated by FGR. Comparison for each group was made to normal controls by Mann-Whitney U test. The overall distribution of s-flt-1:PLGF values in this population ranged from a minimum 4.51 pg/ml to a maximum value of 113.1 pg/ml. There was no significant difference in s-flt-1:PLGF between disease states or BMI class. The distribution is further described in Figures 5.43 & 5.44.

Table 5-19 The median s-flt-1:PLGF ratio by control and disease state.

	N=	s-flt-1:PLGF Median (IQR)	P value
Normal Control	61	37.3 [24.8 – 46.3]	NA
Gestational Hypertension	13	29.8 [21.7 – 41.3]	0.398
Preeclampsia	5	28.7 [18-59.4]	0.875
Fetal Growth restriction	18	32.4 [20.5 – 41.3]	0.257

Abbreviations: s-flt-1: soluble-fms like tyrosine-1; PLGF- placental growth factor; IQR-Inter-quartile range. Values are presented as means medians [Inter-quartile range]. Comparison for each group was made to normal controls and p values were by Mann Whitney U test. A p value <0.05 is considered statistically significant.

Table 5-20 The median s-flt-1:PLGF ratio in the control population by BMI.

	N=	s-flt-1:PLGF ratio Median [IQR]	P value
BMI <20.0	27	37.3 [25.0 -46.2]	0.485
BMI ≥20.0 & <33.0	51	33.7 [21.6 – 48.4]	NA
BMI ≥33.0	19	29.2 [20.4 – 41.1]	0.472

Abbreviations: s-flt-1: soluble-fms like tyrosine-1; PLGF- placental growth factor; IQR- Inter-quartile range. Values are presented as medians [Inter-quartile range]. Comparison for each group was made to normal controls (BMI ≥20.0 & <33.0) and p values were calculated by Mann Whitney U test. A p value <0.05 is considered statistically significant.

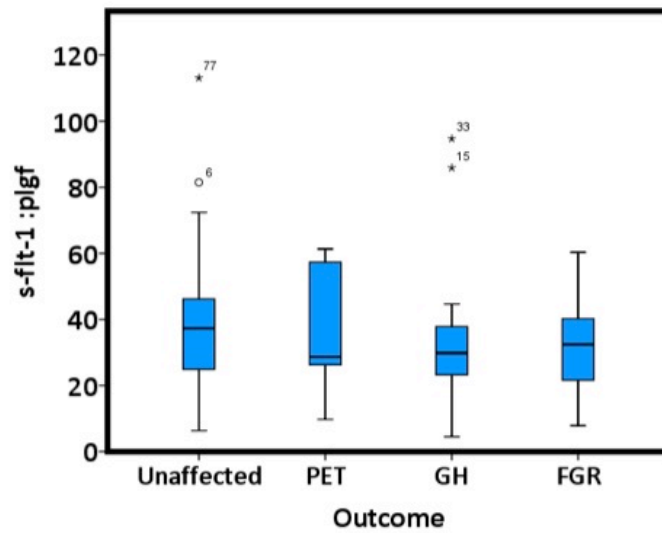


Figure 5-43 The distribution of s-flt-1: PLGF ratios in uteroplacental disease and unaffected pregnancies.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: s-flt-1 – soluble fms-like tyrosine 1, PLGF- placental growth factor, PET- preeclampsia, GH- gestational hypertension, FGR- fetal growth restriction, *- outlier values.

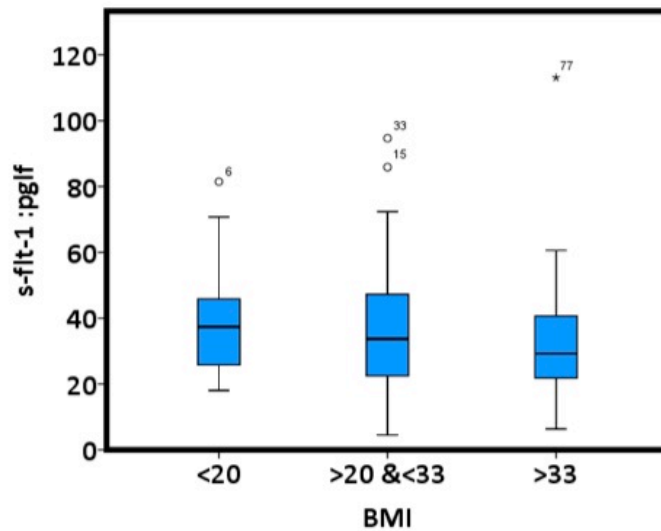


Figure 5-44 The distribution of s-flt-1:PLGF ratios categorised by BMI.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: s-flt-1 – soluble fms-like tyrosine 1, PLGF- placental growth factor, BMI- body mass index, *- outlier values.

5.10 Apelin 13

An Apelin 13 assay was performed on serum samples of 99 participants; two were excluded due to mislabelling. The samples included 61 controls of varying BMI, in addition to five with pregnancy complicated by PE, 13 with pregnancy complicated by GH and 18 with pregnancy complicated by FGR.

The result was generated by reading each plate at an optical density of both 450nm and 570nm. To correct for optical imperfections in each plate, readings at 570nm were subtracted from the reference readings at 450nm. The mean was calculated

from each set of triplicate readings for each standard, control and sample. Using Graphpad Prism (Horsham, PA, USA) a standard curve was generated (see Figure 5.45) and this value was doubled to correct for the 1:1 dilution and apelin serum levels were extrapolated from same.

The R squared value for this curve showed excellent correlation with a value of 0.9909. In addition despite relatively low serum values, the coefficient of variation (CV) for the inter-plate variance sample was 8.9% (<15% is acceptable). The overall distribution of serum Apelin 13 values ranged from a minimum 132.4pg/ml and one sample exceeded the standard curve and was extrapolated to a maximum value of 4934.2pg/ml. The distribution is further described in Figure 5.46 and Figure 5.47 with the majority of samples grouping together around 140pg/ml likely representing “normal” and a minority with elevated levels of >200 pg/ml. Apelin 13 was increased in women with uncomplicated pregnancies and a lower BMI 176.3 [150.7 – 210.9] $p=0.03$. Apelin 13 demonstrated a weak negative correlation with TPRI ($r=-0.29$; $p=0.004$) and a weak positive correlation with COi ($r=0.29$; $p=0.005$).

Table 5-21 The median Apelin 13 values by control and disease state.

	N=	Apelin 13 Median [IQR]	P value
Normal Control	61	155.5 [146.6 – 190]	NA
Gestational Hypertension	13	168.6 [152.5 – 206.5]	0.132
Preeclampsia	5	156.7 [146.3 -182.9]	0.923
Fetal Growth restriction	18	163.6 [146.6 – 214.7]	0.628

Abbreviations: IQR- Inter-quartile range. Values are presented as medians [Inter-quartile range]. Comparison for each group was made to normal controls and p values were calculated by Mann Whitney U test. A p value <0.05 is considered statistically significant

Table 5-22 The median Apelin 13 values in the control population by BMI.

	N=	Apelin 13 Median [IQR]	P value
BMI <20.0	27	176.3 [150.7 – 210.9]	0.03
BMI ≥20.0 & <33.0	51	153.1 [146 – 179.9]	NA
BMI ≥33.0	19	159.1 [147.2 – 179.3]	0.517

Abbreviations: IQR- Inter-quartile range; BMI- body mass index. Values are presented as medians [Inter-quartile range]. Comparison for each group was made to normal controls (BMI ≥20.0 & <33.0) and p values were calculated by Mann Whitney U test. A p value <0.05 is considered statistically significant.

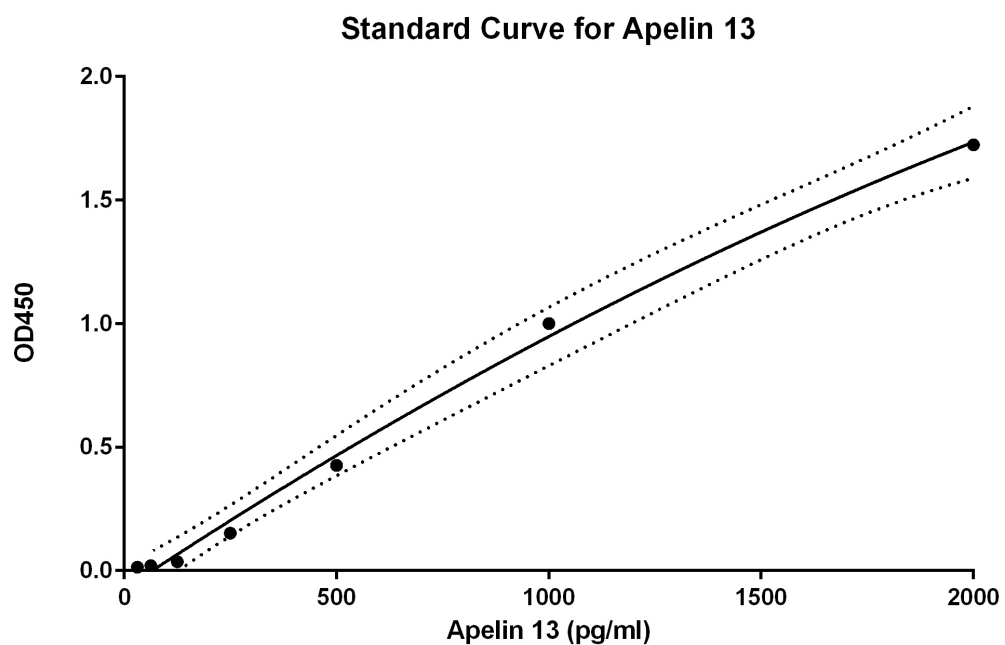


Figure 5-45 Standard Curve for Apelin 13 at Optical Density 450nm.

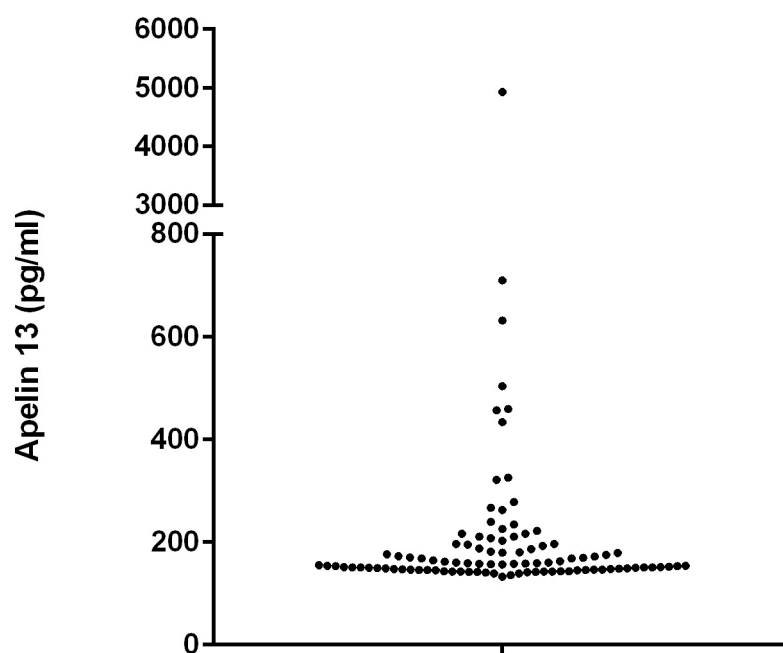


Figure 5-46 Distribution of Apelin in the entire cohort.

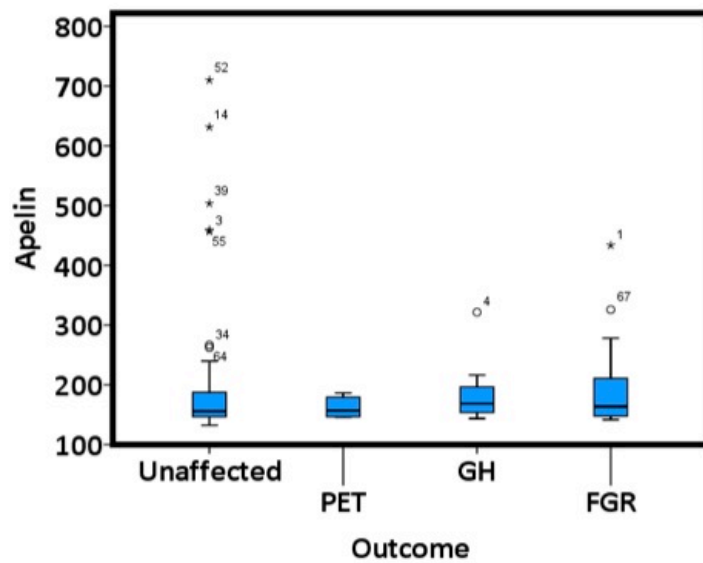


Figure 5-47 Boxplot detailing the distribution of Apelin 13 values between cases of uteroplacental disease and controls.

The boxplot incorporates the median and interquartile ranges with the whiskers demonstrating the 95% Confidence limits. Abbreviations: PET- preeclampsia, GH- gestational hypertension, FGR- fetal growth restriction, *- outlier values.

Chapter 6 RESULTS – PREDICTION

My hypothesis is that a prediction model derived from maternal biomarkers and NICOM® obtained haemodynamic variables will be able to predict the development of preeclampsia.

A univariate logistic regression was performed to produce a Receiver Operating Characteristic (ROC) analysis to determine the ability of individual haemodynamic variables, or individual biomarkers by 16 weeks' gestation to predict the evolution of later PE, GH and FGR. A Multivariate logistic regression analysis was then performed to enhance the predictive capabilities of haemodynamic variables by combining them with systolic and diastolic blood pressures. SPSS version 22 (IBM Corporation, NY, USA) was used to conduct the analysis

6.1 The prediction of uteroplacental disease from individual NICOM variables.

There was no difference in CO between the hypertensive groups and controls. Women with pregnancies affected by FGR had a lower BMI than both those affected by PE/GH and healthy pregnant controls. SBP was significantly higher in women who developed PE/GH compared to the healthy controls and FGR pregnancies. This was mirrored by a similar pattern in TPR. SBP has the best predictive ability for PE/GH (AUC=0.81; 95%CI 0.7 – 0.9; $p<0.001$) when compared with TPR (AUC=0.63; 95%CI 1.02 – 1.30; $p=0.02$) and BMI (AUC=0.6; $p=0.3$).

The best independent predictors for the evolution of uteroplacental disease at 14 weeks' gestation were CO in the prediction of FGR (AUC=0.61; $p=0.002$) and TPR in the prediction of GH (AUC=0.63; $p=0.02$). These are detailed further in Tables 6.1 and 6.2. From

univariate analysis it was not possible to provide cut-offs for variables of interest secondary to the small numbers of disease states.

Table 6-1 Assessment of independent haemodynamic variables in the prediction of uteroplacental disease.

Outcome	Predictor	AUC	Odds ratio	OR95% CL	p-value
FGR	CO	0.61	0.72	0.58-0.89	0.002
FGR	SV	0.58	0.98	0.96-1.0	0.02
GH	TPR	0.63	1.15	1.02- 1.30	0.02

Abbreviations: AUC- Area under curve, OR- Odds ratio, CL- confidence limits, FGR- fetal growth restriction, GH- Gestational hypertension, PE- preeclampsia, CO- cardiac output, SV- stroke volume, HR- heart rate and, SVi- indexed stroke volume.

Table 6-2 Assessment of independent haemodynamic variables at different time points in the prediction of gestational hypertension.

Measure	Time-point	AUC	OR (95% CI)	P-value
HR	14 weeks	0.57	1.03 (0.99,1.08)	0.169
	20 weeks	0.56	1.02 (0.97,1.06)	0.494
	28 weeks	0.63	1.04 (1.00,1.09)	0.045
SBP	14 weeks	0.81	1.10 (1.05,1.16)	0.000
	20 weeks	0.81	1.12 (1.07,1.18)	0.000
	28 weeks	0.77	1.12 (1.06,1.19)	0.000
DBP	14 weeks	0.83	1.23 (1.13,1.35)	0.000
	20 weeks	0.81	1.18 (1.10,1.26)	0.000
	28 weeks	0.80	1.20 (1.11,1.31)	0.000
MBP	14 weeks	0.86	1.21 (1.12,1.31)	0.000
	20 weeks	0.85	1.20 (1.12,1.30)	0.000
	28 weeks	0.80	1.20 (1.11,1.31)	0.000
CO	14 weeks	0.53	0.87 (0.61,1.26)	0.471
	20 weeks	0.49	0.92 (0.63,1.35)	0.659
	28 weeks	0.53	0.84 (0.57,1.23)	0.367
TPR	14 weeks	0.63	1.15 (1.02, 1.30)	0.018
	20 weeks	0.65	1.18 (1.03, 1.35)	0.014
	28 weeks	0.67	1.24 (1.06, 1.45)	0.006
SV	14 weeks	0.55	0.99 (0.96,1.02)	0.341
	20 weeks	0.53	0.99 (0.96,1.02)	0.414
	28 weeks	0.63	0.97 (0.93,1.00)	0.062
COI	14 weeks	0.50	1.00 (0.83,1.20)	0.980
	20 weeks	0.52	1.44 (0.74,2.81)	0.288
	28 weeks	0.51	1.37 (0.75,2.49)	0.304
TPRI	14 weeks	0.61	1.05 (0.95, 1.16)	0.330
	20 weeks	0.61	1.06 (0.96, 1.18)	0.245
	28 weeks	0.65	1.10 (0.99, 1.22)	0.076
SVI	14 weeks	0.47	1.01 (0.95,1.06)	0.835
	20 weeks	0.50	1.02 (0.96,1.08)	0.516
	28 weeks	0.60	0.99 (0.93,1.05)	0.659

Univariate Logistic Regression was used to assess the ability of individual variables to predict disease state. Abbreviations: AUC- Area under curve, OR- Odds ratio, CL- confidence limits, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

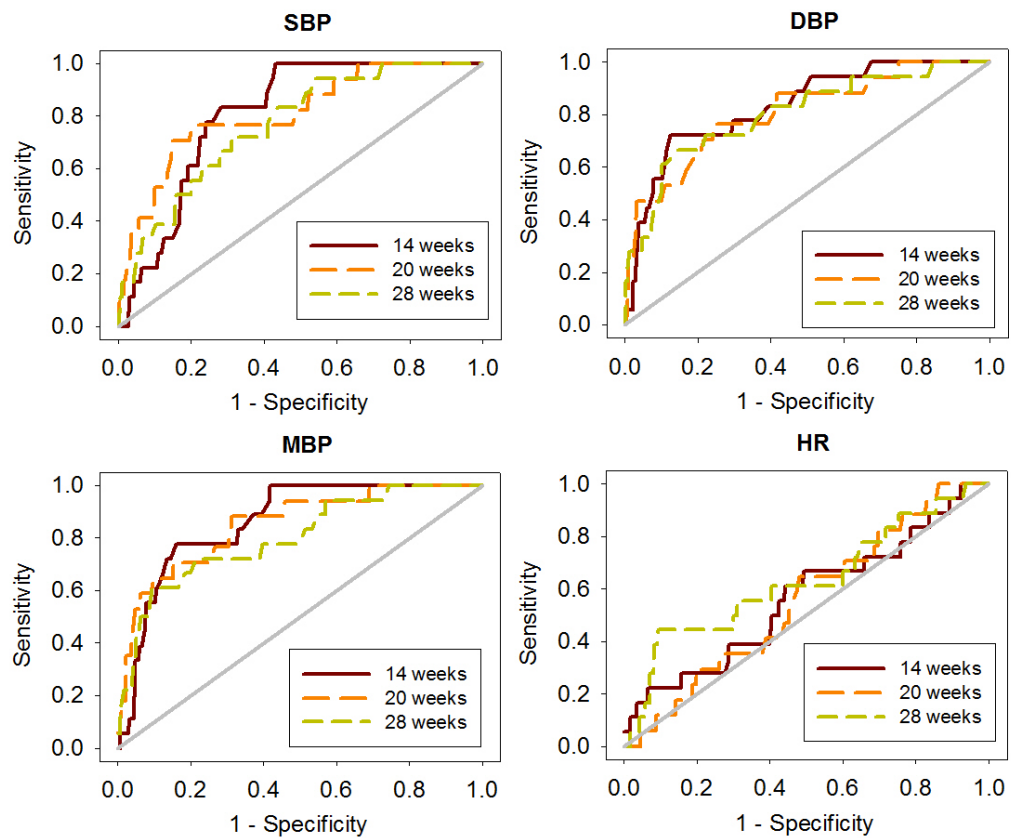


Figure 6-1 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of gestational hypertension.

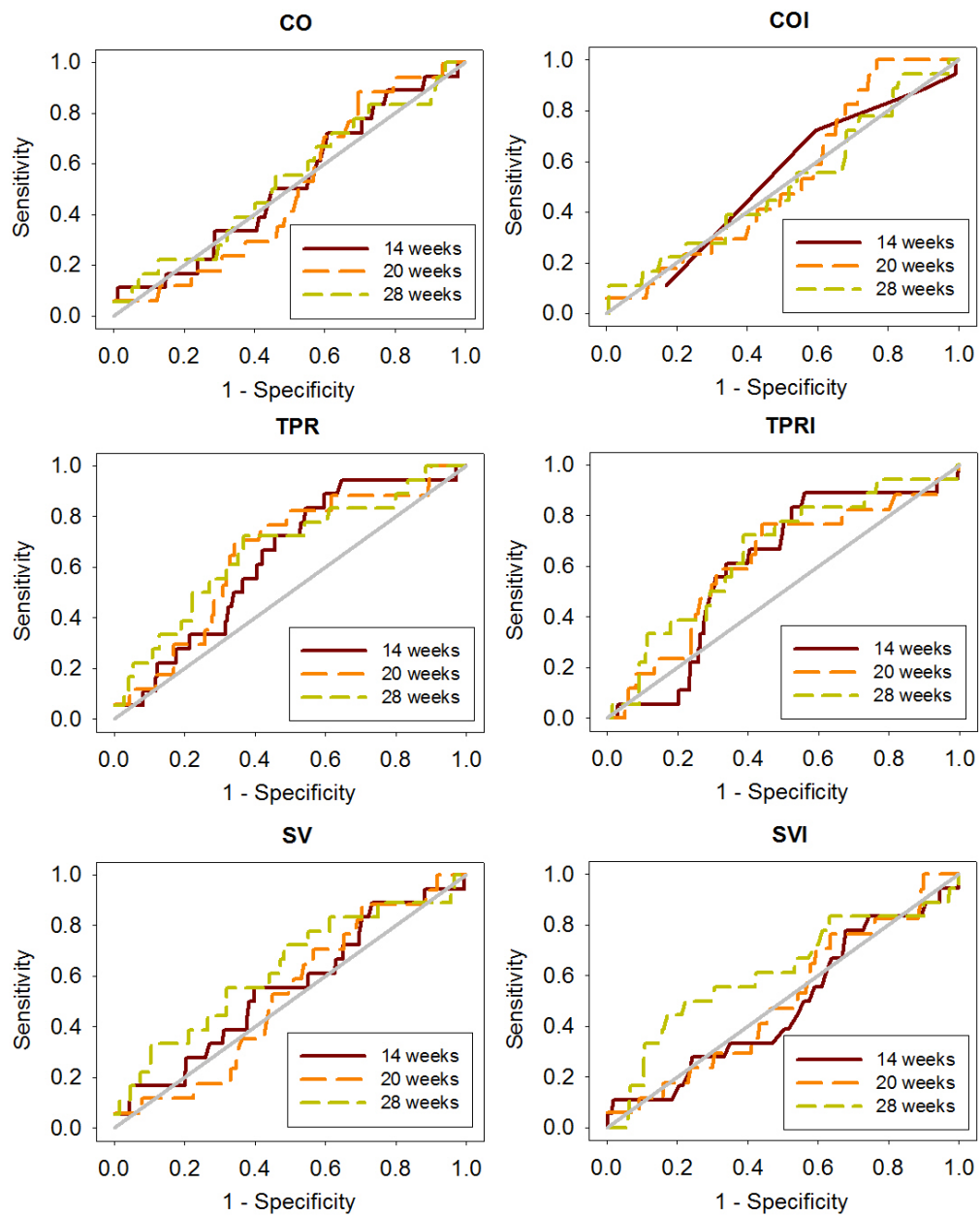


Figure 6-2 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of gestational hypertension.

Table 6-3 Assessment of independent haemodynamic variables at different times points in the prediction of preeclampsia.

Measure	Time-point	AUC	OR (95% CI)	P-value
HR	14 weeks	0.58	0.98 (0.90,1.06)	0.561
	20 weeks	0.65	0.92 (0.84,1.01)	0.068
	28 weeks	0.64	0.96 (0.89,1.03)	0.251
SBP	14 weeks	0.72	1.07 (0.99,1.16)	0.092
	20 weeks	0.64	1.05 (0.97,1.14)	0.233
	28 weeks	0.81	1.15 (1.04,1.28)	0.008
DBP	14 weeks	0.59	1.04 (0.92,1.18)	0.496
	20 weeks	0.64	1.06 (0.95,1.18)	0.293
	28 weeks	0.80	1.21 (1.07,1.37)	0.003
MBP	14 weeks	0.67	1.07 (0.96,1.20)	0.222
	20 weeks	0.64	1.07 (0.96,1.19)	0.220
	28 weeks	0.83	1.25 (1.09,1.44)	0.002
CO	14 weeks	0.51	0.94 (0.50,1.76)	0.850
	20 weeks	0.50	1.06 (0.57,1.97)	0.845
	28 weeks	0.59	1.03 (0.56,1.88)	0.930
TPR	14 weeks	0.52	1.08 (0.81, 1.43)	0.620
	20 weeks	0.45	0.99 (0.71, 1.38)	0.951
	28 weeks	0.57	1.25 (0.95, 1.04)	0.114
SV	14 weeks	0.47	1.00 (0.96,1.05)	0.870
	20 weeks	0.67	1.03 (0.99,1.08)	0.170
	28 weeks	0.58	1.02 (0.97,1.07)	0.555
COI	14 weeks	0.46	0.96 (0.50,1.86)	0.904
	20 weeks	0.58	1.38 (0.42,4.49)	0.597
	28 weeks	0.56	1.24 (0.42,3.67)	0.699
TPRI	14 weeks	0.50	1.05 (0.89, 1.24)	0.601
	20 weeks	0.52	0.97 (0.80, 1.19)	0.800
	28 weeks	0.56	1.11 (0.95, 1.31)	0.198
SVI	14 weeks	0.55	1.02 (0.93,1.11)	0.664
	20 weeks	0.68	1.09 (1.00,1.19)	0.056
	28 weeks	0.62	1.05 (0.95,1.15)	0.335

Univariate Logistic Regression was used to assess the ability of individual variables to predict disease state. Abbreviations: AUC- Area under curve, OR- Odds ratio, CL- confidence limits, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

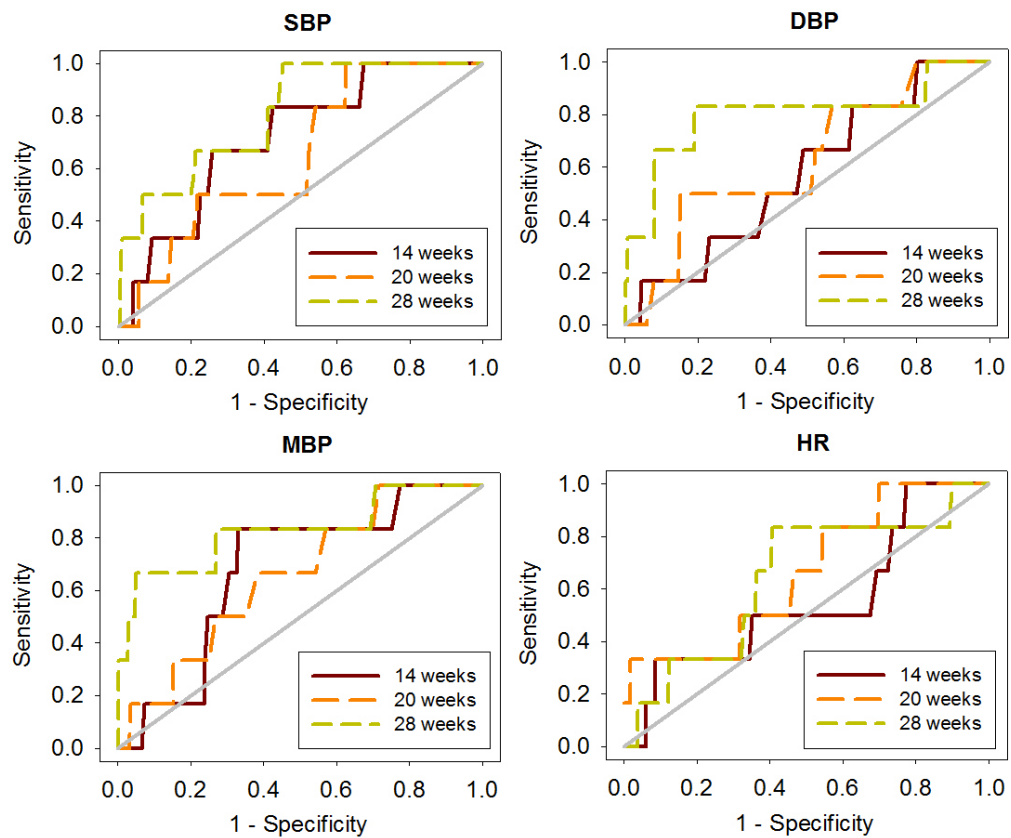


Figure 6-3 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of preeclampsia.

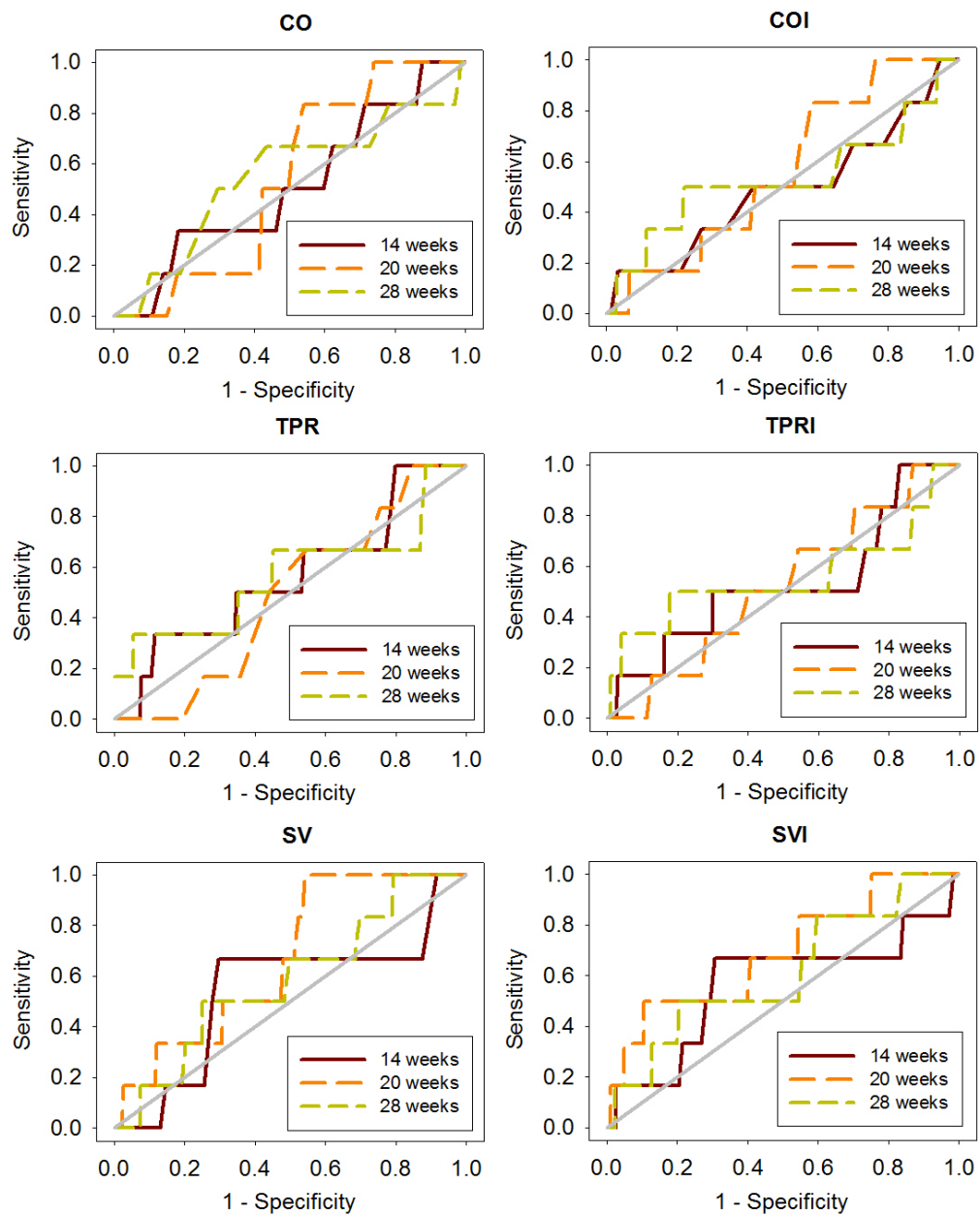


Figure 6-4 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of preeclampsia.

Table 6-4 Assessment of independent haemodynamic variables at different time points in the prediction of fetal growth restriction.

Measure	Time-point	AUC	OR (95% CI)	P-value
HR	14 weeks	0.52	0.99 (0.95,1.04)	0.732
	20 weeks	0.50	0.99 (0.95,1.03)	0.641
	28 weeks	0.52	1.00 (0.96,1.03)	0.833
SBP	14 weeks	0.61	0.95 (0.90,0.99)	0.023
	20 weeks	0.61	0.96 (0.92,1.01)	0.121
	28 weeks	0.59	0.97 (0.93,1.01)	0.199
DBP	14 weeks	0.60	0.95 (0.89,1.01)	0.073
	20 weeks	0.66	0.92 (0.87,0.98)	0.007
	28 weeks	0.51	0.99 (0.93,1.06)	0.828
MBP	14 weeks	0.61	0.94 (0.88,0.99)	0.033
	20 weeks	0.65	0.94 (0.88,1.00)	0.047
	28 weeks	0.56	0.98 (0.92,1.04)	0.447
CO	14 weeks	0.62	0.69 (0.49,0.97)	0.034
	20 weeks	0.59	0.74 (0.52,1.06)	0.104
	28 weeks	0.64	0.61 (0.41,0.90)	0.013
TPR	14 weeks	0.55	1.05 (0.90, 1.22)	0.551
	20 weeks	0.55	1.08 (0.92, 1.27)	0.352
	28 weeks	0.63	1.24 (1.05, 1.45)	0.009
SV	14 weeks	0.59	0.98 (0.95,1.01)	0.148
	20 weeks	0.56	0.98 (0.96,1.01)	0.224
	28 weeks	0.64	0.96 (0.93,1.00)	0.028
COI	14 weeks	0.54	0.79 (0.41,1.50)	0.467
	20 weeks	0.53	0.80 (0.42,1.53)	0.499
	28 weeks	0.59	0.58 (0.28,1.18)	0.133
TPRI	14 weeks	0.52	0.98 (0.89, 1.07)	0.628
	20 weeks	0.52	0.98 (0.89, 1.09)	0.752
	28 weeks	0.58	1.07 (0.97, 1.17)	0.180
SVI	14 weeks	0.53	0.99 (0.95,1.04)	0.719
	20 weeks	0.47	1.00 (0.95,1.05)	0.855
	28 weeks	0.58	0.97 (0.91,1.02)	0.230

Univariate Logistic Regression was used to assess the ability of individual variables to predict disease state. Abbreviations: AUC- Area under curve, OR- Odds ratio, CL- confidence limits, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

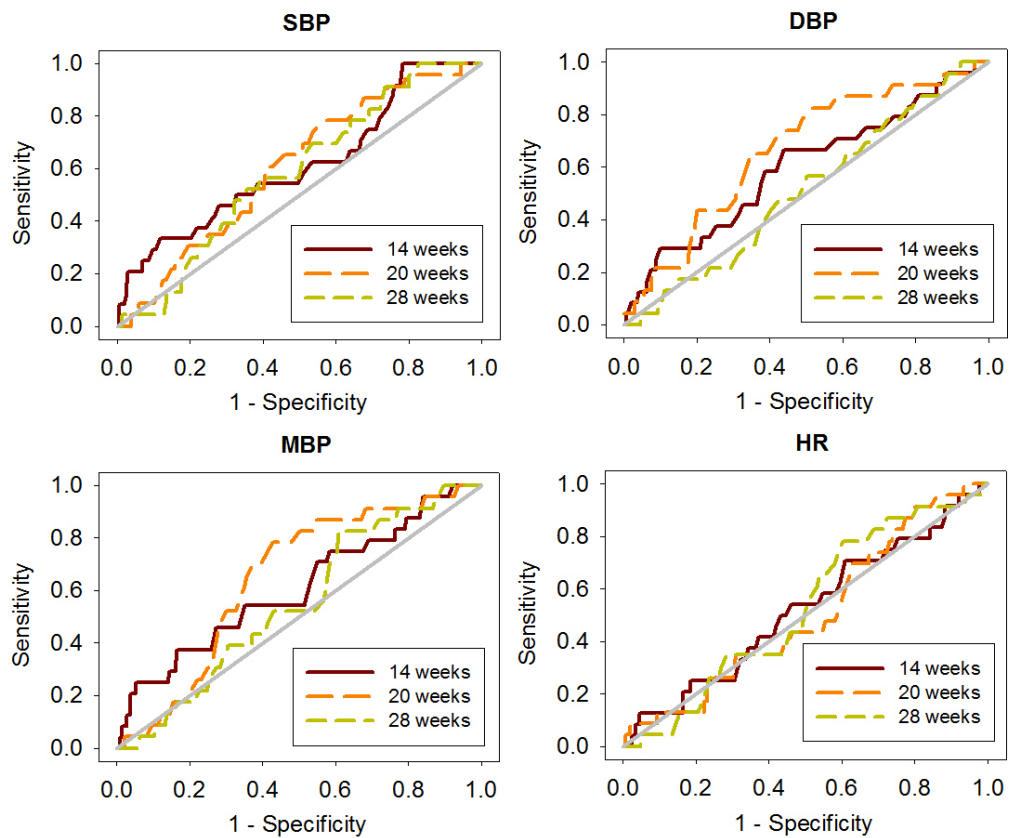


Figure 6-5 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of fetal growth restriction.

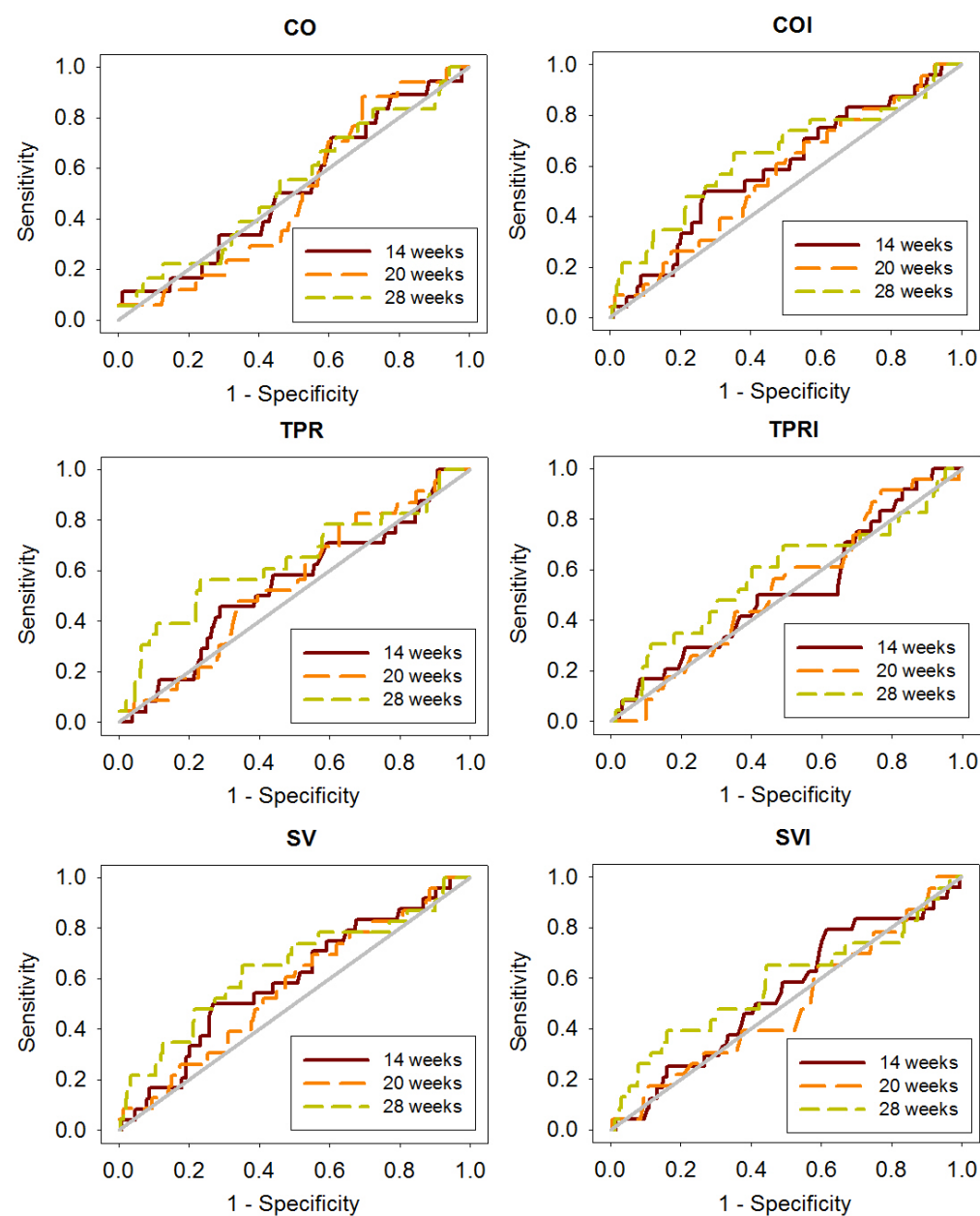


Figure 6-6 ROC detailing predictive capabilities of independent haemodynamic variables at different time points in the prediction of fetal growth restriction.

6.2 Multivariate model combining blood pressure & NICOM variables in the prediction of uteroplacental disease.

The performance of haemodynamic variables was enhanced when combined in a multivariate logistic model as detailed in Table 6.5. We demonstrated that TPR, CO and SV when combined with BP were significant predictors of pregnancies complicated by FGR (AUC=0.64, $p=0.005$; AUC=0.65, $p=0.003$; and AUC=0.65, $p=0.005$ respectively). Whereas in pregnancies complicated by PE, HR and SVi in combination with BP were also statistically significant predictors (AUC=0.75, $p=0.01$ and AUC=0.77, $p=0.009$ respectively).

Table 6-5 The ability of haemodynamic variables (combined with BP and adjusted for gestational age) to predict the evolution of uteroplacental disease.

Outcome	Predictor	AUC	Odds ratio	OR95% CL	p-value
FGR	TPR	0.64	1.0	1.0-1.0	0.005
FGR	CO	0.65	0.7	0.59-0.91	0.003
FGR	SV	0.65	0.98	0.96-0.99	0.005
PE	SVi	0.77	1.1	1.0-1.1	0.009
PE	HR	0.75	0.94	0.9-0.99	0.01
PE/GH	SVi	0.82	1.05	1.02-1.08	0.003
PE/GH	COI	0.81	1.44	1.01-2.05	0.04
GH	TPR	0.84	1.0	0.998-1.001	0.14
PE	TPR	0.71	1.0	0.997 - 1.001	0.54
GH	TPRI	0.85	1.0	0.998- 1	0.12
PE	TPRI	0.77	1.0	0.997 - 1.001	0.30
FGR	TPRI	0.62	1.001	1-1.001	0.21

Abbreviations: AUC- Area under curve, OR- Odds ratio, CL- confidence limits, FGR- fetal growth restriction, PE- preeclampsia, TPR- total peripheral resistance, TPRI- indexed total peripheral resistance, CO- cardiac output, SV- stroke volume, SVi- indexed stroke volume, HR- heart rate and COI- cardiac index.

6.3 The prediction of the development of uteroplacental disease from first trimester biomarkers.

Table 6-6 The prediction of the development of uteroplacental disease from first trimester s-flt-1.

Uteroplacental Disease	AUC	OR (95% CI)	p-value
PE	0.69	1.00 (1.00, 1.00)	0.153
GH	0.60	1.00 (1.00, 1.00)	0.262
FGR	0.61	1.00 (1.00, 1.00)	0.099

Abbreviations: s-flt-1: soluble fms like tyrosine-1; AUC- Area under the curve, OR- Odds ratio, CI- confidence interval, PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction.

Table 6-7 Logistic regression results for PLGF in the prediction of uteroplacental disease.

Uteroplacental Disease	AUC	OR (95% CI)	p-value
PE	0.56	1.00 (0.96, 1.04)	0.933
GH	0.49	1.00 (0.98, 1.03)	0.881
FGR	0.48	1.00 (0.98, 1.02)	0.716

Abbreviations: AUC- Area under the curve, OR- Odds ratio, CI- confidence interval, PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction.

Table 6-8 Logistic regression results for s-flt-1:PLGF in the prediction of uteroplacental disease.

Uteroplacental Disease	AUC	OR (95% CI)	p-value
PE	0.52	1.26 (0.42,3.75)	0.68
GH	0.58	1.32 (0.66,2.62)	0.43
FGR	0.59	1.50 (0.78,2.89)	0.22

Abbreviations: s-flt-1: soluble fms-like tyrosine 1; PLGF- placental growth factor; AUC- Area under the curve, OR- Odds ratio, CI- confidence interval, PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction.

Table 6-9 Logistic regression results for Apelin in the prediction of uteroplacental disease.

Uteroplacental Disease	AUC	OR (95% CI)	p-value
PE	0.52	0.99 (0.97, 1.02)	0.553
GH	0.62	1.00 (1.00, 1.00)	0.282
FGR	0.46	1.00 (0.99, 1.00)	0.964

Abbreviations: AUC- Area under the curve, OR- Odds ratio, CI- confidence interval, PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction.

Table 6-10 Logistic regression results for MPV in the prediction of uteroplacental disease.

Uteroplacental Disease	AUC	OR (95% CI)	p-value
PE	0.51	1.03 (0.61, 1.76)	0.902
GH	0.59	1.24 (0.97, 1.60)	0.090
FGR	0.50	1.05 (0.81, 1.38)	0.697

Abbreviations: AUC- Area under the curve, OR- Odds ratio, CI- confidence interval, PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction.

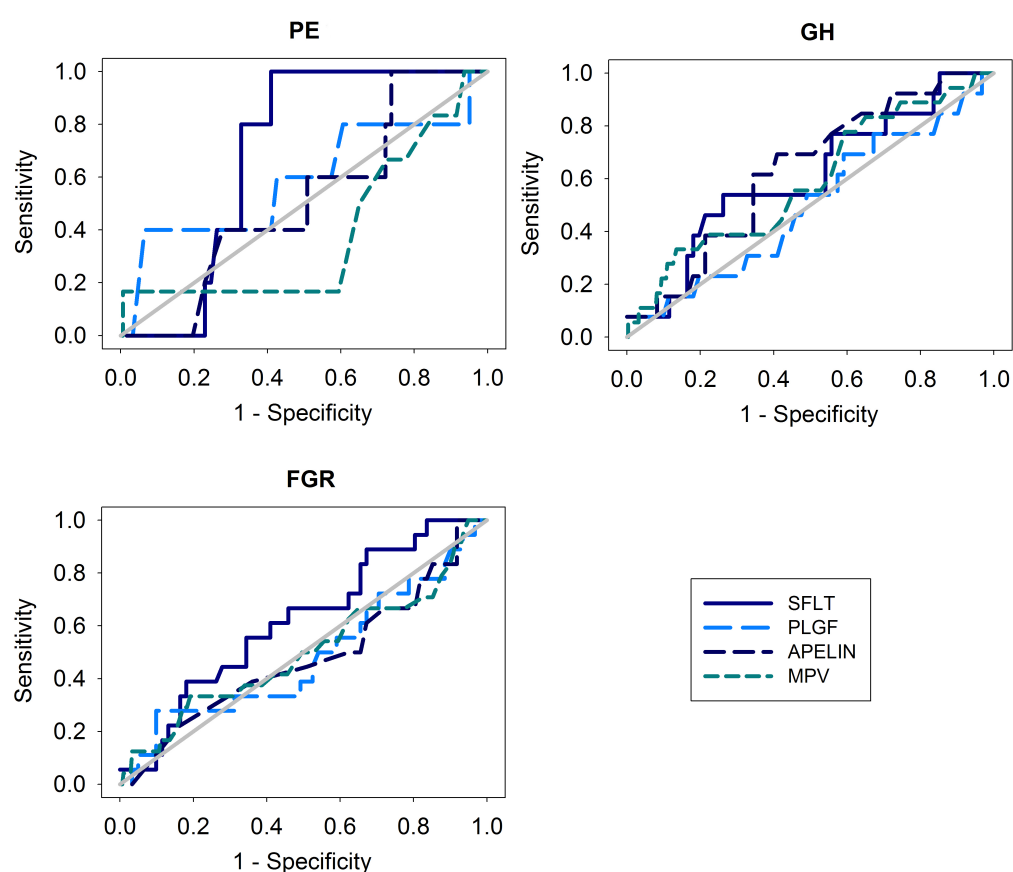


Figure 6-7 ROC curve detailing the predictive capabilities of uteroplacental disease at 14 weeks' gestational age by independent biomarkers.

Abbreviations: PE- preeclampsia, GH- gestational hypertension and FGR- fetal growth restriction. SFLT- soluble fms-like tyrosine-1; PLGF- placental growth factor; MPV- Mean platelet volume.

6.4 The prediction of uteroplacental disease using combinations of haemodynamic variables and first trimester biomarkers.

The biomarkers and first NICOM haemodynamic measures (total: 15 predictors) were used to determine patterns associated with outcomes. Given the relatively few adverse outcomes in our study (6 PE, 18 GH and 24 FGR), traditional modelling strategies such as logistic regression are difficult to apply. As a consequence of the low number of disease states I undertook an unplanned, exploratory secondary statistical analysis (discriminant analysis (DA)) to identify trends amongst disease states.

This different modelling approach, DA allows correlations within sets of predictors. The objective was pattern recognition as opposed to outcome prediction. It is often used, successfully, to determine sets of predictors, which have potential value for further investigation. Dimension reduction (reduced models) may be obtained using the partial- r^2 . Wilks Lambda is a measure of discrimination (0=perfect discrimination and 1=no discrimination).

Table 6-11 Discriminant analysis of combined biomarkers and first trimester cardiac variables in preeclampsia.

Full Model		Reduced Model		
Variable	Partial R-square	Partial R-Square	Walds Lambda	P < Lambda
s-flt-1	0.0319	0.0351	0.95329695	0.0838
PLGF	0.0001			
APELIN	0.0073			
MPV	0.0467	0.0467	0.91987821	0.0751
PLGF:s-flt-1	0.0023			
HR	0.0108			
SBP	0.0167			
DBP	0.0036			
MBP	0.0087			
CO	0.0000			
TPR	0.0005			
SV	0.0048			
COI	0.0012			
TPRI	0.0009			
SVI	0.0109			

Abbreviations: s-flt-1 - soluble fms-like tyrosine 1, PLGF- placental growth factor, MPV- Mean platelet volume, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

Table 6-12 Discriminant analysis of combined biomarkers and first trimester cardiac variables in gestational hypertension.

Full Model		Reduced Model		
Variable	Partial R-square	Partial R-Square	Walds Lambda	P < Lambda
s-flt-1	0.0154	0.0363	0.75561366	0.0002
PLGF	0.0004			
APELIN	0.0570	0.0493	0.78409750	0.0002
MPV	0.0077			
PLGF:s-flt-1	0.0080			
HR	0.0556			
SBP	0.1357			
DBP	0.1676			
MBP	0.1753	0.1753	0.82472978	0.0002
CO1	0.0001			
TPR	0.0286			
SV1	0.0035			
COI	0.0008			
TPRI	0.0005			
SVI	0.0074			

Abbreviations: s-flt-1 - soluble fms-like tyrosine 1, PLGF- placental growth factor, MPV- Mean platelet volume, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

Table 6-13 Discriminant analysis of combined biomarkers and first trimester cardiac variables in fetal growth restriction.

Full Model		Reduced Model		
Variable	Partial R-square	Partial R-Square	Walds Lambda	P < Lambda
s-flt-1	0.0328	0.0328	0.96715751	0.1123
PLGF	0.0020			
APELIN	0.0001			
MPV	0.0060			
PLGF:s-flt-1	0.0186			
HR	0.0034			
SBP	0.0211			
DBP	0.0049			
MBP	0.0117			
CO	0.0196			
TPR	0.0015			
SV	0.0206	0.0318	0.93642921	0.0852
COI	0.0041			
TPRI	0.0061			
SVI	0.0022			

Abbreviations: s-flt-1 - soluble fms-like tyrosine 1, PLGF- placental growth factor, MPV- Mean platelet volume, HR- Heart rate, SBP- systolic Blood pressure, DBP- diastolic blood pressure, MBP- Mean Blood pressure, CO- cardiac output, TPR- total peripheral resistance, SV- stroke volume, COI- Cardiac index, TPRI- indexed total peripheral resistance and SVI- indexed stroke volume.

Following identification via discriminate analysis a multi-logistic regression analysis was performed on variables of interest. The result of this showed that in the prediction of PE the combination of s-flt-1 and MPV had strong capabilities with an AUC 0.88 and $p=0.011$. Similar improvements in the prediction of FGR was noted by the combination of s-flt-1, SV and TPRi with an AUC=0.76 and $p=0.007$. See Table 6.14 for further details.

Table 6-14 Multi-logistic regression analysis in the prediction of uteroplacental disease.

		Individual Predictors		Combined Predictors	
		AUC	p-value	AUC	p-value
PE	s-flt-1	0.69	0.155	0.88	0.011
	MPV	0.76	0.103		
FGR	s-flt-1	0.61	0.098	0.76	0.007
	SV	0.59	0.148		
	TPRi	0.52	0.628		

Abbreviations: AUC- area under the curve; PE- preeclampsia, s-flt-1- soluble fms-like tyrosine 1; MPV- mean platelet volume; FGR- fetal growth restriction; SV- stroke volume; TPRi- indexed total peripheral resistance.

Chapter 7 THE EFFECT OF LABETALOL ON THE NEONATAL MYOCARDIUM

To allow for assessment of neonatal myocardial performance a small cross sectional study of 30 infants from the patient cohort of 318 unaffected pregnancies underwent echocardiographic assessments. There were a total of forty-five infants who underwent neonatal echocardiogram as part of the HANDLE study, 15 infants born to mothers with Gestational Hypertension (GH) and 30 controls. It was not possible to perform neonatal echocardiogram in an additional three infants born to mothers with GH due to the brevity between delivery and discharge. All infants assessed in this cross-sectional study were well at delivery and had no indication for neonatal echocardiography outside of the research setting. My hypothesis is that maternal antenatal labetalol use causes altered neonatal cardiac function in addition to the known consequence of neonatal bradycardia.

Continuous data were presented as either means (standard deviation) and compared using a Student independent t-test, or as medians [inter-quartile ranges- IQR] and compared using the Mann Whitney U test. Categorical variables were presented as absolute numbers and percentages and compared using a Chi square test (or Fisher exact test as appropriate). Correlations were tested using Pearson's correlation coefficient. Multi-variate linear regression was used to test the independent association between important variables and the functional parameters. A p-value <0.05 was considered significant. SPSS version 23 (IBM, Corporation, NY, USA) was used to conduct the statistical analysis.

Table 7.1 illustrates the maternal and infants' characteristics in the two groups. Mothers with GH were slightly older than controls but had similar weight and body mass index, ethnic origin and smoking status between the two groups. Women who developed gestational

hypertension were more likely to have a university education and had a longer hospital stay. Infants born to mothers with GH were similar to infants born to healthy mothers with no differences in gestation, birthweight, 5-minute Apgar score or cord pH. There was a higher rate of caesarean section in the GH group (Table 7.1). There were no differences in systolic or diastolic blood pressure between the two groups at the first antenatal visit (Table 7.1). All mothers in the GH group were in receipt of labetalol with maximum dosage ranging between 200mg - 900mg daily and one patient was receiving additional 20mg nifedipine daily at the time of delivery. There were no instances in either the control or gestational hypertensive group whereby mothers received Magnesium Sulphate.

Table 7-1 Maternal characteristics and infant birth demographics.

	Gestational Hypertension n=15	Control n=30	p
Maternal age (years)	32 [30 – 35]	29 [25 – 32]	0.03
Maternal weight (kg)	71 [61 – 81]	63 [58 – 78]	0.61
Maternal BMI (kg/m²)	26 [24 – 28]	24 [22 – 28]	0.30
BP at booking (mmHg)			
<i>Systolic</i>	128 [115 – 133]	125 [113 – 129]	0.06
<i>Diastolic</i>	73 [68 – 85]	69 [66 – 76]	0.07
<i>MAP</i>	92 ± 9.1	87.4 ± 6.2	0.17
White European	13 (86.7)	27 (90)	0.82
Tertiary education	11 (73.3)	12 (38.7)	0.03
Single	7 (46.7)	21 (67.6)	0.17
Smoker	0(0)	6 (19.4)	0.16
Gestational age (weeks)	39.4 [38.8 – 40.5]	40.0 [39.7 – 41.2]	0.19
Birthweight (g)	3390 [2910 – 3560]	3515 [3210 – 3900]	0.21
Male	7 (47)	18 (60)	0.53
Caesarean Section	8 (53)	4 (13)	0.01
5 Minute Apgar Score	10 [10 – 10]	10 [10 – 10]	0.78
Venous Cord pH	7.31 [7.26 – 7.35]	7.29 [7.26 – 7.33]	0.81
Neonatal hypoglycaemia	3 (20)	0	0.03
NICU admission	3 (20)	6 (19.4)	0.96
NICU LOS	1 [1.0 -1.0]	1.5 [1-2.25]	0.564

Data presented as medians [inter-quartile range] or absolute value (%). * between 14 – 16 weeks gestation. Abbreviations: BMI- body mass index; BP- blood pressure; MAP- mean arterial pressure, LOS- length of stay; NICU- Neonatal intensive care unit.

Echocardiography was performed at a median of 27 [17 – 44] hours following delivery. Infants born to mothers with GH had a lower incidence of patent ductus arteriosus (PDA) at the time of the scan (Table 7.2). There was no difference in left atrium:aorta ratio or left ventricular end-diastolic diameter between the two groups and no difference in left ventricle dimensions. Infants in the GH group had a lower ejection fraction [54 (6) vs. 61 (6)%, $p<0.01$], lower left ventricular global longitudinal strain [-20 (2) vs. -25 (3)%, $p<0.01$], and a lower twist [11 (8) vs. 16 (6) degrees, $p=0.04$]. Strain rate, basal rotation, twist rate and untwist rate were similar between the two groups. There were no differences in any of the right ventricle functional or dimension measurements between the two groups (Table 7.2).

The effect of potential confounding variables (maternal age, infant birthweight, mode of delivery and presence of PDA) on functional parameter was examined in linear regression. Group assignment (GH vs. Control) remained independently associated with left ventricular global longitudinal strain and ejection fraction but not left ventricle twist which became a trend (Table 7.3).

Table 7-2 Myocardial function in the two groups.

	Gestational Hypertension n=15	Control n=30	p
Time of Scan (hours after birth)	27 [22 – 34]	27 [14 – 42]	0.94
Heart Rate	128 (9)	119 (15)	0.07
PDA and Preload Surrogates			
PDA Presence	1 (7%)	9 (31%)	0.13
PDA Diameter (mm)	2.8	2.0 (0.6)	0.22
LA:Ao	1.4 (0.3)	1.2 (0.1)	0.1
Mitral Inflow E:A ratio	1.1 (0.2)	1.1 (0.2)	0.98
Mitral Inflow VTI	8.4 (1.2)	8.3 (1.9)	0.94
LV Dimensions			
MV annular diameter (mm)	9.5 (1.3)	9.4 (1.1)	0.77
LVEDD (mm)	18 (2)	18 (2)	0.39
Septal wall thickness (mm)	2.7 (0.5)	2.6 (0.4)	0.41
LV Posterior wall thickness (mm)	2.2 (0.6)	2.3 (0.6)	0.53
LV Length (mm)	27 (2)	28 (2)	0.28
LV Function			
Ejection Fraction (%)	54 (6)	61 (6)	<0.01
Global Longitudinal Strain (%)	-20 (2)	-25 (3)	<0.01
Global Longitudinal systolic SR (1/s)	-1.9 (0.4)	-2.0 (0.3)	0.25
Apical Rotation (°)	15 (5)	17 (5)	0.31
Basal Rotation (°)	4 (8)	0.9 (4.3)	0.25
Twist (°)	11 (8)	16 (6)	0.04
Twist Rate (°/s)	145 (58)	151 (47)	0.74
Untwist Rate (°/s)	-170 (84)	-188 (53)	0.43
RV Function and Dimensions			
RV Length (mm)	26 (3)	27 (2)	0.44
TV annular diameter (mm)	9.7 (1.5)	9.9 (1.4)	0.55
RV mid cavity diameter (mm)	12.6 (1.9)	12.9 (1.4)	0.54
TAPSE (mm)	8.7 (1.7)	8.4 (1.1)	0.57
RV Fractional Area Change (%)	26 (7)	25 (4)	0.18
RV Longitudinal Strain (%)	-23 (4)	-25 (4)	0.18
RV Longitudinal SR (1/s)	-2.0 (0.8)	-2.3 (0.8)	0.30

Values are presented as medians [inter-quartile range], means (SD) or absolute value (%). Abbreviations: PDA: patent ductus arteriosus; LA:Ao: left atrial to aortic root ratio; MV: mitral valve; LVEDD: left ventricular end diastolic diameter; LV: left ventricle; SR: strain rate; RV: right ventricle; TV: tricuspid valve; TAPSE: tricuspid annular plain systolic excursion.

Table 7-3 Association between group assignment (Maternal GH vs. Control) and functional measurements adjusting for maternal age, infant birthweight, mode of delivery and patent ductus arteriosus.

Dependent Variable	Group assignment β coefficient*	p value
Global Longitudinal Strain	0.42	0.02
Ejection Fraction	0.54	0.003
LV Twist	0.37	0.09

Abbreviations: LV- left ventricle. * Standardised β

Chapter 8 DISCUSSION.

Data presented in this thesis, which originate from the prospective single centre **Haemodynamic Assessment in pregnancy and neonatal Echocardiography assessment (HANDLE)** study, consolidate knowledge regarding haemodynamics in pregnancy and the potential for prediction of uteroplacental disease in the first trimester. Given the significant morbidity and mortality with this condition these concepts have been demonstrated as important at both national and international levels.(221, 349, 350, 378-389)

Following finalisation of the study protocol, funding through the Medical Research Charities Group and The Rotunda Foundation (formerly known as Friends of the Rotunda- MRCG-2013-9) and ethical approval from the Rotunda Research and Ethics committee, the HANDLE study commenced recruitment in May 2014. The last participant was recruited in January 2016 and participants completed study protocol by October 2016.

A total of 366 women completed the study protocol. Of these, six pregnancies were complicated by PE, eighteen by GH, twenty-four by FGR and 318 were unaffected. The resultant incidence of PE in this cohort was 1.6%, much lower than the anticipated 5-7% in nulliparous women. To assess for potential selection bias I performed a 13-year review of the Rotunda Annual Clinical Reports between 2004-2016. From the hypertension chapter in the clinical reports I calculated that the mean PE rate in nulliparous women between 2004-2016 was 4.3%, in keeping with the expected 5%. However, when assessed over time there was a significant reduction in nulliparous PE after 2009 seen in Figure 4.2 ($p<0.001$). This fell from a peak of 5.3% in 2005 to a trough of 2.4% in 2015, which incorporates the population presented in this thesis.

The reasons for the declining rate of PE in the hospital population remain unclear. Despite anticipating potential increases in the PE rates I have observed the converse. This is despite a changing obstetric population where there are increases in known risk factors for PE such as increasing maternal age, increasing maternal BMI, increasing complex comorbidities and pregnancies resulting from assisted reproduction techniques. When addressing potential reasons for this observed declining PE rate, I considered whether it was a change in documentation or Hospital in-patient enquiry (HIPE) coding. This was not deemed to be the case as local coding practice was improved over the reported time period. The exclusion of documentation as a causative factor is further supported by similar reductions observed in the rates of eclampsia ($p=0.02$), induction of labour indicated by hypertension ($p=0.03$), lower segment caesarean section indicated by PE ($p=0.03$) and admission to High Dependency Unit indicated by hypertension ($p=0.03$).

Consideration was also given to the increased prescription of aspirin in the obstetric population and the evidence regarding its benefit in the prevention of uteroplacental disease. However, the use of aspirin was an exclusion criterion in this cohort and does not explain the lower than anticipated PE rate. I also considered whether there was a decline in later onset PE as there is a trend to deliver women sooner. However, this was not the case with no significant difference between gestational age at delivery between 2004-2016 as detailed in Figure 4.3. It has been established that a maternal history of smoking is protective against PE.(4) The prevalence of smoking is highest in young adults with more than one in four of adults of reproductive age found to be smoking. This high background prevalence and the poor self reporting of smoking status in pregnancy are possible contributors to our declining PE rates.(173)

8.1 Key Findings

8.1.1 Validation

While recruitment was underway a small sub cohort underwent simultaneous echocardiography and NICOM® assessment. This echocardiography data allowed for validation of bioreactance based NICOM® measures of cardiac output and stroke volume within this unique pregnant population and completion of a secondary objective of the HANDLE study (Chapter 3.1). BRT-measured CO and SV obtained during the second trimester of pregnancy demonstrated acceptable agreement with echocardiography-measured values. A higher maternal BMI appeared to have a negative impact on measures of diastolic function using echocardiography.

The use of non-invasive CO monitoring by the bioreactance technique has gained considerable interest recently. The use of NICOM was first described by Keren et al in animal studies in 2007.(239) Following on from this Squara et al validated its use the adult population by correlation with thermodilution in 2007.(233) Following these early studies NICOM® received its approval by the American Food and Drug Administration (FDA) in 2008.

Early work on NICOM® has focused on establishing its reliability and validity in a variety of populations spanning neonates to adults.(390-393) Much of the data surrounding the use of NICOM® has been positive, suggesting it is a valid non-invasive alternative to cardiac output monitoring. However, there are also conflicting studies surrounding its reliability across the measured variables. Kupersztych-Hagege et al undertook a validation study assessing COI in critically ill patients via transpulmonary thermodilution in that study NICOM® failed to achieve level of acceptance with a mean percentage error of 82% (n=144).(394) Another study by Kober in gynaecology oncology patients similarly demonstrated a

high percentage error of 50.7% over 84 measurements.(395)

Use of NICOM® in the obstetric population is increasing. Recent studies have demonstrated that NICOM® can identify distinct haemodynamic profiles associated with placental disease, consistent with the findings of more invasive methods.(243)

Haemodynamic assessment in the high risk population using NICOM® can predict the evolution of clinical preeclampsia and detect different evolving haemodynamic profiles in women with preeclampsia and intrauterine growth restriction vs. normal control subjects.(208) In addition, NICOM® has also been used to devise an optimal dosing regimen of phenylephrine to prevent hypotension during spinal anaesthesia in patients undergoing Caesarean section, demonstrating no clinical difference from administering phenylephrine as an infusion vs. a bolus regimen in a randomized controlled setting.(396) Despite its emerging use in the obstetric population, there was a lack of studies assessing its validity in this setting. The challenge to validating NICOM® in pregnant women stems from the impracticality and risk associated with recognized gold standards, such as thermodilution. As a result, I chose to use echocardiography (echo), which is validated against thermodilution in pregnancy and has been suggested as a reference for the validation of other CO techniques in pregnant women.(397)

My results (Chapter 3.1) suggest that NICOM® is a valid method for assessing CO in the pregnant population when compared with echocardiography measured CO. The mean percentage error obtained in this study was 26%, which is less than the recommended cut-off of 30%. It is important to emphasize that while LOA ranging between -1.3 and 1.7litres may be acceptable when the mean CO in the study population is 6.2litres, those LOAs would be too wide for a population with a lower average CO. Therefore, the validation results of this study are only applicable to pregnant women of a similar BMI and CO profile.

My findings differ from that presented by Vinayagam et al where NICOM did not achieve an acceptable mean percentage difference with values of 70.6%, 61.0%, 32.2% and 44.1% in the first, second, third trimesters and postnatal periods respectively.(222) The different findings in their study might be explained by a different platform (GE Vivid E9) used to perform the echocardiograph for comparison and greater numbers assessed in their study (n=98). Similarly in the third trimester they also demonstrated a wide LOA ranging from -1.099 to 2.26 L/min in the third trimester. The precision of the NICOM® device was assessed when obtaining repeated measures in subjects at rest, when their CO is assumed to be stable. We found relatively low CE, suggesting that the bioreactance method demonstrated very good precision in pregnant women during the second and third trimesters.

Although echocardiography is a low-risk and non-invasive method of CO and SV measurement in the obstetric population it requires expert training to acquire appropriate images and expertise gathered over many years to read the images and measure SV and CO. In stark contrast, NICOM® requires minimal training in order to acquire live data. It has the benefit of being completely non-invasive, essentially operator independent and can provide continuous SV and CO measurement. As a result, NICOM® can provide useful haemodynamic data on low-risk pregnant women in both the inpatient and outpatient settings. Vinayagam et al recently assessed 98 women in all three trimesters using NICOM® and echocardiography. However, they only found its use acceptable in the third trimester and suggested it was primarily of use at advanced gestations and intrapartum.(222) With conflicting findings to my results, larger studies are required to confirm the validity of NICOM® in the obstetric population.

In addition to assessing the clinical validity of this novel modality, I distributed a questionnaire, which for the first time has evaluated patient acceptability of this new method for CO measurement (Chapter 3.2). Ninety-five women were invited to participate in the questionnaire and the resultant uptake was excellent at 83%. The most frequent challenge to completion of all three antenatal assessments were a change of the coinciding hospital appointment or work commitments requiring a prompt exit from the department. These challenges most frequently arose at the time of fetal anomaly scan. Overall patient perception of the NICOM® was positive with 97% reporting willingness to participate again. The patient median [IQR] acceptable number of assessments was 4 [3-4], which is in keeping with this study's 4 assessments. The majority of responses stated they were comfortable during the NICOM® assessments and 12% reporting the sensors as being painful to remove. The data gained from this survey of patient experience will prove invaluable during conceptualisation and planning of any further study methodologies.

8.1.2 Haemodynamic Profiles

The primary objective of the HANDLE study was to assess the serial changes in haemodynamic variables, in particular total peripheral resistance (TPR) throughout pregnancy and the postpartum (Chapter 5). Among the 422 women recruited, 366 completed the study protocol of whom 318 had uncomplicated normal pregnancy, six pregnancies were complicated by preeclampsia (PE), 18 complicated by gestational hypertension (GH) and 24 by normotensive fetal growth restriction.

There have been many studies detailing the altered cardiovascular profile of pregnancy in the presence of co-existing uteroplacental disease.(203-207) The majority of these studies have employed

transthoracic maternal echocardiography (TTE) as the methodology of choice for evaluation of the cardiac profile in the presence of uteroplacental disease. Previous longitudinal studies via TTE have described a unique haemodynamic profile in PE specifically that of a lower CO and increased TPR when compared to unaffected pregnant controls. Using the novel automated NICOM® device I have similarly observed these altered profiles in the setting of PE and FGR (Chapter 5.2).

In this cohort, pregnancies complicated by PE had the profile of an unchanged cardiac output (CO) and increased blood pressure (Chapter 5.4). There were observed trends suggesting a reduction in HR, increased SV and increased TPR. However, this did not achieve significance and was likely secondary the small sample size. Therefore I was unable to prove my hypothesis that the first trimester NICOM® obtained TPR in the setting of PE would be 200dyne.s.cm^{-5} when compared to unaffected pregnancies. Pregnancies complicated by gestational hypertension (GH) had relatively unchanged CO, an expected elevated blood pressure, increased heart rate and increased total peripheral resistance combined with a reduction in stroke volume in comparison to unaffected controls (Chapter 5.3). The trend of differences in the SV and HR between the two hypertensive states suggests that GH may in fact be a separate cardiovascular entity. Women with GH maintained their CO by increasing their HR when their SV fell due to an increase in TPR.

In the setting of fetal growth restriction, a fourth haemodynamic profile of uteroplacental disease exists as affected pregnancies are found to have a CO lower than the hypertensive counterparts and a moderate elevation in TPR when compared to pregnant controls but not reaching that of the hypertensive groups (Chapter 5.5). (207-213) A similar NICOM® derived haemodynamic profile has also

been described in the late third trimester by Guy et al and was similarly characterized by a relatively unchanged HR, lower CO, reduced SV and elevated TPR.(338) My findings are also in keeping with Mahendru et al who have reported a significant association between pre-pregnancy to mid-pregnancy changes in CO and fetal weight gain.(339)

8.1.3 Bloods

The investigation into the role of first trimester biomarkers was added to the HANDLE study protocol after recruitment had commenced and additional funding was secured. As a result the first 70 recruits did not have serum blood banked and not all disease states were captured in this aspect of my thesis.

8.1.3.1 s-flt-1:PLGF

There were no significant differences in serum s-flt-1, PLGF or s-flt-1:PLGF ratio between unaffected pregnancies and those with either gestational hypertension or fetal growth restriction (Chapters 5.7; 5.8 & 5.9). This finding is consistent with what is known for the later stages of pregnancy and at time of diagnosis of these uteroplacental diseases.

In the setting of PE there was a significant reduction observed in s-flt-1 but no differences in PLGF or s-flt-1:PLGF ratio in comparison to unaffected controls. This finding should be interpreted with caution given my small sample size in PE (n=5) and the conflicting previous data. In addition, the mean s-flt-1 value of 1612.9 (662.4)pg/ml this cohort is higher than the reported median for normal pregnancy in the first (1107pg/ml) and second trimesters (1437pg/ml).(398) A previous study by Saxena et al similarly demonstrated a reduction in s-flt-1 in the first trimester in women who developed PE.(399) This reduction in s-flt-1 is in contrast to other reports that s-flt-1 is up regulated, PLGF decreased and an

increased s-flt-1:PLGF ratio in preeclampsia, indicating an overall anti-angiogenic state.(264, 400)

There was no correlation between s-flt-1; PLGF; or their ratio between haemodynamic variables or BMI. However, within this cohort there was an observed trend of a reducing s-flt-1 value as BMI increased however, this failed to reach significance. Similar findings of a negative correlation between s-flt-1 and BMI have been reported by von See et al.(401)

8.1.3.2 Apelin

Apelin 13 is an inodilator produced by the placenta in pregnancy. Levels of Apelin in uncomplicated pregnancy have previously been reported by Kourtis et al.(402) Cobellis et al previously detailed declining Apelin levels in placentas of normotensive women between the first to third trimesters. Placental levels of Apelin 13 and 36 in PE has subsequently been shown to be significantly decreased in comparison to those of normotensive women.(403, 404) Similar to the downregulation of placental Apelin in PE, there is a growing body of evidence demonstrating lower levels of serum Apelin 36 from 20 weeks' gestation in PE. This reduction of Apelin levels has been observed with significant differences between GH, mild PE and severe PE.(299, 404) However, the reported reduction in Apelin in the setting of PE was not observed at 14 weeks' gestation as presented in this study (Chapter 5.10). Similarly I did not observe a difference in circulating levels of Apelin 13 at 14 weeks' gestation in women whose pregnancies were complicated by FGR and those unaffected. This is in keeping with current conflicting data regarding Apelin levels in the setting of FGR, with both increased and decreased levels reported.(298, 405)

The haemodynamic correlation with Apelin by Van Mieghem et al demonstrated that mean Apelin between 20-26/40 correlates with TPR ($r=0.57$ $p=0.01$) and showed a trend to an inverse correlation

with SV ($r=-0.42$ $p=0.08$).⁽²⁹⁸⁾ This thesis now demonstrates the haemodynamic effects of Apelin 13 are present from as early as 14 weeks' gestation. In contrast to the study by Van Mieghem et al I have observed a weak inverse correlation between Apelin 13 and TPRi ($r=-0.29$; $p=0.004$), no correlation with SV and a weak correlation to COi ($r=0.29$; $p=0.005$) at 14 weeks gestation.

There is strong data, outside of pregnancy, surrounding the positive correlation between Apelin and BMI. In this thesis I have demonstrated the converse with a significant increase of Apelin 13 in women with a BMI less than 20kg/m^2 , whilst no differences were observed in those with a high BMI ($>33\text{kg/m}^2$). Ziora et al have previously assessed serum Apelin 36 and 12 in adolescent girls between 11-19 years with a diagnosis of Anorexia Nervosa. In contrast to my findings they demonstrated that those with a BMI <18 had significantly lower levels of Apelin 36 and 12 in comparison to both healthy controls and obese participants.⁽⁴⁰⁶⁾ Those findings were also in keeping with findings by Heinonen et al who observed significant reductions in Apelin in obese individuals following gastric banding surgery.⁽⁴⁰⁷⁾ However, when Heinonen assessed those with Metabolic syndrome no difference was observed in serum Apelin in those who achieved weight loss via a calorie restrictive diet.⁽⁴⁰⁸⁾

The previously studies in Apelin have occurred in the non-pregnant population and may explain my differing results. In this study serum blood was collected in the late first/ early second trimester when there is altered hormonal levels e.g. increased oestrogen. This discrepancy from the norm is further supported by other studies at times of hormonal flux. Tapan et al evaluated the role of Apelin in obese children who had significantly lower levels than their non-obese counterparts. However, in this study the differences were only present in the pubertal period.⁽⁴⁰⁹⁾ A recent animal study by Abedinzade similarly they noted an increase in Apelin and weight in

oophorectomised rats in comparison to normal controls. This effect was reversed following estradiol treatment. (410)

8.1.3.3 MPV

There is increasing evidence supporting that a diagnosis of preeclampsia results in increased platelet consumption as detailed by falling platelet counts and rising MPV. However, data from both the first trimester and postpartum remain conflicting. Altinbas et al and Dogan et al have reported that preeclampsia is significantly associated with increased MPV. I have detailed similar findings in the early-onset PE cohort in our local population. However, this rise in MPV did not translate to an ability to discriminate between pregnancies with mild versus severe preeclampsia.(328, 329) Several authors have provided a “cut-off” value of MPV as a significant predictor for preeclampsia with values ranging from 8.65- 9.95 and dependant on gestational age with varying sensitivities and specificities.(320, 328, 330) The data presented in this thesis is conflicting with what is reported above as I have detailed a reduction in MPV in the first trimester in the setting of PE (Chapter 5.6).

8.1.4 Prediction

Given my previous presumption that PE is associated with an increased TPR of 200 dynes.sec (208) and my power calculation being performed on this basis, the second HANDLE study objective was to evaluate the ability of maternal haemodynamics and in particular TPR to predict the evolution of PE and other uteroplacental disease (Chapter 6). Interrogation of maternal haemodynamics via NICOM® presents us with a novel, non-invasive and easily applied opportunity to identify women at increased risk of uteroplacental disease. These differing maternal haemodynamic variables are evident prior to the clinical

emergence of disease. In this study I demonstrate four different haemodynamic profiles among women with pregnancies complicated with PE, GH and FGR and unaffected controls (Chapters 5.2- 5.5). From the logistic regression analysis it was not possible to provide cut-offs for variables of interest secondary to the small numbers of disease states (Chapter 6.1).

Given the poor predictive performance of the individual NICOM® variable and serum biomarkers I undertook a discriminate analysis to identify which variables would perform best in a multi-logistic regression analysis. This discriminate analysis proposed the following models warranted further evaluation via logistic regression - PE (s-flt-1 & MPV); GH (s-flt-1, MBP & Apelin) and FGR (s-flt-1, SV and TPRi).

8.1.4.1 Prediction of hypertension

When assessing individual variables and excluding maternal blood pressure, the best haemodynamic predictor for the evolution of GH at 14 weeks' was TPR (AUC=0.63; $p=0.018$). This did not reach the same level of significance as maternal blood pressures SBP (AUC=0.81), DBP (AUC=0.83) and MBP (AUC= 0.86) with $p<0.0001$ for each (Chapter 6.1). In the prediction of PE no parameters in isolation were predictive of disease at 14 or 20 weeks' gestation and only maternal blood pressure was predictive at the later 28 weeks' gestational age prior to emergence of disease. This poor performance in predictive capability is likely in part due to the unexpected low incidence of PE disease states in this cohort. The performance of haemodynamic variables was enhanced when combined in a multivariate logistic model as detailed in Table 6.5 (Chapter 6.2). In pregnancies complicated by preeclampsia, HR and SVi when combined with BP became significant predictors at 14 weeks' gestation (AUC=0.75, $p=0.01$ and AUC=0.77, $p=0.009$ respectively).

A logistic regression analysis was performed on the reported biomarkers s-flt-1; PLGF; Apelin 13; MPV and s-flt-1:PLGF. None of which, in isolation were predictive of disease states (Chapter 6.3). In regard to first trimester s-flt-1:PLGF this is in keeping with previous findings.(411, 412) However, a report by Myatt et al suggested that the change in values between first and second trimesters had predictive capabilities with AUC=0.86, sensitivity 77% and specificity 80%.

This resulted in a discriminate analysis being employed to model variables of interest across NICOM® haemodynamics and serum biomarkers. In the multi-logistic regression analysis, a model using s-flt-1 and MPV had the best predictive capability for PE with an AUC=0.88 and $p=0.011$ (Chapter 6.4). The multi-logistic regression analysis (s-flt-1, Apelin and MBP) assessing the prediction of GH did not achieve significance.

Preeclampsia has traditionally been regarded as a disease of increased total peripheral resistance (TPR) resulting in hypertension. A reduction in cardiac output (CO) is associated with the development of PE with changes apparent as early as the first trimester.(209, 253) Khaw et al have previously demonstrated that higher SV is an independent predictor of PE.(212) They similarly had small numbers of PE ($n=8$) and reported a model employing SV, Uterine artery pulsatility index and MAP. The AUC was not reported but the best point was at the probability of $>5.4\%$ with a sensitivity of 77.8%, specificity of 79.1% and a 20% screen positive rate. Comparison with my PE model was not possible as the number of women developing PE was too small ($n=5$ with serum bloods) to provide meaningful sensitivities and specificities.

8.1.4.2 Prediction of Fetal growth restriction

In this study I observed a difference in the BMI of women with pregnancies affected by FGR than both those affected by PE/GH and healthy pregnant controls (Chapter 4.1). This is in keeping with previous studies as low maternal weight has been demonstrated as a risk factor for development of FGR. The best haemodynamic predictor for the evolution of FGR at 14 weeks' was CO (AUC=0.62; $p=0.03$). This was similar to the predictive abilities achieved by maternal blood pressure with SBP (AUC=0.61; $p=0.02$) and DBP (AUC=0.61; $p=0.03$) as detailed in Chapter 6.1. The performance of haemodynamic variables was enhanced when combined in a multivariate logistic model as detailed in Table 6.5 (Chapter 6.2). I demonstrated that TPR, CO and SV when combined with BP were significant predictors of pregnancies complicated by FGR (AUC=0.64, $p=0.005$; AUC=0.65, $p=0.003$; and AUC=0.65, $p=0.005$ respectively). However, this is only moderately better than maternal BP alone.

A logistic regression analysis was performed on the reported biomarkers s-flt-1; PLGF; Apelin 13; MPV and s-flt-1:PLGF. None of which in isolation were predictive of disease states (Chapter 6.3). This resulted in a discriminate analysis being employed to model variables of interest across NICOM® haemodynamics and serum biomarkers. In the multi-logistic regression analysis a model using s-flt-1, SV and TPRi had the best predictive capability for FGR with an AUC=0.76 and $p=0.007$ (Chapter 6.4). This was stronger than previous combinations of haemodynamic variables and maternal BP.

As part of the Wilson and Jungner Criterion a screening test should also be economically balanced in relation to possible expenditure on medical care as a whole.(413) The first trimester biomarker aspect did not demonstrate a difference between controls and disease states (Chapter 5.6- 5.10). The costs excluding laboratory overhead costs, time and staffing to perform the assays was €3000 for Apelin 13 ELISA, €3105 for the Roche s-flt-1 reagent kits and €3105 for the Roche PLGF reagent kits. Once a NICOM® monitor is purchased at a cost €6500, the haemodynamics of each patient can be assessed using the single use sensors at a cost of €100 per patient.

8.1.5 Postnatal Adaption

As preeclampsia is an independent risk factor for future cardiac disease the third primary objective of the HANDLE study was to evaluate for resolution or persistence of these aberrant changes in the hypertensive disease states (Chapter 5.1).

A diagnosis of preeclampsia (PE) has a significant impact on the pregnancy of a woman but also has long-term ramifications. There is a reported increase in the overall mortality in women with pregnancies complicated by PE in comparison to those with unaffected pregnancy.(92, 93) The reason for this increased risk remains unclear but studies are emerging detailing cardiac dysfunction and enhanced platelet responsiveness in women whose pregnancies were complicated by PE.(414-416) One possible mechanism for this increased risk is that PE and cardiovascular disease share common risk factors and aetiologies such as obesity, endothelial dysfunction, dyslipidaemia, inflammation, hyper coagulability, diabetes and renal disease(41, 55, 417, 418). Alternatively, this increased risk may be secondary to PE placental released mediating factors causing metabolic and

vascular damage; or a combination of both leading to the future manifestation of cardiovascular disease.

A growing area of concern is that many primary care physicians and indeed obstetricians are unaware of the positive correlation between PE and cardiovascular, cerebrovascular and renal diseases. It was also reported that few practitioners counselled patients regarding lifestyle modification in the postpartum.(419) A study by van Kesteren et al in The Netherlands reported suboptimal levels of annual BP checks, low intervention rates for dyslipidaemia/ impaired glucose tolerance and only one third of women having previously been identified as hypertensive being commenced on antihypertensives.(420) Both the Institute of Obstetricians and Gynaecology and the American Heart Association advocate for lifestyle modification and annual BP checks with pregnancy complicated by PE being to be viewed as a failed physiological “stress test”.(421-423) Preventative strategies for cardiovascular disease are well established in the setting of metabolic disease however, in the setting of postpartum women with PE the evidence is lacking regarding their efficacy.(102, 424)

8.1.6 Neonatal

A secondary HANDLE objective was the evaluation of the impact of gestational hypertension and antihypertensives on neonatal myocardial performance in the immediate postnatal period (Chapter 7). In this study, I demonstrated that infants born to mothers with GH and treated with labetalol exhibited lower LV function illustrated by lower EF, LV global longitudinal strain, and twist. These differences remained significant following adjustment for maternal age, infant birthweight and mode of delivery. There was preservation of RV function with no differences in any of the RV functional parameters.

Previous research has primarily focused on the assessment of infants born to mothers with overt evidence of uteroplacental disease such as pre-eclampsia and FGR, demonstrating reduced left ventricle diastolic function in premature infants born to mothers with PE in the first week after delivery (425). The presence of this dysfunction is further supported by the elevation in certain cardiac enzymes which have been identified at birth (104). In addition, infants born SGA exhibit significantly reduced LV global longitudinal strain (-15.9% vs. -21.3%) compared to appropriately grown infants during the early neonatal period, and this was related to the degree of arterial stiffness (426). Other studies have demonstrated that myocardial dysfunction in SGA infants involves both ventricles in systole and diastole (427). This cardiac dysfunction may represent the effects of both utero-placental insufficiency and prematurity. Adverse loading conditions experienced by the myocardium of SGA infants manifesting as increased aortic stiffness also contributes to dysfunction. In my study, all infants in both the GH and the control arm, were born at term and were of normal birthweight. In the absence of evidence of utero-placental disease, it is therefore likely that any differences in function identified in my study are due to the drug exposure. As the majority of mothers with GH receive treatment, it is difficult to assess the independent effect of the condition on myocardial performance.

My study utilises novel markers which more accurately reflect cardiac performance. Normative values for global longitudinal strain are becoming established in neonatology and form a basis for comparison with disease states. The values obtained in my normal infants are similar to those obtained in other studies by our group and are in keeping with the recent systematic review of normative deformation values in children and neonates (428, 429). Variations in reference ranges between different normal

populations maybe explained by the time of the scans, the type and version of measurement software used, and measurement techniques (430). I found a reduction in LV function measures using ejection fraction and deformation in infants born to mothers with GH who were in receipt of labetalol. In addition, net LV twist is reduced in this group of infants. Those measurements are influenced by a combination of loading conditions in addition to inherent contractility. The relative lack of difference in load independent parameters (strain rate, twist rate and untwist rate) suggest that labetalol exposure may alter neonatal loading conditions rather than inherent myocardial contractility (431). None of the surrogates of preload measurements (LA:AO, LVEDD, MV E:A and MV VTI) were different between the two groups; and the observed relationship between GH/labetalol use and the function measurement remained significant when controlling for PDA. Therefore, the beta-receptor blocker effect cannot be completely discounted as a contributor to the reduced function. RV function is less impacted by beta blockade and this may explain the lack of difference in RV performance between the two groups. I can speculate that maternal GH and/or labetalol treatment may induce a subtle LV-specific morphological changes. This relationship warrants further exploration in a larger cohort.

Our research group has recently seen a similar pattern of dysfunction in infants born with neonatal encephalopathy who were treated with therapeutic hypothermia where apical rotation was reduced and basal rotation unaffected (113). The subepicardial layer of the myocardium promotes apical rotation and may be more affected during neonatal cardiac dysfunction. In elderly patients, subendocardial dysfunction occurs as part of physiological aging, resulting in an increased rotation which is felt to be a compensatory mechanism for decreased conventional "contraction" (432). The lack of basal change may also be due to its inherent lack of contribution to rotation in early neonatal life.

Fetal exposure to labetalol is associated with several neonatal complications. Labetalol crosses the placenta with significant plasma levels found on day one and has a half-life of approximately 24 hours (433). The commonest side effects appear to be secondary to beta-receptor blockade and include bradycardia and hypoglycaemia in term infants (108), and hypotension in premature infants (434). Those consequences appear to be self-limiting and resolve within the first 24 to 48 hours, probably reflecting the medication half-life and drug clearance. In addition, labetalol exposure can affect cerebral autoregulation and perfusion during the early neonatal period assessed by near infra-red spectroscopy (NIRS). Ritcher et al demonstrated that infants exposed to labetalol (with or without magnesium sulphate) have lower cerebral fractional tissue oxygen extraction (FTOE) representing either increased cerebral perfusion or depressed neurological function. There was concomitant high splanchnic FTOE and unchanged renal FTOE. However, the majority of the infants in the study were born to mothers with PE and associated placental insufficiency and it is possible that those changes were related to the disease process rather than the drug exposure (435).

8.2 Strengths

A major strength of the HANDLE study is its prospective study design and the ability to plot serial haemodynamics across four time points in a low risk nulliparous population. It took less than two years to recruit 422 nulliparous pregnancies in a single tertiary obstetric centre. The haemodynamic profile of participants was not revealed to their clinicians and so it did not influence their antenatal care or open to the potential of bias. Recruited pregnancies underwent serial monitoring and a valuable strength is that over 80% of women returned for the postnatal monitoring (Chapter 4.2).

The emergence of the novel NICOM® bioreactance technology has the benefit of being easier to implement than the traditional echocardiograph. Although echocardiography is low-risk and non-invasive it requires expert training in order to acquire appropriate images and expertise in interpretation of images obtained. In contrast NICOM® has the benefit of requiring minimal training and being independent of both a skilled technician to perform and interpret the exam. This easy to use technology also has the advantage of providing rapid minute-by-minute output of maternal haemodynamic variables, which can be utilised in both the inpatient and outpatient setting.

There has been much research via alternative modalities to assess haemodynamics in pregnancy. Another strength of this study was the ability to validate the use of NICOM® in comparison to echocardiography in the obstetric population (Chapter 3.1). The NICOM® device also demonstrated good precision over repeated measurements at rest with a relatively low CE during both the second and third trimesters. Another strength is that via my patient questionnaire, I gained the patient perspective of this device. From this I can conclude that NICOM® is an acceptable alternative from the patient perspective (Chapter 3.2).

8.3 Limitations

A limitation of the HANDLE study was that it was not feasible to perform pre-pregnancy NICOM® monitoring. This was in part addressed by the recruitment of 30 nulliparous non-pregnant women to provide a comparison for both baseline first trimester values and comparison for postnatal resolution (Chapter 5.1). The postnatal element of this study is also limited by the comparison to this cohort for the non-pregnant values. Due to the brevity between delivery and postnatal review, the findings of a persistent

hypertensive state and blunted postnatal adaption should be interpreted with caution. By six weeks postpartum it is expected that the CO has decreased to midway between pregnant and non-pregnant values. Further analyses extending past the six weeks in this methodology to beyond six months will be required to confirm that my finding of a blunted postnatal response persist beyond the early postpartum.

In addition it was not feasible to perform a simultaneous echocardiographic assessment in all 422 participants, which resulted in a small sample size of 30 being used for comparison with NICOM® (Chapter 3.1). Furthermore, my validation analysis was limited by the absence of a concurrent full blood count (FBC) being performed. Comparisons between echocardiography and thermodilution have shown increasing levels of bias when haemoglobin concentrations dropped to $<140\text{g litre}^{-1}$.(391) I did not measure haemoglobin concentration at time of echocardiography and as such was unable to explore this association.

A potential criticism of this study is the lower than expected number of PE cases (Chapter 4.2). My power calculation was based on the assumption of a 5% PE rate in the nulliparous population (Chapter 2.2.5). This assumption was based both on international and locally published data at the time of my study design. (209, 436-440) This had a negative effect on the predictive performance of both the haemodynamic variables and serum biomarkers. Whilst differing from normal controls they did not reach statistical significance (Chapter 6.1; Chapter 6.2; Chapter 6.3 & Chapter 6.4).

This lower than anticipated PE rate of 1.6% was in keeping with the local Rotunda PE rates of 3.3%, 2.4% and 2.8% the years 2014, 2015 and 2016 respectively and was therefore a reflection of the local overall declining PE rates within the hospital population at the

time of the study (Chapter 4.2). There was a reduction in the mean nulliparous PE rate from $5.1\% \pm 0.3\%$ between the years 2004-2009 to $3.7\% \pm 0.9\%$ between the years 2010- 2016 ($p>0.001$), reaching a trough of 2.4% in 2015 (Chapter 4.2). Following interrogation of the Rotunda Hospital Clinical Annual Reports between 2004-2016 I have demonstrated similar reductions in rates of eclampsia, delivery indicated by hypertension and admittance to high dependence unit indicated by hypertension. The reasons for this reduction remain unclear as the obstetric population are ageing, have a higher incidence of obesity and are not delivering at an earlier gestational age.

An additional larger study adequately powered in keeping with my reported lower PE rates would need to be undertaken before any recommendations with regards to a change in practice could be made. In addition to the lower than expected area under the ROC, another study would need to be undertaken to compare current risk stratification from maternal history using NICE or ACOG guidelines to the haemodynamic prediction model prior to implementation.(1, 441) These predictive capabilities should be reviewed in the context of improved risk stratification and may not translate to improved perinatal outcomes in both the hypertensive and growth restricted cohorts.

The neonatal aspect of my study is limited by the small number of infants, as more subtle differences between the groups may not have been identified as significant (Chapter 7). The neonatal echocardiograms were carried out at only one time point in the early neonatal period. As such I cannot elude to changes in cardiac performance over time. It would have been interesting to assess the change in those functional parameters when labetalol had been cleared. The rate of clinical events in both groups was low. As

such, I could not assess the relationship between myocardial function and clinical outcomes.

8.4 Future research

The positive findings but low PE prevalence in this study substantiates the need for a larger multicentre prospective study interrogating the use of NICOM® derived maternal haemodynamics as a predictor of uteroplacental disease in the obstetric population. Once these findings are replicated in the general obstetric population it would be important to perform a further study randomising patients to either traditional screening methods via NICE, ACOG protocols, or to those presented in this thesis to investigate for superiority. Prior to implementation into routine practice, further evaluation of this tool would be required to assess the impact of any intervention (e.g. prescription of aspirin) in those deemed to be high risk. This would also require a cost-analysis of screening & intervention in high-risk pregnancies vs. universal intervention alone.

Additional longitudinal studies are also needed to interrogate the haemodynamics of women prior to embarking on pregnancy to address whether these observed aberrations are a direct result of placental mediated factors or a pre-existing cardiac risk prior to pregnancy. This would not be feasible to perform in the general low risk population. However, the possibility for investigation would be present in those women known to be at increased risk of PE attending other clinics e.g. women undergoing infertility investigation or treatment and women attending the recurrent miscarriage clinic.

Following a single assessment in the early postpartum this study has demonstrated differing haemodynamic changes in the early postnatal period in women with pregnancies complicated by hypertensive disease. As detailed in the limitations of this thesis, a

further study investigating the haemodynamics in the late postnatal period is required to evaluate whether the changes, which I have observed, ultimately persist or resolve by 26 weeks' postpartum. Given the reported increased risk of cardiovascular disease in this subgroup of women, further longitudinal research is needed to identify potential early intervention strategies and their resultant impact on long-term cardiovascular disease.

Now that NICOM® has been validated for use in the pregnant population this presents an opportunity for further interrogation of maternal haemodynamics with relative ease. Using this novel technology in the hypertensive population it would allow real time interpretation of the impact of antihypertensive therapies e.g. comparison of intravenous infusion of labetalol vs. oral nifedipine; comparison of intravenous infusion of hydralazine vs. intravenous infusion of labetalol; comparison of cardiovascular effects of intravenous magnesium sulphate when used in hypertensive women vs. administration for fetal neuroprotection and the individualisation of fluid restriction/ resuscitation in severe PE.

With respect to fetal growth restriction, this thesis again highlights the need for improved prenatal detection of FGR. As demonstrated in this thesis still only one in three cases were suspected antenatally. Given the significant association between stillbirth and undiagnosed FGR there is a need for better screening tools and this remains a research priority. The link between FGR and maternal cigarette smoking is well established. There is a growing use of e-cigarettes as a “healthier” alternate to cigarette smoking. However, the safety surrounding their use remains uncertain with long-term data lacking in both the traditional adult and obstetric populations. As such correlations between e-cigarette smoking and uteroplacental disease cannot yet be made.

8.6 Conclusion

In conclusion, uteroplacental disease is a multifactorial complication of pregnancy. Data originating from the HANDLE study has firstly contributed by being one of the first studies to validate the use of NICOM® in the obstetric population. Following validation of this novel technology I have added to the knowledge surrounding the haemodynamics in this unique cohort as I was able to plot the serial changes in both normal pregnancy and those complicated by uteroplacental disease. Data from the HANDLE study has demonstrated an altered postnatal adaption characterised by persistently higher total peripheral resistance, coupled with persistently elevated blood pressures. This blunted postnatal response may explain why women with hypertensive disease of pregnancy have a life long increased risk of cardiovascular disease.

In the neonatal aspect this thesis presents the use of novel echocardiography techniques in infants born to mothers receiving antihypertensive therapy for gestational hypertension and suggests evidence of left ventricular dysfunction when compared to healthy controls. Right ventricular function appears to be spared. Further research is warranted to explore the impact of an abnormal in utero haemodynamic environment and exposure to cardiotropic medication on both fetal and neonatal myocardial performance, to confirm this association with a larger cohort and to evaluate the potential long-term implications of this.

This thesis further highlights the need for the development of prediction models especially in the case of FGR as within this cohort only one third of FGR pregnancies were suspected antenatally. In addition further large multicentre trials are required in both the prediction of PE and earlier diagnosis of PE as currently there is insufficient evidence to recommend the routine adoption of previously reported strategies.

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APPENDICES

PUBLICATIONS