UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Human Development and Health

Measurement of and Relationship between Placental Size and Fetal Cardiac Development

by

Jillian Connor

Thesis for the degree of Doctor of Medicine

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ABSTRACT

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Introduction- Fetal programming is the concept by which a fetus adapts to the intrauterine environment by altering blood flow to various organs which may induce permanent structural and/or functional change in those organs, altering disease susceptibility in later life. We hypothesised that the heart is a susceptible organ and that alterations in blood flow from the placenta in relation to maternal factors may have different effects on each side of the heart.

We therefore aimed to assess the feasibility of measuring placental volume and growth, cardiac structure and cardiac function and assess the relationships between these measurements and also to maternal characteristics.

Methods- We undertook a prospective, cross-sectional cohort study of low-risk women. 144 scans were performed on 127 women, 89 in the first trimester (10^{+6}-13^{+6} weeks gestation) and 55 in the second trimester (18^{+0}-20^{+6} weeks gestation). 17 of these women were scanned in both trimesters. Measurements performed were the left and right myocardial performance index to assess cardiac function and 3D placental volume. Second trimester cardiac structure was assessed by 2D measurements of total cardiac circumference, total ventricular circumference and internal left and right ventricular circumferences. These measurements were then correlated with each other, standard fetal biometry, maternal body composition, and birth and placental weights.

Results- We developed reliable and reproducible techniques for measuring placental volume in the first and second trimester and relative cardiac chamber

sizes in the second trimester, but had difficulty reliably measuring cardiac function by means of the myocardial performance index. We did not find any significant relationships between maternal body composition and fetal cardiac function or structure within our small cohort.

Conclusions- Assessment of fetal cardiac function and structure in relation to maternal nutritional status and stress has the potential for detecting fetuses adapting to an adverse intra-uterine environment. This could assist identification of the maternal factors which lead to increased risk of disease in adult life and facilitate the development of targeted diet and lifestyle interventions.

Contents

ABSTRACT i

Contents iii

List of tables vii

List of figures xi

DECLARATION OF AUTHORSHIP xv

Acknowledgements xvii

Definitions and Abbreviations xix

1. Chapter 1- The Developmental Origins of Disease: Influence of the Fetal Circulation 1

1.1 The Developmental Origins of Adult Disease Concept 2
1.2 Fetal Circulation- Overview 5
1.3 Changes in arterial flow in response to placental function 6
1.4 Changes in Venous Flow 10
1.5 Role of the Placenta 12
1.6 Fetal Cardiac Development 15
1.7 Hypothesis 18
1.8 Aims 18

2. Chapter 2- Methods- Study cohort and design 19

2.1 Recruitment and the Salmon in Pregnancy Study 19
2.2 Additional ultrasound examinations 21
2.3 Pregnancy outcome measures 27
2.4 Statistics applied 27
2.4.1 Ultrasound parameters 27
2.4.2 Fetal biometry 28
2.4.3 Maternal Characteristics 28
2.4.4 Limitations and sources of error 29

3. Chapter 3- Development and Assessment of Placental and Fetal Cardiac Measurement Techniques 32

3.1 Placental Volume 32
3.2 Cardiac Structure 36
3.2.1 2D Cardiac Measurements Performed at 20 Weeks Gestation 37
3.2.2 Statistical Analysis of Cardiac Chamber Measurements 39

3.3 Cardiac Function 52
3.3.1.1 Measuring the Myocardial Performance Index 53
3.3.1.2 Exploring measurement error in the Myocardial Performance Index 63
3.4 Conclusions 75
4. Chapter 4- Relationships Between Fetal Cardiac Function And Structure, Placental Volume And Fetal And Maternal Anthropometry 77
Measurements obtained 77
4.1.1 Myocardial Performance Index 77
4.1.2 Placental Volume 78
4.1.3 Cardiac chamber measurements 78
4.2 Pregnancy outcomes 78
4.3 Analysis of measurements obtained 79
4.3.1 Gestation at time of scan 81
4.3.2 Summary statistics- Placental Volume, Myocardial Performance Index and Cardiac Structure 82
4.3.3 Associations with gestation 83
4.3.3.1 First trimester (10^{+0}-13^{+6}) 83
4.3.3.2 Second Trimester (18^{+0}-20^{+6}) 84
4.3.4 Relationship between fetal cardiac function on the left and right side of the heart 85
4.3.5 First trimester v. Second trimester 86
4.3.6 Relationships between cardiac circumferences 87
4.3.7 Relationships between MPI and placental volume 88
4.3.8 Relationships between cardiac circumferences and placental volume 89
4.3.9 Relationships between cardiac circumferences and MPI 90
4.3.10 Ratio of left to right internal ventricular circumferences 91
4.3.11 Relationship between rate of placental growth and cardiac structure and function 92
4.4 Fetal biometry on ultrasound related to cardiac structure and function 93
4.4.1 Relationship between placental volume and fetal biometry 95
4.4.2 Ratio of fetal to placental size in first trimester related to cardiac function and structure 96
4.4.3 Relationship between fetal biometry and cardiac function 98
4.4.4 Relationship between fetal biometry and cardiac chamber size 100
4.5 Maternal characteristics 102

4.5.1 Relationship between fetal anthropometry and maternal characteristics 104
4.5.2 Relationship between placental volume and maternal characteristics 105
4.5.3 Relationship between fetal cardiac function and maternal characteristics in the first trimester 107
4.5.4 Relationship between fetal cardiac function and maternal characteristics in the second trimester 108
4.5.5 Relationship between cardiac chamber size and maternal parameters 109
4.5.6 Relationship between the ratio of the left to right ventricular circumferences and maternal characteristics 111
4.5.7 Summary Statistics- Birth weight and placental weight 112
4.5.8 Relationship between fetal cardiac function and birth outcomes 112
4.5.9 Relationship between fetal cardiac structure and birth outcomes 113
4.6 Birth Outcomes 114
4.6.1 Relationship between placental volume and birth outcomes 114
4.6.2 Relationship between fetal:placental ratio and pregnancy outcomes 115
4.6.3 Relationship between maternal characteristics and birth outcomes 116
4.7 Measurement of placental volume in the second trimester 117
4.7.1 Placental volume from projected areas and circumferences 118
5. Chapter 5- Discussion 125
5.1 Measurement of Fetal Cardiac Function 126
5.2 Measurement of Cardiac Architecture using 2D Ultrasound 130
5.3 Placental Volume 132
5.4 Conclusions 137
Appendices 141
5.5 Appendix 1- SiPS Consent form for eligibility screening 141
Glossary 145
References 147
Bibliography 154

List of tables

Table 1- Scan measurements obtained 26

Table 2 - Sources of variance in Cardiac Chamber Measurements 49

Table 2- Correlation of cardiac chamber measurements based on number of images and repeat measurements 51

Table 3- Right ventricular myocardial performance index in the second trimester adjusted for heart rate 74

Table 4- Summary statistics- gestation at time of scan 81

Table 5- Summary statistics for placental volume, myocardial performance index and cardiac chambers 82

Table 6- Associations with gestation in the first trimester 83

Table 7- Association with gestation in the second trimester 84

Table 8- Association between the left and right MPI in the first and second trimester 85

Table 9- Relationship between placental volume in the first and second trimester and MPI in the first and second trimester 86

Table 10- Associations between cardiac cirumferences 87

Table 11- Association between placental volume and cardiac function 88

Table 12- Association between placental volume in the second trimester and cardiac circumferences 89

Table 13- Relationship between cardiac function and structure 90

Table 14- Associations between the ratio of ventricular circumferences, placental volume and cardiac function in the first and second trimesters..... 91

Table 15- Relationship between placental growth rate and cardiac function in the first and second trimester and cardiac structure 92

Table 16- Summary statistics of fetal biometry in the first, second and third trimesters 93

Table 17- Association between placental volume and fetal biometry 95

Table 18- Relationship between fetal:placental ratio and cardiac function, structure and placental volume 97

Table 19- Association between fetal biometry and cardiac function in the first and second trimester..... 98

Table 20- Associations between fetal size and cardiac chamber size . 100

Table 20- Maternal characteristics in the first trimester 102

Table 21- Maternal characteristics in the second trimester 102

Table 22- Associations between maternal anthropometry and fetal size 104

Table 23- Association between maternal characteristics and placental voluume in the first and second trimesters 105

Table 24- Relationship between placental growth rate and maternal characteristics 106

Table 25- Association between maternal characteristics and fetal cardiac functon in the first trimester..... 107

Table 26- Association between maternal characteristics and fetal cardiac functon in the second trimester..... 108

Table 27- Association between total cardiac circumference and maternal characteristics 109

Table 28- Association between total ventricular circumference and maternal characteristics..... 109

Table 29- Association between internal right ventricular circumference and maternal characteristics 110

Table 30- Association between internal left ventricular circumference and maternal characteristics 110

Table 31- Correlation between the ratio of the left to right ventricular circumferences and maternal characteristics 111

Table 32- Summary statistics for birth weight and placental weight... 112

Table 33- Association between fetal cardiac function in the first and second trimester and pregnancy outcomes 112

Table 34- Association between cardiac circumferences and pregnancy outcomes 113

Table 35- Association between placental volume and pregnancy outcome..... 114

Table 36- Association between fetal:placental ratio and pregnancy outcomes 115

Table 37- Association between maternal characteristics and pregnancy outcomes 116

Table 37- Summary statistics of second trimester placental volume measureuments according to technique 120

Table 38- Relationship between second trimester placental volume measurements by different techniques..... 120

Table 38- Associations between second trimester placental volume measured by 3D ultrasound and fetal cardiac function, cardiac structure and size..... 122

List of figures

Figure 1- Description of cohort scanned by JC 22

Figure 2- First trimester placental volume measurements (cm^2), variation with different slice thickness 35

Figure 3- Second trimester placental volume measurements (cm^2), variation with different slice thickness 35

Figure 4- Measurement of cardiac chamber circumferences in the second trimester 38

Figure 5-Total cardiac area, within and between subject variation 40

Figure 6- Total ventricular area, within and between subject variation . 41

Figure 7- Internal left ventricular area, within and between subject variation 42

Figure 8- Internal right ventricular area, within and between subject variation 43

Figure 9- Total cardiac circumference, within and between subject variation 44

Figure 10- Total ventricular circumference, within and between subject variation 45

Figure 11- Internal right ventricular circumference, within and between subject variation 46

Figure 19- First trimester right myocardial performance index, standard deviation of measurements by subject over time 64

Figure 21- Second trimester right myocardial performance index, standard deviation of measurements by subject over time 66

Figure 22- First trimester left myocardial performance index, identification of learning curve plateau 67

Figure 23- First trimester right myocardial performance index, identification of learning curve plateau 69

Figure 24- Second trimester left myocardial performance index, identification of learning curve plateau 71

Figure 25- Second trimester right myocardial performance index, identification of learning curve plateau 71

DECLARATION OF AUTHORSHIP

I, Jillian Margaret Connor

declare that the thesis entitled

Fetal Adaptations to Intra-uterine Environment and Risk of Disease in Adult Life

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
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- where I have consulted the published work of others, this is always clearly attributed;
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Definitions and Abbreviations

CHD-	coronary heart disease
DNA-	deoxyribonucleic acid
2D-	two dimensional
3D-	three dimensional
4D-	four dimensional
pO2-	partial pressure of oxygen
SD-	standard deviation
PI-	pulsatility index
IGF-	insulin like growth factors
PAPP-A-	pregnancy associated plasma protein A
B hCG-	beta human chorionic gonadotrophin
STIC-	spatio-temporal image correlation
TCC-	total cardiac circumference
TCA-	total cardiac area
TVC-	total ventricular circumference
TVA-	total ventricular area
IRVC-	internal right ventricular circumference
IRVA-	internal right ventricular area
ILVC-	internal left ventricular circumference
ILVA-	internal left ventricular area
MPI-	myocardial performance index

LVMPI-	left ventricular myocardial performance index
RVMPI-	right ventricular myocardial performance index
ICT-	isovolumetric contraction time
IRT-	isovolumetric relaxation time
ET-	ejection time
E/A wave-	diastolic flow velocity waveform across the a-v valve
a-v valve-	atrio-ventricular valve
MHz-	mega hertz
SiPS-	Salmon in Pregnancy Study
NHS-	National Health Service
PVOL1-	placental volume in the first trimester
PVOL2-	placental volume in the second trimester
ZPVOLDIFF-	placental growth rate calculated by devising a Z-score
ILRRTIO-	ratio of internal left:internal right ventricular circumference measurements
CRL1ag-	crown rump length 1st trimester adjusted for gestation
NT1ag-	nuchal translucency 1st trimester adjusted for gestation
BPD2ag-	biparietal diameter 2nd trimester adjusted for gestation
HC2ag-	head circumference 2nd trimester adjusted for gestation
AC2ag-	abdominal circumference 2nd trimester adjusted for gestation
FL2ag-	femur length 2nd trimester adjusted for gestation
BPD3ag-	biparietal diameter 3rd trimester adjusted for gestation
HC3ag-	head circumference 3rd trimester adjusted for gestation

AC3ag-	abdominal circumference 3rd trimester adjusted for gestation
FL3ag-	femur length 3rd trimester adjusted for gestation
TC3ag-	thoracic circumference 3rd trimester adjusted for gestation
MAGE1-	maternal age in the first trimester
MAGE2-	maternal age in the second trimester
MHT1-	maternal height in the first trimester
MHT2-	maternal height in the second trimester
MPFAT1-	maternal percentage body fat in the first trimester
MPFAT2-	maternal percentage body fat in the second trimester
ZLMWT1-	maternal weight in the first trimester (standardised and log converted)
ZLMWT2-	maternal weight in the second trimester (standardised and log converted)
ZLMBMI1-	maternal body mass index in the first trimester (standardised and log converted)
ZLMBMI2-	maternal body mass index in the second trimester (standardised and log converted)
Birthwtag-	birth weight adjusted for gestation
Placwtag-	placental weight adjusted for gestation
UV flow-	blood flow in the umbilical vein
DV flow-	blood flow in the ductus venosus
DV ratio-	percentage blood flow shunted through the ductus venosus
TAMX-	time-averaged maximum velocity
ICC-	interclass co-efficient of agreement
OR-	odds ratio

1. Chapter 1- The Developmental Origins of Disease: Influence of the Fetal Circulation

It is well recognised that the intra-uterine environment a fetus experiences affects not only the growth and development ante-natally but also susceptibility to disease in later life. Babies born with a low-birth weight have an increased risk of coronary heart disease, hypertension, type 2 diabetes and other chronic diseases (Barker, Winter et al. 1989, Hales, Barker et al. 1991). The exact mechanisms for this are being elucidated. Certain adaptations the fetus makes in response to a sub-optimal environment may underlie this increased risk, especially if there is a mismatch between the intra- and extra-uterine environments (Hanson and Gluckman 2005). Poor maternal nutrition or reduced placental function will affect the fetal supply of oxygen and nutrients and the fetus can adapt to this with altered blood flow to various organs in order to optimise the supply of nutrients and oxygen to vital organs, particularly those served by the left side of the heart, i.e. heart and brain (Wladimiroff, vd Wijngaard et al. 1987, al-Ghazali, Chita et al. 1989, Vyas, Nicolaides et al. 1990, Baschat, Gembruch et al. 1997).

Where an intra-uterine stimulus or insult has a lasting effect on the structure or function of an organ this can lead to long-term health effects and is known as fetal programming. These adaptations may have a permanent effect on the structure of organs such as the heart by altering the haemodynamics (Pinson, Morton et al. 1991, Barbera, Giraud et al. 2000) and alterations in the blood supply to the liver can also have an impact on the growth of other organs (Tchirikov, Kertschanska et al. 2001, Tchirikov, Kertschanska et al. 2002). Alterations in certain hormone axes, particularly the hypothalamic-pituitary-adrenal axis, resulting in increased cortisol levels also occur in response to a sub-optimal environment and impact on organ development (Giraud, Louey et al. 2006). It is now recognised that these adaptive mechanisms occur in infants whose birth-weight falls within the

normal range and who are not recognised to be growth restricted (Haugen, Hanson et al. 2005).

Different placental phenotypes have also been reported in association with various situations where the fetus may experience a sub-optimal environment, including pre-eclampsia, which has a well-established link with intra-uterine growth restriction and fetal circulatory adaptations, maternal anaemia and pregnancy at altitude (Burton, Reshetnikova et al. 1996).

Our aim is to investigate whether alterations in fetal cardiac structure and function can be detected in association with placental volume and maternal body composition with a view to identifying fetuses that may be clinically normally grown, but are demonstrating adaptive responses to a sub-optimal environment.

1.1 The Developmental Origins of Adult Disease Concept

There is now a wealth of epidemiological evidence demonstrating the link between low birth weight and an increased risk of coronary heart disease, stroke, hypertension and type 2 diabetes in later life. This association exists across a range of birth weights and is also affected by growth in infancy. As growth is closely related to nutrition, variations in maternal nutrition within the normal range may have long-term consequences for the fetus. This developmental model proposes that nutrition in fetal life and infancy alters gene expression affecting metabolic function and responses to environmental factors in later life.

The initial evidence for the association between birth weight and disease in later life came from a cohort study (Barker, Winter et al. 1989) of almost 11000 men born in Hertfordshire between 1911 and 1930. These subjects were well characterised with their birth weight recorded by a midwife and weight at one year recorded by a health visitor. Relating this to the later risk of developing coronary heart disease (CHD) there were significantly increased

hazard ratios for CHD in those men with lower birth weights and also lower weight at one year of age. Similar trends with regard to birth weight were observed in women, but there was no association with weight at one year. These results have since been reproduced in studies examining cohorts in Europe, North America and India (Frankel, Elwood et al. 1996, Stein, Fall et al. 1996, Forsen, Eriksson et al. 1997, Rich-Edwards, Stampfer et al. 1997).

In those subjects who had glucose tolerance tests in later life the incidence of impaired glucose tolerance or Type 2 diabetes fell with increasing birth weight and subsequent studies (Hales, Barker et al. 1991) have established that low birth weight can predict altered glucose tolerance in adulthood.

There are many other factors associated with an increase in risk of diseases such as CHD and Type 2 diabetes with a possible argument for the persistence of the poor environment the fetus is exposed to predisposing an individual within that environment to an ongoing increased risk of disease. The association between low birth weight and increased risk of CHD, type 2 diabetes and hypertension have been demonstrated (Leon, Lithell et al. 1998) to be independent of factors such as smoking, employment, social class and alcohol consumption. This suggests that poor growth in-utero and infancy lead to inherent changes increasing the risk of certain diseases. Adult lifestyle factors can add to these effects with the prevalence of impaired glucose tolerance being highest in those who were of low birth weight and then became obese in adult life.

The biological basis for the alteration in risk of developing disease is the concept of developmental plasticity (West Eberhard 1989). This describes a phase in the development of a system where that system is ‘plastic’ and can adapt to external influences to which it is sensitive. After a period of time the plasticity is lost and the function of that system or organ is then fixed and unable to adapt further to environmental influences. Periods of plasticity largely occur in-utero and early infancy so that beyond this time the ability to respond effectively to either a scarcity or an excess of a particular environmental factor is lost.

The evolutionary advantage of this ability to respond to the environment in which a fetus finds itself is that it allows expression of a phenotype which is

well matched to that environment. On the other hand, should there be a mis-match between the environment predicted by the fetus and the actual environment experienced throughout life the individual may not be able to adapt accordingly. This can explain how a fetus that does not grow well in utero as a result of poor maternal nutrition adapts itself for a relatively sparse environment but when faced with increasing availability of food rich in nutrients such as fat and sugar may be more susceptible to the diseases associated with that type of diet. This has certainly been the case in the typical Western diet over the last half-century with increased availability of cheaper food which is more highly processed and contains more fat and sugar to improve texture and flavour.

In humans the main influences on fetal growth are the size of the mother (a baby that grows too big for the maternal proportions cannot be born normally), the nutrition available in utero, oxygen availability and stress. Availability of nutrition will in turn be influenced by the mother's nutrition stores, metabolism and diet in pregnancy. In the developing world fetal malnourishment is generally a direct effect of chronic malnourishment in the mother. In the Western world, even with widely available, affordable food a fetus may be malnourished as a result of an imbalance of available nutrients.

A fetus which is exposed to a nutrient-poor environment in utero will prioritise its energy allocation, typically to spare brain growth and development, at the expense of other processes such as tissue repair capacity. There are three main processes by which poor fetal growth and a low birth weight increase disease vulnerability. Functional capacity in key organs, such as the kidney may be reduced as they are not prioritised in a sparse environment. This may lead to reduced numbers of glomeruli and an increased risk of hypertension. There is also alteration in the settings of hormonal axes. A fetus will maintain glucose delivery to the brain at the expense of other organs such as muscle and will develop relative insulin resistance predisposing to diabetes in later life. Finally lower birth weight babies who have experienced a poor intra-uterine environment have a permanently reduced capacity to cope with adversity in later life. For example in men with lower incomes in a cohort from Helsinki, those with lower ponderal indexes, a measure of the relationship between weight and length, and were therefore thinner at birth

had higher rates of CHD (Forsen, Eriksson et al. 1997). Those with higher ponderal indexes were apparently more resilient to the effects of low income.

Growth patterns in infancy also influence risk of disease. In the Helsinki cohort those identified with CHD could be predicted by low birth weight and ponderal index, poor growth in the first year and subsequent increased weight gain after the age of 2 where the body mass index at age 11 was then high. Thinness at birth is associated with a low muscle mass and, as there is little muscle replication after birth subsequent weight gain may lead to a disproportionately high fat mass. Similar associations are seen with type 2 diabetes and hypertension.

There is increasing understanding of the underlying molecular basis for these observations. Adaptation to a particular intra-uterine environment involves epigenetic mechanisms where a single genotype can give rise to a range of phenotypes dependant on what is likely to give the best survival advantage. This involves an alteration to gene structure without a change in the nucleotide sequence which can occur as a direct result of environmental influences. For example maternal protein restriction has been shown to lead to reduced methylation of DNA (Lillycrop, Phillips et al. 2005) which alters gene activity while histone modification will affect gene transcription and expression. These changes have also been demonstrated to be transmissible to the next generation (Bertram, Khan et al. 2008) and thus the adaptatory responses of a fetus to its particular environment can not only modify its own risk of disease, but these risks can be passed from one generation to the next.

1.2 Fetal Circulation- Overview

As the fetus receives its oxygen and nutrient supply from the placenta rather than its lungs and gastrointestinal tract there are great differences between the fetal and adult circulation (Kiserud 2005). Fetal heart development is dependent on intra-uterine environmental factors, including blood flow. The myocardium grows by hyperplasia, i.e. cell division, until after birth when the cardiomyocytes terminally differentiate and are no longer capable of cell division. Subsequent growth is by hypertrophy, or cell enlargement. In the fetus both systolic and diastolic pressure increase with gestation, but no significant difference is seen between the right and left ventricles. The two

ventricles pump in parallel with the right ventricle having a slightly greater output than the left. The ventricles have a limited capacity to increase their stroke volume to a pressure challenge by increasing end-diastolic filling pressure. The fetal myocardium is relatively stiff as it is constrained by the pericardium, lungs and chest wall but adrenergic drive in response to stress will shift the ventricular function curve to increase stroke volume. However increased heart rate is the main means of increasing their cardiac output. The right ventricle has less capacity to increase its stroke volume than the left as it is already operating closer to the top of the Starling curve whereby further increases in end-diastolic filling will not improve the contractility and therefore ejection force of the ventricle.

Oxygen and nutrient-rich blood from the placenta returns via the umbilical vein with a proportion supplying the liver and the rest passing through the ductus venosus to the right ventricle of the heart. This is one of three shunts within the fetal circulation, which allows the fetus to alter flow to various organs according to need. Another shunt is the foramen ovale, connecting the right and left atria. Oxygenated blood from the ductus venosus preferentially flows through the foramen ovale into the left atrium and onto the left ventricle, which supplies the heart and upper part of the body. The third shunt is the ductus arteriosus, connecting the right ventricle, which supplies the lungs in adult life, to the descending aorta and so supplying the lower body and returning blood to the placenta in fetal life. It is these shunts allow the fetus to adapt to altered supply of oxygen and nutrients.

1.3 Changes in arterial flow in response to placental function

Adaptive responses in the fetus have been extensively studied in both animal models and in human fetuses. In humans these investigations centre around measurement of alterations in organ size and blood flow using 2D ultrasound, Doppler flow measurements and, more recently 3D/4D ultrasound. These changes were identified in growth-restricted fetuses with an increased head to abdominal circumference ratio (Campbell and Thoms 1977) suggesting preservation of brain growth compared to the liver. Subsequent studies using

Doppler waveforms demonstrate the fetus redistributing its blood flow to essential organs such as the brain, heart and adrenal glands (Wladimiroff, vd Wijngaard et al. 1987, al-Ghazali, Chita et al. 1989, Vyas, Nicolaides et al. 1990, Mari, Uerpairojkit et al. 1996, Baschat, Gembruch et al. 1997).

In 1987 Wladimiroff compared Doppler waveforms in the internal carotid artery and umbilical artery in 156 normal pregnancies and 42 growth restricted pregnancies in the third trimester (Wladimiroff, vd Wijngaard et al. 1987). They compared 40 normal pregnancies with 9 pregnancies complicated by fetal growth restriction, confirmed post-natally. In the growth-restricted pregnancies the pulsatility index in the umbilical artery was raised compared with the normal pregnancies, indicating increased resistance within the placenta. This was associated with reduced pulsatility index in the internal carotid artery demonstrating reduced resistance, which would lead to increased flow. This suggested the presence of a 'brain-sparing' effect.

Al-Ghazali then compared 16 symmetrically growth restricted fetuses with 15 who were asymmetrically growth-restricted, and measured blood flow velocity in the pulmonary artery and aorta (al-Ghazali, Chita et al. 1989). In comparison with normal fetuses, those with symmetrical growth restriction had greater output from the right heart than the left. In the asymmetrically growth-restricted fetuses, with an increased head to abdominal circumference ratio above the 95th centile, there was higher mean and maximum velocity in the aorta just above its origin from the heart, significantly different from normal and consistent with the theory of brain sparing.

These alterations were shown to be present in hypoxaemic fetuses (Vyas, Nicolaides et al. 1990). Doppler waveforms in the middle cerebral artery were obtained in 81 small for gestational age fetuses and compared with the fetal pO2 obtained by cordocentesis. All fetuses had reduced pulsatility index and higher mean blood velocity in the middle cerebral artery compared to reference ranges for normal fetuses, and there was a significant relationship between fetal hypoxaemia and the degree of reduction in the pulsatility index,

with the maximum reduction reached when the fetal pO2 was 2-4 SD below the mean.

A similar increase in flow to the heart itself has been demonstrated (Baschat, Gembruch et al. 1997). This study compared 55 normal fetuses with 54 growth-restricted fetuses. Median coronary peak blood flow velocities in the right coronary artery were higher in intrauterine growth-restricted than appropriate-for-gestational-age fetuses. The ability to detect the coronary flow at an earlier gestation was associated with poorer perinatal outcomes including a higher mortality rate, lower birth weight and more acidosis in the umbilical artery at birth.

In a study comparing 131 normal fetuses with 21 who were small for gestational age, Mari found that in ten small-for-gestational-age fetuses the adrenal artery pulsatility index (PI) was below the 95% confidence interval, demonstrating reduced impedance. Small-for-gestational-age fetuses with abnormal adrenal artery PI had a significantly higher incidence of fetal heart rate decelerations, preterm delivery and Cesarean sections when compared to small-for-gestational-age fetuses with normal adrenal artery PI (Mari, Uerpairojkit et al. 1996).

While increasing its flow to these vital organs the fetus has been shown to reduce flow to other areas. Vielle found increased resistance in the renal artery in growth restricted fetuses with oligohydramnios compared to normal fetuses (Veille and Kanaan 1989).

In keeping with these findings Stigter found that peak systolic velocities in the renal artery showed a significant reduction in growth-restricted fetuses and significant correlation with low birth weight in 16 fetuses (Stigter, Mulder et al. 2001). The same group also found a significant correlation between low birth weight and reduced PI in the middle cerebral artery and the ductus venosus, which shunts blood away

from the liver towards the heart. Those fetuses with reduced impedance in the ductus venosus were also found to have significantly lower umbilical artery pH at birth.

Rizzo studied flow in the peripheral pulmonary arteries in182 normal fetuses and 61 growth-restricted fetuses (Rizzo, Capponi et al. 1996). They found in growth-retarded fetuses the pulsatility index values were significantly elevated compared to those of normal fetuses. A significant relationship was observed between the severity of hypoxia and pulsatility index values from the peripheral pulmonary arteries in 29 fetuses in which Doppler recordings were obtained immediately before cordocentesis.

These alterations favour structures that are supplied by the left side of the heart (coronary circulation and upper body) over those supplied by the right (lungs, placenta and lower body). The fetus can achieve this by shunting blood through the ductus arteriosus and aortic isthmus, where flow is reversed directing more blood towards the cerebral circulation in the presence of increasing placental resistance and cerebral vasodilatation (Fouron, Zarelli et al. 1994, Fouron, Gosselin et al. 2005). This reversal of flow is associated with poorer developmental outcomes. The effects in tissue of changes in arterial flow are accentuated by alteration of flow through the third circulatory shunt, the ductus venosus. As will be discussed in more detail below, the ductus venosus is an important shunt in the fetal circulation, connecting the intra-abdominal umbilical vein and inferior vena cava. In hypoxaemia this vessel relaxes allowing more nutrient rich blood to be directed directly towards the heart and through the foramen ovale towards the cerebral circulation at the expense of the liver (Kiserud, Eik-Nes et al. 1994).

These clinically identifiable fetuses are at an extreme end of the spectrum of fetal adaptation to a sub-optimal intra-uterine environment and have been extensively studied because they represent a challenge to modern obstetrics (Romero, Kalache et al. 2002). They are generally recognised to be growth-restricted secondary to ‘placental insufficiency’ characterised by decreased angiogenesis within the placenta at histology and abnormal

umbilical artery Doppler waveforms on ultrasound due to increased placental resistance (Giles, Trudinger et al. 1985), leading to reduced maternal blood flow to the placenta and reduced oxygen delivery.

It is now recognised that fetal adaptive responses can be demonstrated within the normal range of birth weight (Haugen, Hanson et al. 2005) related to the maternal nutritional status rather than hypoxaemia. In a study of 381 low-risk pregnancies slimmer mothers with low body fat stores and those eating an unbalanced diet, with consequent altered nutrient content availability in the umbilical vein, there was decreased flow through the ductus venosus, increasing flow to the liver to allow increased hepatic nutrient interconversion, the so-called liver sparing effect.

1.4 Changes in Venous Flow

At mid-gestation on average 30% of blood from the umbilical vein is shunted through the ductus venosus, falling to 20% by thirty weeks gestation under normal conditions. Because this blood enters the right atrium in a vertical stream and has a higher kinetic energy it presses open the foramen ovale sending oxygenated blood to supply the upper body. This vessel is under adrenergic control and flow through it will increase in hypoxaemia (Kiserud, Eik-Nes et al. 1994, Tchirikov, Eisermann et al. 1998), providing an important compensatory mechanism in acute hypoxaemia or hypovolaemia as well as a more prolonged adaptatory role in chronic hypoxaemia. In situations where there is reduced nutrient availability, in slim mothers with lower body fat stores and those eating an imprudent diet- characterised by low intakes of fruit, vegetables and wholemeal bread and high intakes of white bread, confectionery, chips and roast potatoes- it has been demonstrated that shunting through the ductus venosus is decreased, directing a greater proportion of blood flow to the liver (Haugen, Hanson et al. 2005).

It can be seen that 70-80% of oxygenated blood passes straight to liver under normal conditions, suggesting a high priority for this organ. The effects of reducing that supply by increasing ductus venosus shunting in a hypoxic environment are unknown but may result in permanent alteration of liver

function. Supply to the liver is also reduced by increased blood viscosity, for example an increased haematocrit in response to chronic hypoxia, as the vasculature of the liver has a huge capillary cross-section and is therefore a low blood velocity and high resistance circulation. For this reason liver flow is also more reduced in comparison to ductus venosus flow when umbilical vein pressure is reduced. The liver vasculature is also more sensitive to the effects of catecholamines with the intrahepatic veins demonstrating six-times more forceful constriction in response to noradrenaline and adrenaline in fetal sheep compared to the ductus venosus (Tchirikov, Kertschanska et al. 2003). This will decrease liver flow and increase shunting in conditions in which circulating catecholamines are increased such as in fetal hypoxia.

Although the ductus venosus appears to have an important role in fetal responses to adverse conditions its physiological role is not well understood. Normal growth can occur where there is congenital agenesis of the ductus venosus (Kiserud, Rasmussen et al. 2000). In experiments in sheep where the ductus venosus was obliterated with an embolisation coil (Tchirikov, Kertschanska et al. 2001) there was increased hepatic flow and increased cell proliferation in liver, heart, skeletal muscle and kidneys compared to controls while placental proliferation was reduced. Absolute and relative liver weights were significantly increased in fetuses with an obstructed ductus venosus. The liver is also responsible for the production of important growth-factors insulin-like growth factors (IGF) I and II and their binding proteins. The expression of these was increased in association with increased blood flow. Reduced hepatic flow is recognised in growth-restricted fetuses (Tchirikov, Rybakowski et al. 1998) caused by the effects of catecholamines and adaptations made by the fetus to sub-optimal conditions, i.e. increased ductus venosus shunting.

In further studies where fetal lambs either had the ductus venosus obliterated, increasing liver flow, or stented, reducing liver flow (Tchirikov, Kertschanska et al. 2002) the same results as above were seen in the embolised group while the stented group demonstrated a 50% reduction in hepatic, heart and skeletal muscle proliferation. It appears that hepatic flow plays an important role in regulation of fetal growth and that where hepatic

blood supply is reduced in growth restricted fetuses, this in itself could lead to growth restriction.

1.5 Role of the Placenta

As the supplier of fetal oxygen and nutrients the placenta plays the key role in fetal growth with alterations in placental vasculature and flow having a major impact. The placenta is also an important metabolic and endocrine organ producing growth factors and enzymes which affect the fetal hormonal environment (Robinson, Chidzanja et al. 1995).

In epidemiological studies placental size relative to the birth weight has been associated with increased risk of disease in adult life, including hypertension, coronary heart disease, stroke and type 2 diabetes (Godfrey 2002). The relationship is not straightforward and varying associations have been reported. For example both a low and high placental ratio have been associated with an increase in coronary heart disease within the same cohort (Martyn, Barker et al. 1996). Larger placental weight relative to the birth weight has also been associated with hypertension in adult life (Barker, Bull et al. 1990)

The placenta is constantly growing and differentiating throughout gestation with an increase in fetal capillary occupation of villi and trophoblast surface area and a decrease in trophoblast thickness (Myatt 2006). This reflects ongoing periods of branching angiogenesis, non-branching angiogenesis, trophoblast differentiation and syncytium formation. The effect of any disruption of placental development will depend upon the predominant developmental process at that time. Placentas from growth-restricted fetuses are smaller and show altered placental vasculogenesis (Krebs, Macara et al. 1996), enzyme activity and hormone production (McMullen, Osgerby et al. 2004)

The barrier between the mother and fetus is the syncytiotrophoblast, which covers the placental villi containing the fetal capillaries. These project into the intervillous spaces containing maternal blood and are abnormally developed in growth-restricted pregnancies (Sibley, Turner et al. 2005). In severe early onset growth restriction with abnormal umbilical artery Doppler waveforms on ultrasound the villi are straighter, with a simpler vascular network and fewer interconnections. In lesser degrees of growth restriction the surface area of the syncytiotrophoblast is reduced and the exchange barrier is thickened. In late-onset growth restriction there will be increased tortuosity of the villi with more interconnections. Increased apoptosis is seen in all types of growth restriction.

The placenta also demonstrates differing response to differing forms of hypoxia (Burton, Reshetnikova et al. 1996) such as that seen at altitude, in anaemia and in pre-eclampsia. The principle adaptation at altitude, where there is reduced oxygen tension in the maternal circulation, is thinning of the diffusion barrier with dilatation of the fetal capillaries. There is also an increased total capillary volume with increased length and diameter. In contrast this is unchanged in anaemia, where there is reduced oxygen carrying capacity, and pre-eclampsia where there is relative ischaemia secondary to reduced uterine artery blood flow, although an increase in capillary diameter was seen without an increase in length. In both anaemia and pre-eclampsia, there is a reduction in the total villous volume. Possible underlying mechanisms for these changes include up-regulation of placental angiogenic cytokines secondary to reduced oxygen tension at altitude, while in anaemia and pre-eclampsia where the oxygen tension is normal, but oxygen flux is reduced, increased oxygen uptake resulting in relative maternal venous hypoxia may not be enough to stimulate the same angiogenic response. Instead placental circulatory dynamics may be altered with a redistribution of fetal blood flow from the peripheral placental circulation to the umbilical circulation, resulting in locally increased flow rates and pressure. This is supported by experiments demonstrating the same alterations in diameter but not length in response to increasing pressure differentials (Karimu and Burton 1994).These also showed that the capillary dimensions are governed by the pressure differential between the fetal and maternal circulation. It is unclear if these changes in capillary

diameter in pre-eclampsia and anaemia are caused by hypoxia or are secondary to reduced villous surface area.

In contrast large placentas have been recognised in association with maternal anaemia and pregnancy at high altitude (Godfrey, Redman et al. 1991). These larger placentas have increased intervillous spaces, reduced volume of villi and increased peripheral capillary development, all of which enhances oxygen transfer with no rise in resistance within the placental vasculature (Jackson et al, 1987).

Restriction of placentation will result in reduced fetal growth as reduced oxygen supply down-regulates DNA synthesis. Where there is growth restriction with a compromised placenta there is increased pulsatility in the umbilical artery identifiable using Doppler ultrasound. Less of the fetal cardiac output is distributed to the placenta and there is increased recirculation of umbilical blood in the fetal body. This increases the load on the right ventricle (Kiserud, Ebbing et al. 2006). The size of the placenta in mid-gestation measured on ultrasound is significantly correlated with birth-weight, with lower birth-weight babies having smaller placentas. (Hafner 1998, Kinare AJOG 2000) Placental volume may potentially be used to predict later fall in fetal growth.

As well as supplying oxygen to the growing fetus the placenta is responsible for transfer of nutrients from the maternal circulation. Placental transport capacity is reduced in growth restricted pregnancies with reduced activity of amino-acid transport systems such as System A resulting in reduced placental protein transfer and reduced plasma amino-acid concentrations in the fetus (Sibley, Turner et al. 2005).

In animal studies alterations in maternal nutrition at specific points in pregnancy have been shown to have different effects on placental size, also dependant on the mother's nutritional status prior to and around

conception. In poorly nourished ewes high nutrient intakes in early pregnancy increased placental size, while in well nourished ewes high intakes reduced placental size (Robinson, Chidzanja et al. 1995). There is also evidence in humans that high dietary intakes in early pregnancy can suppress placental growth, with mothers with a high intake of carbohydrate having smaller placentas, altered placental weight to birth weight ratio and lower ponderal index in the neonate, a phenotype recognised to be associated with increased risk of disease in adulthood (Godfrey, Robinson et al. 1996, Godfrey, Barker et al. 1997).

1.6 Fetal Cardiac Development

As mentioned the fetal heart grows by hyperplasia of the cardiomyoctes. In humans terminal differentiation of these cells does not normally occur until late gestation when the cells lose their ability to divide and further growth is by hyperplasia of the cells. The rate of proliferation in intra-uterine life and occurrence of terminal differentiation will determine the cardiomyocyte number for life.

This process of terminal differentiation can occur in-utero in response to altered pressure and flow within the heart (Pinson, Morton et al. 1991). In instrumented sheep, a species like humans in which the cardimyocytes do not terminally differentiate until late gestation, increasing the pressure load on the right heart by inflating a vascular occluder around the pulmonary artery led to a significant increase in weight of the heart, heart weight:fetal weight ratio, myocyte length and number of binucleated, i.e. terminally differentiated, myocytes (Barbera, Giraud et al. 2000). The effects are more pronounced on the right ventricle due to its different geometry. It has an increased radius of curvature, leading to higher wall stress against any given pressure rise. This makes it more difficult for the right ventricle to increase contractility. In situations of hypoxaemia and consequent raised arterial pressure secondary to increased resistance within the placental bed the load on the right ventricle is increased more than on the left (Reller, Morton et al. 1989) with potential effects on the architecture of the heart.

In fetal sheep infused with sterile plasma in order to increase cardiac loading (Jonker, Faber et al. 2007) the early response to this was increased cardiomyocyte proliferation, with increased cell cycle activity markers and increased in mononucleated cardiomyocytes, i.e. those cells capable of proliferation. This was followed by an increase in binucleated cells and increased length and width of the cardiomyocytes in keeping with growth by hypertrophy. Heart weight was increased by 30% in keeping with these findings. The initial surge of proliferation enabled the fetus to maintain its stroke volume against an increased afterload but this was quickly followed by terminal differentiation reducing the proliferative potential prematurely.

Cardiomyocyte development is also affected by alterations in placental function. Inadequate placental function will lead to reduced fetal growth. As the placenta provides downstream resistance to fetal ventricular ejection, alterations in placental vasculature leading to increased resistance will alter the fetal haemodynamics and consequently cardiomyocyte development. Other factors may also contribute to altered cardiac development, including hypoxaemia and hypercortisolaemia which are associated with poor placental function. These changes are seen in fetal lambs when small vessels in the placenta are embolised. At later gestations embolisation provokes only a mild, transient increase in arterial blood pressure with no alteration in fetal heart weight (Cock, Albuquerque et al. 2001), but earlier embolisation leads to persistent arterial hypertension and an increase in heart weight relative to body weight (Murotsuki, Challis et al. 1997).The renin-angiotensin system may also be implicated as increased renin activity is seen in newborn lambs where the placenta has been embolised (Louey, Cock et al. 2000) and angiotensin II has been shown to stimulate cardiomyocyte proliferation in vitro (Sundgren, Giraud et al. 2003).

In a study where fetal lambs underwent placental embolisation for either 10 or 20 days (Louey, Jonker et al. 2007) body weight and heart weight were both reduced, although the ratio remained unaltered. These fetuses showed

only a transient small increase in blood pressure so haemodynamic load was not significantly altered. However the embolised fetuses were hypoxaemic and had increased plasma cortisol levels and renin activity. On examining the myocardium cardiomyocyte dimensions were unaltered but there was reduced cell-cycle activity in embolised fetuses and the proportion of binucleated cardiomyocytes was less by 20 days indicating a less mature myocardium. Placental insufficiency suppressed the proliferation and maturation of the cardiomyocytes. It was also noted that after 20 days there was an increase in the brain:liver ratio, indicating a degree of brain-sparing, a recognised fetal adaptation.

In fetal sheep where uterine caruncles were removed preconceptually (Morrison, Botting et al. 2007), reducing the area available for placentation there was an increase in the proportion of mononucleated cardiomyocytes with cardiomyocytes that were smaller in absolute terms but larger relative to heart size. Although there were more mononucleated cardiomyocytes the proportion of proliferating cells was the same between the placentally restricted fetuses and controls. These findings were consistent with an overall reduction in cardiomyocyte number in the growth restricted fetal heart. There was also an inverse relationship between the oxygen content of the fetal blood and the proportion of mononucleated cardiomyocytes suggesting a delay in terminal differentiation related to reduced oxygen delivery.

The increased cortisol levels found in relation to chronic hypoxia in placental restriction have also been found to affect cardiomyocyte development in conditions where an exogenous infusion was administered to fetal sheep in doses too low to affect blood pressure (Giraud, Louey et al. 2006). The hearts in the treated fetuses were significantly larger with no change in size of the cardiomyocytes, but an increase in cell-cycle activity, indicating that cortisol stimulated growth by hyperplasia rather than hypertrophy.

1.7 Hypothesis

Our hypothesis is that in fetuses destined to show recognised signs of adaptation to a suboptimal intrauterine environment there will be evidence early in the pregnancy of alterations in placental volume to fetal size and changes in cardiac function and size that may favour the left side the heart. The structural development of the heart may be permanently altered by differences in flow early in pregnancy and may explain the long-term increase in cardiovascular disease seen in these fetuses. We reasoned that such alterations may have effects on the structure and function of the heart which may affect the left and right side of the heart differently.

In order to address this hypothesis our research question were-

1. Could placental volume be reproducibly measured in 1st and 2nd trimester using 2D and 3D ultrasound?
2. Could the structure of the fetal heart be reproducibly measured in 2nd trimester?
3. Can cardiac function be reproducibly measured in 1st and 2nd trimester
4. Do these measurements inter-relate and are they related to maternal characteristics?

1.8 Aims

We had two principal aims in our study- firstly to establish the feasibility, reliability and reproducibility of measuring cardiac function in the first and second trimester and two-dimensional measurements of cardiac structure in the second trimester and, secondly, relating these measurements along with fetal biometry and placental volume to basic maternal anthropometry in order to detect any differences in fetal heart function and structure related to maternal body composition.

2. Chapter 2- Methods- Study cohort and design

2.1 Recruitment and the Salmon in Pregnancy Study

The cohort for our study was selected from a group of women who were invited to the University Ultrasound department for a first trimester scan. The women were randomly selected from the population referred for a routine first trimester scan by their midwife at booking. Women deemed high risk at booking by the midwife and therefore planned for Consultant led care were excluded. The women received an information leaflet about the Salmon in Pregnancy Study (SiPS) (Miles, Noakes et al. 2011) when they received their postal invitations to attend the hospital for their routine ultrasound appointment.

The SiPS is a single-blind, randomized controlled trial of increased consumption of farmed salmon by pregnant women from week 20 of gestation until the end of their pregnancy. Eligibility criteria included a family history of atopy, allergy or asthma and the primary outcome of the SiPS relates to atopy and its manifestations in infants born to women enrolled in the study. Women who expressed an interest in the SiPS provided written informed consent (Appendix A) at their week 12 appointment to enable screening for habitual consumption of fish and family history of atopy, allergy, or asthma to assess eligibility for the SiPS, plus additional scanning for cardiac function (all performed by JC) and a 3D scan for placental volume (performed by either PM or CN).

The study was conducted according to the principles of the Declaration of Helsinki, and all women gave written informed consent. All procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (approval no. 07/Q1704/43), including additional scanning for organ size and blood flow measurement with JC as a named Research Member responsible for performing ultrasound scans. The SiPS trial is registered at www.clinicaltrials.gov (clinical trials identifier NCT00801502).Those women who were not interested in undergoing screening for eligibility for the trial were offered a standard first trimester scan confirming viability, number of

fetuses, dating by crown-rump length measurement and a nuchal translucency measurement as part of screening for Trisomy 21if they wished to have this done. Only those women who had provided consent to be screened for eligibility for the trial had the additional scanning performed as outlined above.

Women who agreed to be screened were then recruited to the SIPs trial if eligible and had follow-up second and third trimester scans, assessing fetal growth by measuring the biparietal diameter, head circumference, abdominal circumference and femur length. In addition in the third trimester the thoracic circumference was measured and Doppler measurements of the umbilical artery maximum flow and pulsatility index and middle-cerebral artery maximum velocity were performed. All of these scans were performed by the sonographers with in the University Department, PM and CN.

In the initial stages of the trial all women who had consented to be screened for eligibility were offered a second trimester scan within the University department, rather than the NHS ultrasound department, regardless of their actual participation in the study. As recruitment increased it was no longer possible to accommodate all of the ineligible women and second trimester scans in the University department were then restricted to women who were recruited and randomised within the SIPs protocol. Third trimester scans were only ever available to women in the SIPs trial. For this reason a number of women who had consented to be screened for eligibility had scans performed for cardiac function and placental volume in the first trimester, but if they proved ineligible after screening and would therefore not be recruited to the SiPS they returned to the NHS department for their second trimester scans meaning there was no opportunity to collect further scan data on those women.

All women who agreed to be screened for eligibility had basic biometry collected. These included maternal age, height, weight, body mass index and the percentage of body fat as measured by the Tanita MC180 body composition analyser.

2.2 Additional ultrasound examinations

Our study, performing ultrasounds and collecting data on women from within the SiPS cohort was a prospective, crossectional study. For the women who had consented to screening for eligibility for the SiPS and thus to additional scanning, this consisted of a measurement of cardiac function, the myocardial performance index (MPI), which were all performed by JC. For all the women scanned by JC at any time point a 3D sweep of the placenta was obtained by University Department sonographers CN or PM in the first trimester for offline measurement of the placental volume by JC. In the second trimester women scanned by JC had either a 3D sweep of the placenta obtained by PM or CN, again for offline measurement of the placental volume by JC, or, if it was not possible to fit the entire placenta within the volume box, a 2D measurement obtained by PM or CN. In addition in the second trimester a 2D cineloop of the fetal heart was obtained by either PM or CN for offline measurement by JC.

144 scans were performed by JC on 127 women between September 2007 and June 2008. Of these 89 were scanned in the first trimester (10+6-13+6 weeks gestation) and 55 in the second trimester (18+0-20+6 weeks gestation). 17 of these women were scanned in both trimesters. Of those scanned in the first trimester four women subsequently withdrew and their data was not available for analysis and in a further two women the data was not available at the time of performing the analysis. Three women attended for scan but did not participate in any other aspect of the SiPS protocol and we excluded them as we did not have adequate scan data or any maternal characterisation. From the second trimester cohort one woman withdrew when her baby was found to have an undetected anomaly at birth and for four women data was not available at the time of performing the analysis. One woman had a second trimester scan but no other SiPS data available and so was excluded as above. There were therefore 112 women included in the final analysis as outlined in Figure 1 below

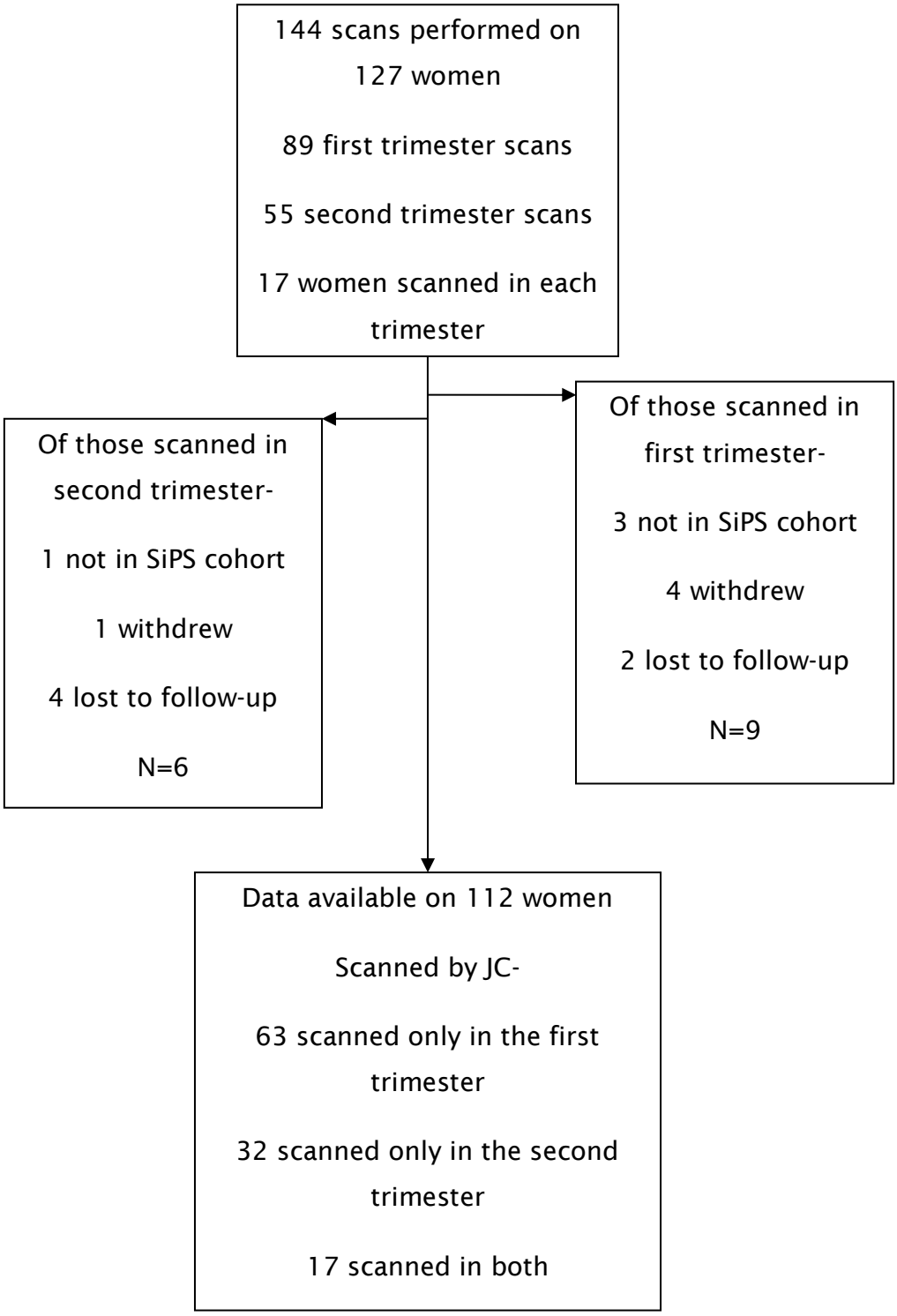

Figure 1- Description of cohort scanned by JC

It was not always possible to obtain every scan measurement in every woman and the reasons for this varied as summarised in Table 1.

The placental volume in the first trimester was obtained in all women. In the second trimester where the entire placenta could be fitted inside the volume box a 3D sweep was obtained and measured offline by JC. 79 placental volumes were obtained in this way. In a further 26 women where the placenta did not fit in the volume box, usually because it was anterior and in the narrower upper segment of the image, a 2D placental volume measurement was performed by PM or CN using a previously described technique outlined in Section 4.7.1. There were therefore 7 women who did not have a placental volume obtained in the second trimester. 5 women did not have a second trimester scan in the University department as they proved ineligible for the SiPs. In 2 women who had a placental volume stored in 3D it was apparent during off-line analysis that the image quality did not allow for adequate resolution in the reconstructed planes to be able to differentiate the placental borders with certainty and therefore an accurate measurement could not be obtained.

For the myocardial performance index, the measurement on the left was obtained in all but 4 women scanned by JC in the first trimester. All of these women did have a measurement on the right, and so were included in our final analysis. The reason that the measurement could not be obtained was fetal activity in every case. For the right MPI in the first trimester the measurement was not obtained in 17 women for a variety of reasons including fetal activity, position and raised BMI. It is worth noting that a number of the cases where the right MPI was not obtained were close to the start of the study, e.g. it was not obtained in any of the first 4 women scanned, and there was also a tendency to measure the left first so if the fetus was very active and was only transiently in a good position it was more likely that it would be the left sided measurement that would be obtained. In the second trimester the MPI on the left was measurable in 47 out of 49 women and on the right in 45, with similar reasons for non-ascertainment.

The cardiac chamber measurements described in Section 3.2 were obtained in 93 women out of the 112. 5 women did not have a second trimester scan, but were included in the final analysis as they had first

trimester data which could be related to other data obtained, i.e. maternal characteristics. In 12 women the correct image could not be obtained due to fetal position and in 2 the images that were obtained were of insufficient quality for analysis due to being at slightly the wrong level in the heart.

As not all scan parameters were measured in every woman when the relationships between these parameters and routine fetal biometry, maternal body composition and pregnancy outcomes were examined some of the datasets were quite small. Second and third trimester fetal biometry were also not available for all women as scans at these times were only available to women who were actually recruited into the SiPS and as the study progressed and numbers recruited increased, there was no longer capacity within the department for on-going scanning of all women initially screened.

Of our cohort of 112 women, 75 proved ineligible for the SiPS after screening and 37 were recruited. 17 were randomised to the salmon branch of the study and 20 were in the control group. Given these small numbers it is unlikely that the intervention offered influenced our results especially as the intervention did not start until after the 20 week scan.

Measurement obtained	Number obtained	By whom	Number not obtained	Reason
Placental volume first trimester	112	Images- PM and CN Offline measurement JC	0	-
Placental Volume second trimester	105	Images- PM and CN Offline measurement 3D- JC	7	Where 3D image not obtainable 2D measurement performed
Placental volume second trimester 3D	79	Images- PM and CN Offline measurement JC	7 (as above)	5- no second trimester scan 2-Image quality
Placental volume second trimester 2D	26	Image and measurement PM and CN	-	2D measurement only taken if 3D thought not feasible
Left Myocardial Performance index first trimester	74	All JC	5	5-Fetal activity
Right Myocardial Performance index first trimester	62	All JC	17	6-Fetal activity 4- fetal position 6- raised BMI 1-Multiple fibroids
Left Myocardial Performance index second trimester	47	All JC	2	1-fetal bradycardias 1-Fetal position

Right Myocardial Performance index second trimester	45	All JC	4	4- fetal position
Total Cardiac Circumference	93	Images- PM and CN Offline measurement JC	19	5-No second trimester scan 12- not obtained, fetal position 2 image quality, not measurable
Internal Ventricular Circumference	93	Images- PM and CN Offline measurement JC	19	5-No second trimester scan 12- not obtained, fetal position 2 image quality, not measurable
Internal Left Ventricular circumference	93	Images- PM and CN Offline measurement JC	19	5-No second trimester scan 12- not obtained, fetal position 2 image quality, not measurable
Internal Right Ventricular Circumference	93	Images- PM and CN Offline measurement JC	19	5-No second trimester scan 12- not obtained, fetal position 2 image quality, not measurable

Table 1- Scan measurements obtained

2.3 Pregnancy outcome measures

Of the 112 women who had a measurement of fetal cardiac function performed at any point in the pregnancy by JC and were therefore part of our cohort, there was outcome data available on 109. 3 women, who had proved ineligible for the SiPS following screening, went on to deliver elsewhere. The obstetric outcomes for the 109 women we could follow up are described in section 4.6.

Birth weight was available in 108 women for the final analysis. Placental weight was available in 57. Of these 28 were measured within the SiPs protocol in a laboratory setting with the placentas trimmed and clot extruded. As this number was very limited for a very important parameter we also included those placentas measured in the Labour ward setting. Again this was not available for all women as placentas are no longer routinely weighed after birth. This obviously introduced significant potential for error as the methods would be non-standardised.

2.4 Statistics applied

Our aims were to assess the reliability and reproducibility of various scan parameters as well as examining the relationship between a number of continuous variables.

2.4.1 Ultrasound parameters

We undertook three different novel scan assessments- placental volume, myocardial performance index and cardiac chamber size. All the data within these groups was normally distributed.

When establishing the reproducibility of placental volume, myocardial performance index and cardiac chamber sizes we used scatter plots to demonstrate clustering of the measurements within and between subjects. For placental volume and cardiac chamber size these demonstrated tight clustering of the measurements indicating good reproducibility. For the myocardial performance index measurements there was a much wider scatter of

observations. We therefore went on to formally calculate the variance according to potential different sources of error as outlined in section 3.3.

Both the placental volume and cardiac chamber sizes were associated with gestation and so were adjusted for the ongoing analyses. The myocardial performance index was not associated with gestation. When comparing data from the first and second trimesters we wanted to establish if the first trimester measurements of placental volume and MPI could predict the second trimester values and so performed linear regression analyses, rather than parametric tests such as *t*-tests which would only establish an association.

2.4.2 Fetal biometry

In the first trimester crown-rump length was measured. In the second trimester biparietal diameter, head circumference, abdominal circumference and femur length were measured and for those women who had a third trimester scan these were repeated along with thoracic circumference. Again all measurements were normally distributed and adjusted for gestation.

Comparing fetal biometry to placental volume, cardiac function and cardiac structure we again used linear regression to test if one set of variables was dependent on the other, rather than a straightforward association.

2.4.3 Maternal Characteristics

The maternal demographics and anthropometry collected were maternal age, height, weight, body mass index and the percentage of body fat as measured by the Tanita MC180 body composition analyser.

All of these variables were normally distributed and were again related to placental volumes, fetal cardiac function and structure and biometry using a regression analysis to determine if maternal parameters could predict fetal.

2.4.4 Limitations and sources of error

As we were principally exploring the feasibility of obtaining reproducible and reliable measurements using novel ultrasound techniques, such as 2D cardiac chamber measurements we didn't calculate a sample size as we couldn't know the mean or standard error of these measurements in advance. However this then limited our ability to draw meaningful conclusions when relating the ultrasound parameters to maternal characteristics as demonstrated by the retrospective power calculation based on the correlation co-efficient performed on one of the largest data sets we had, correlating first trimester placental volume with birth weight.

```
. pwcorr birthwtag pvol1ag, sig obs

             | birthw~g  pvol1ag
-------------+------------------
  birthwtag |   1.0000
            |
            |      108
            |
    pvol1ag |   0.1954   1.0000
            |   0.0427
            |      108      112
            |

. sampsi 0.1954 0 , n(108) sd(0.9807) onesam

Estimated power for one-sample comparison of mean
  to hypothesized value

Test Ho: m =  .1954, where m is the mean in the population

Assumptions:

        alpha =   0.0500  (two-sided)
```

alternative m = 0
sd = .9807
sample size n = 108

Estimated power:

power = 0.5441

Even with one of our larger data sets we only had 54% power to detect a correlation coefficient of 0.20 with 108 subjects at the 5% significance level.

Although the women invited for ultrasound within the University department were deemed low-risk by their midwife at booking there was still a large degree of heterogeneity amongst the cohort as they were unselected for age, parity, smoking status etc, and we did not exclude those that then became high risk as the pregnancy progressed.

Another potential source of bias was having scans being performed by more than one sonographer. Although all the MPI measurements were performed by one examiner (JC), the stored placental volumes and cardiac cineloops were obtained by two different practitioners, PM and CN. The fact that all of these measurements were then analysed offline meant that there was no opportunity to optimise images found to be of poorer quality, although this was not a big factor in our analysis, with the majority of images meeting the criteria for measurements in terms of identifiable landmarks.

The lack of umbilical and middle cerebral artery Doppler measurements also limited our ability to detect fetuses showing recognised signs of adaptation to the in-utero environment. However as our cohort were essentially unselected low-risk and the numbers small then we would not have been able to come to any meaningful conclusions.

Among our multiple analyses there were findings of statistical significance, with a *p*-value of <0.05 and some which were highly significant, with a *p*-value of <0.01 or even<0.00. It is recognised that in testing a null

hypothesis using a *p*-value of <0.05 as significant then for every 20 analyses run 1 would be expected to be significant, i.e. 20x0.05. One method to control for this is to use the Bonferroni method which essentially multiplies the observed *p*-value by the number of tests performed and if the answer is still less than 0.05 then the answer is still significant. As our variables were not likely to be completely independent of each other and our numbers were small this method would alter the threshold of significance too much for us to be able to draw any conclusions.

3. Chapter 3- Development and Assessment of Placental and Fetal Cardiac Measurement Techniques

In this chapter we will examine the development of the techniques for measuring placental volume, cardiac structure and cardiac function, discussing reproducibility and how measurement technique was streamlined for placental volume and cardiac chamber measurements in order to minimise the time taken to perform these without losing accuracy.

3.1 Placental Volume

Placental volume has been successfully measured on ultrasound using both 2D and 3D ultrasound (Howe, Wheeler et al. 1994, Metzenbauer, Hafner et al. 2001). Howe et al used a parallel planimetric technique, measuring the length and area of 5 equally spaced transverse planes on 2D ultrasound at 18 weeks in 568 women. They found this method to be reproducible with a low margin of error. Hafner et al measured the placental volume in the second trimester using 3D ultrasound (Hafner, Philipp et al. 1998). Scans were performed between 16 and 23 weeks gestation with a mean gestation of 19 weeks. They scanned in a plane parallel to the placental attachment with the largest placental surface area visualised and then calculated the volume by tracing placental borders in 1cm slices. They used more slices where the shape was irregular, with a minimum of 12 slices per placenta. Out of 400 women scanned 18 images were unsuitable for measurement, either because the whole placenta was not captured or the borders were indistinct. Of the 382 measured there was good reproducibility and low intra- and inter-observer variability. They found significant associations between birth weight and placental volume on ultrasound and also maternal weight and placental volume.

Metzenbauer et al investigated the feasibility of measuring placental volume in the first trimester using the same 3D ultrasound technique described above (Metzenbauer, Hafner et al. 2001). They could obtain a

measurement of placental volume in 98% of subjects scanned, with poor image quality the reason for not obtaining the volume. They found the placental volume significantly correlated with crown-rump length and also noted an association with placentally derived serum markers PAPP-A and free β hCG. The placental volume measured was 41.3ml at 11 weeks of gestation up to 59.2ml at 13 weeks.

Subsequently Deurloo et al assessed the feasibility of measuring fetal and placental volume using 3D ultrasound in the first and second trimester (Deurloo, Spreeuwenberg et al. 2007). They noted that beyond 18 weeks it was often not possible to fit the whole placenta into the volume box and therefore the measurement could not be performed. For pregnancies between 11 and 18 weeks they used the same technique as Hefner and Metzenbauer, tracing the placental border on parallel slices with a maximum distance of 10mm between slices. A minimum of 15 slices were measured for each placenta. They scanned 34 women and failed to obtain a measurement in one with poor image quality due to maternal obesity. They also demonstrated good intra- and inter-observer variability. The same measurement technique was used by Michailidis to assess placental growth in the first and second trimester, except they chose to measure parallel slices no thicker that 5mm (Michailidis, Morris et al. 2002). Again high intra- and inter-observer correlations were found. They performed serial scans at 3 weekly intervals from 11 weeks up to 21 weeks and did not comment on whether there was any difficulty with obtaining the volumes at later gestations related to the size of the placenta as previously reported. Typical volume measurements in the second trimester ranged from 150cm^3 to 300cm^3.

Within our department placental volumes had previously been measured on different cohorts (Inskip, Godfrey et al. 2006) (Holroyd, Harvey et al. 2012) using 2D ultrasound at the 19-21 week scan due to the difficulties with fitting the whole placenta in the volume box for 3D volume measurement. This is particularly true for anterior placentas in the upper part of the scan sector, a factor which has not been previously commented

on. Our study provided an opportunity to compare these 2 methods with the anterior placentas which did not fit into the volume box being measured by 2D ultrasound in the second trimester and the rest being measured by 3D scan. In the first trimester 3D scanning was used to measure all placental volumes.

Placental volume measurements were obtained in 112 women in the first trimester using 3D scanning. In the second trimester volumes were measured in 105 women: of these 79 were measured on 3D scanning and 26 using the 2D method. 3D volumes were stored on disc and uploaded to a computer and measured using 3D SonoView 2000 software (Medison, Inc.) which allows for tracing of irregular shapes. The lateral borders of the placenta were identified and the placental circumference measured in 3mm slices until the opposite lateral border was reached. As there had been different slice thicknesses used in the previously published literature we reasoned that the smallest distance between slices (i.e. the thinner the slice) the greater the accuracy of the measurement would be, particularly given the generally irregular shape of the placentas. In the first trimester placentas this was more feasible with typical measuring times of approximately 10-15 minutes, but in the second trimester placentas this number of slices was taking 45 minutes on average to measure. We therefore took the opportunity to compare the impact of measuring different slice thicknesses on the final volume result to see if thicker slices introduced more variation as we had reasoned.

We performed repeat measurements on 10 first trimester placentas and 6 second trimester placentas, measuring in 3mm slices, then 6mm slices and finally 10mm slices. The following graphs show the results of these

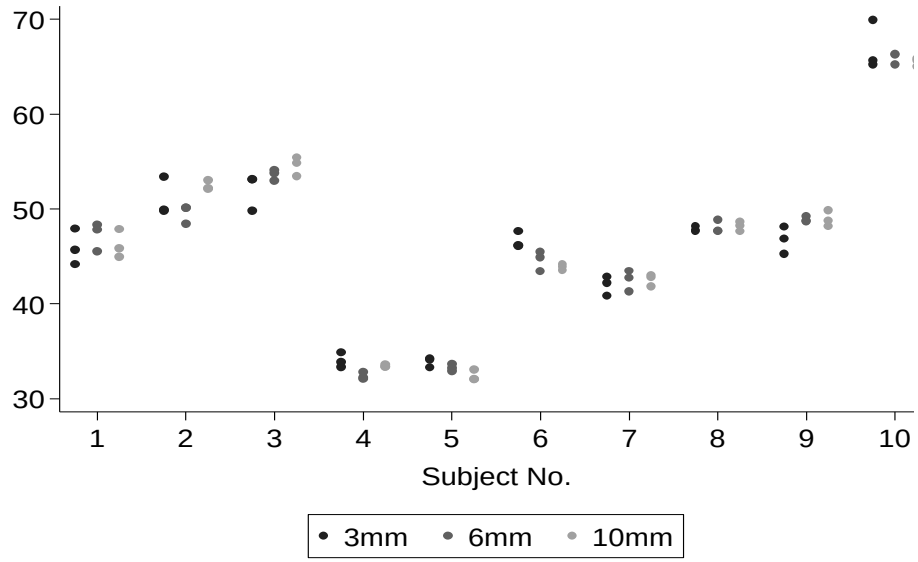

Figure 2- First trimester placental volume measurements (cm^2), variation with different slice thickness

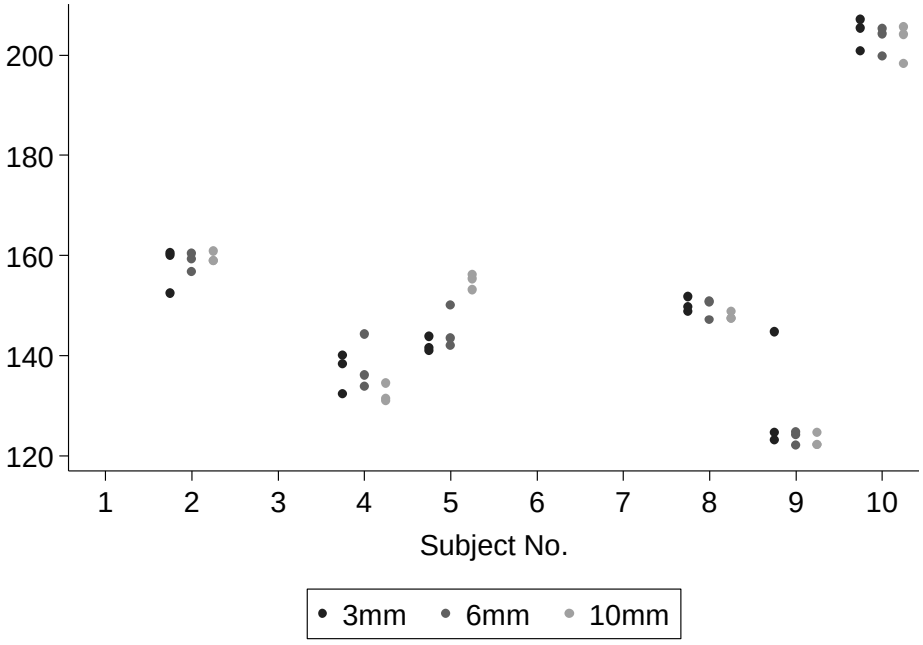

Figure 3- Second trimester placental volume measurements (cm^2), variation with different slice thickness

These graphs demonstrate that there is tight clustering of the volume measurements which does not show any increase in scattering with the larger slice thicknesses, confirming good reproducibility. There is good correlation between the volumes measured using either 3mm, 6mm or 10mm slice thicknesses and so it appears that there is no loss of accuracy, but a great time-saving in measuring in 10mm thick increments.

3.2 Cardiac Structure

Cardiac structure can also be assessed using 2D and 3D techniques (Schmidt, Silverman et al. 1995, Firpo, Hoffman et al. 2001, Bhat, Corbett et al. 2004, Bhat, Corbett et al. 2004).

In studies using 2D measurements the most obtainable measurements were the width and length of the cardiac chambers, successfully measured in the four-chamber view in 52-57% of 60 fetuses at 14 weeks gestation and 100% of 80 fetuses at 20 weeks. (Firpo, Hoffman et al. 2001). The authors attempted a long-axis view of the heart in order to obtain the third dimension but found this to be attainable in only 26% at 14 weeks and 70% at 20 weeks.

Using 3D ultrasound the ventricular mass was measured in 90 pregnancies between 15 weeks gestation and full term (Bhat, Corbett et al. 2004). The authors validated their measurement technique using small balloon models and in vivo and ex vivo small animal experiments. They found that measuring the ventricular myocardial mass was reproducible but were unable to determine at what point in the cardiac cycle their measurements were being taken. This difficulty can be overcome using 4D spatiotemporal image correlation (STIC), which allows acquisition of a fetal heart volume as a 4D cine sequence, therefore allowing off-line analysis to choose a set point in the cardiac cycle (Goncalves, Lee et al. 2006). However difficulties may be encountered in defining cardiac outlines in the reconstructed planes.

As access to STIC technology would be limited for our population we reasoned that 2D measurements of total cardiac circumference, total ventricular circumference and internal left and right ventricular circumferences in the four-chamber view would be easily obtainable and may provide some insight into subtle alterations in cardiac structure. The images would be saved as a cine sequence so that accurate and consistent determination of the point in the cardiac cycle could be made by the positioning of the valve leaflets. End-diastole, just after the mitral and tricuspid valves had closed, when the ventricles are at their maximum size would be an easily identifiable point and the larger size would minimise measurement error. Off-line measurements would then be performed.

3.2.1 2D Cardiac Measurements Performed at 20 Weeks Gestation

Between February and May 2007 18 patients were scanned at 19-21 weeks for a routine anomaly scan. These women consented to having additional cardiac measurements done. Out of these 18 women cardiac measurements as detailed above were obtained in 10. The reasons for not obtaining measurements were fetal position (4), raised maternal BMI (2), limited scan time (1) and recurrent fetal bradycardias (1). On reviewing the images obtained there was an obvious wide variation and the reasons for this were explored. The measurements were dependant on the angle of beam relative to the septum, the point in the cardiac cycle and the level in the heart at which the measurements were obtained. There was also some pressure of time to choose the best images and perform the measurements as this was being done on-line during the scan.

In order to minimise these sources of error the technique for obtaining images was revisited and the criteria redefined. The fetal cardiac setting was used and the res box placed over the heart to obtain maximum zoom. The septum was to be at 90 degrees to the beam and the pericardium identified as an easily definable landmark. The insertion of the valve leaflets into the ventricular wall was also an identifiable landmark to assess the level of the section within the heart and the point in the cardiac cycle at which to

perform measurements. The papillary muscles were to be included in the measurement of the internal right ventricular circumference, which was felt to give greater consistency.

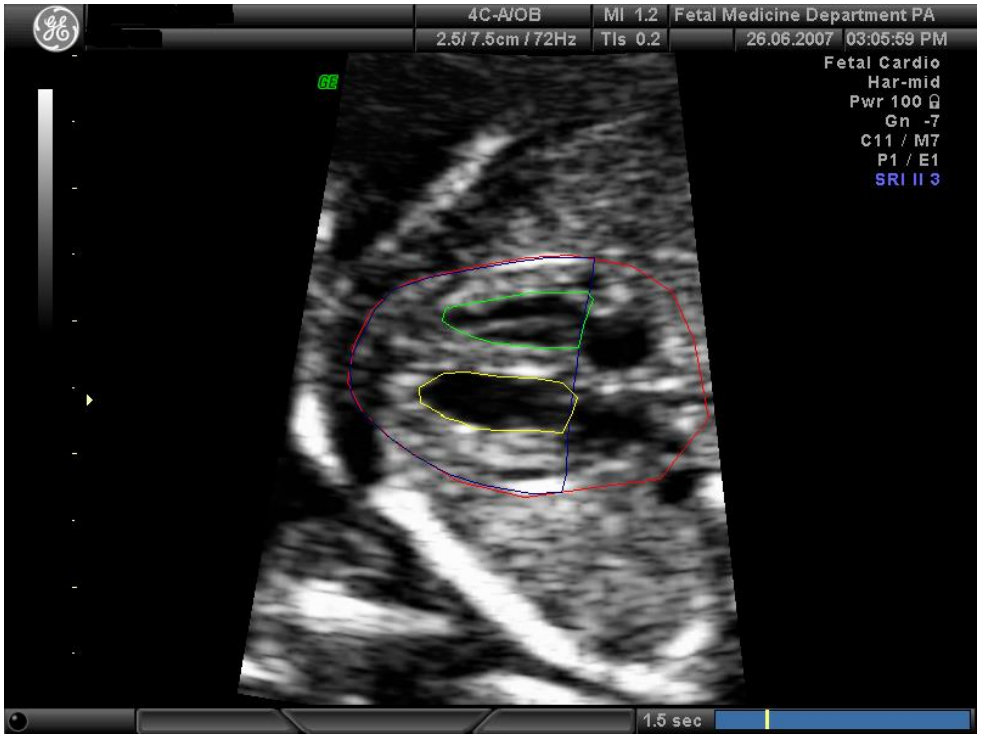

Figure 4- Measurement of cardiac chamber circumferences in the second trimester

The above image demonstrates the measurements performed. The red line represents the total cardiac circumference, the blue line is the total ventricular circumference, the yellow is the internal left ventricular circumference and the green is the internal right ventricular circumference.

Using these criteria a further 10 women were scanned up to July 2007. Measurements were obtained in 8 of these with fetal position the reason for not being able to obtain measurements. Where the fetus was lying with the

apex of the heart pointing anteriorly it was difficult to get the ventricular septum at 90°. In the meantime Escape Medical Viewer software was purchased in order to allow offline analysis of cine sequences obtained. In the next 19 women scanned optimal images were obtained in 17, with fetal position again the reason for not obtaining images. Cine sequences were obtained and analysed. On review of one of these cine sequences it was felt that the correct landmarks could not be identified and measurements were not performed. In the remaining sequences 3 images at the same point, i.e. end-diastole in different cardiac cycles were chosen and measurements of each of the parameters (total cardiac circumference, total ventricular circumference and internal left and right ventricular diameters) performed 5 times.

Using the Escape software the default measurement when measuring an irregular polygon, such as a fetal heart, is the area in mm^2. Ten sets of measurements as described above were performed for area. The method for obtaining the circumference was then worked out and so this was measured in the same 10 patients plus an additional 3. These results were analysed in order to establish the variability within subjects, the variability between subjects and the reproducibility of the measurements.

3.2.2 Statistical Analysis of Cardiac Chamber Measurements

These tables demonstrate the variance in the 4 parameters within and between each subject. The first four are for the area and the second four are for the circumference. Five measurements were performed on three separate images, but where two measurements were the same the dots are superimposed on each other explaining why five dots do not appear for each image. Each subject has an individual identifying 'JC number'. The gestational age ranged from 19 weeks and 4 days to 21 weeks and 5 days. The measurements were not adjusted for gestational age.

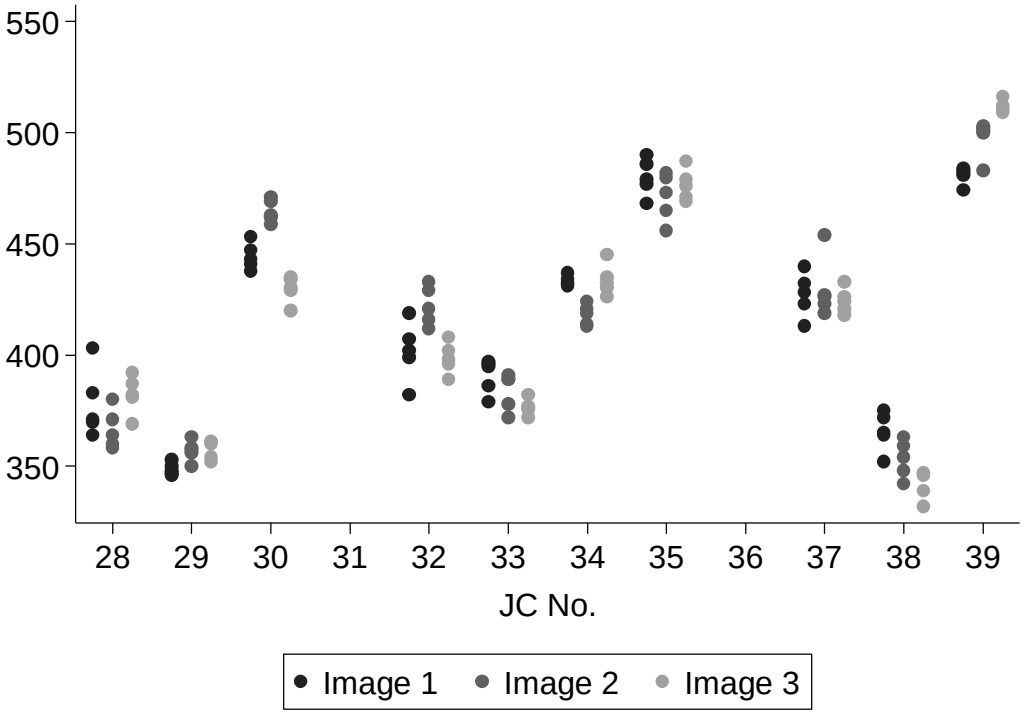

Figure 5-Total cardiac area, within and between subject variation

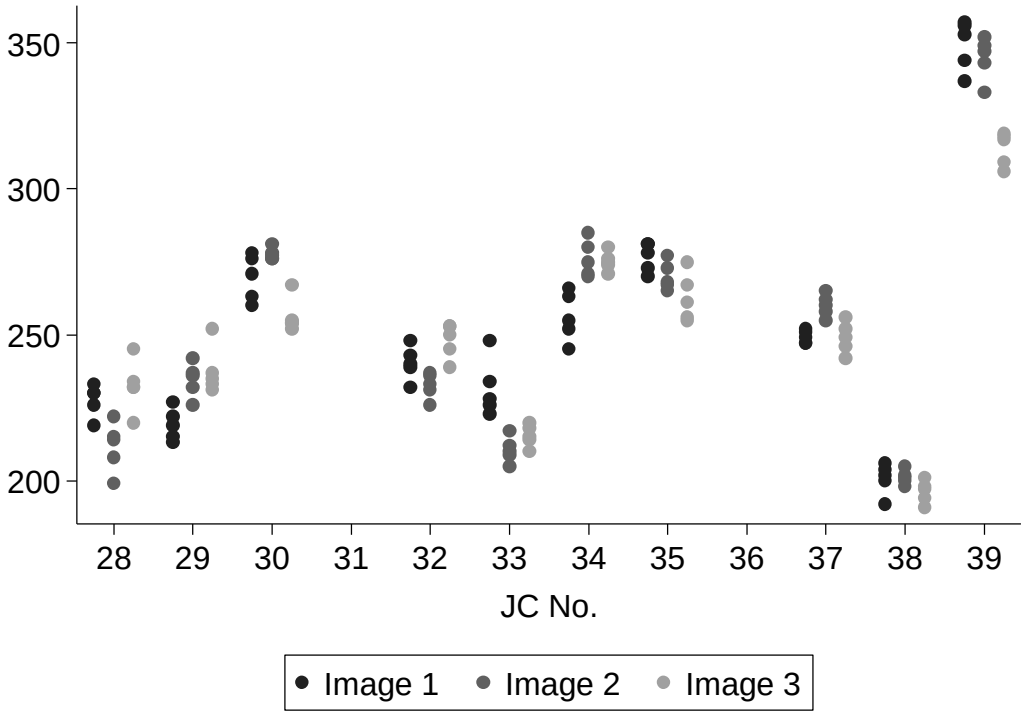

Figure 6- Total ventricular area, within and between subject variation

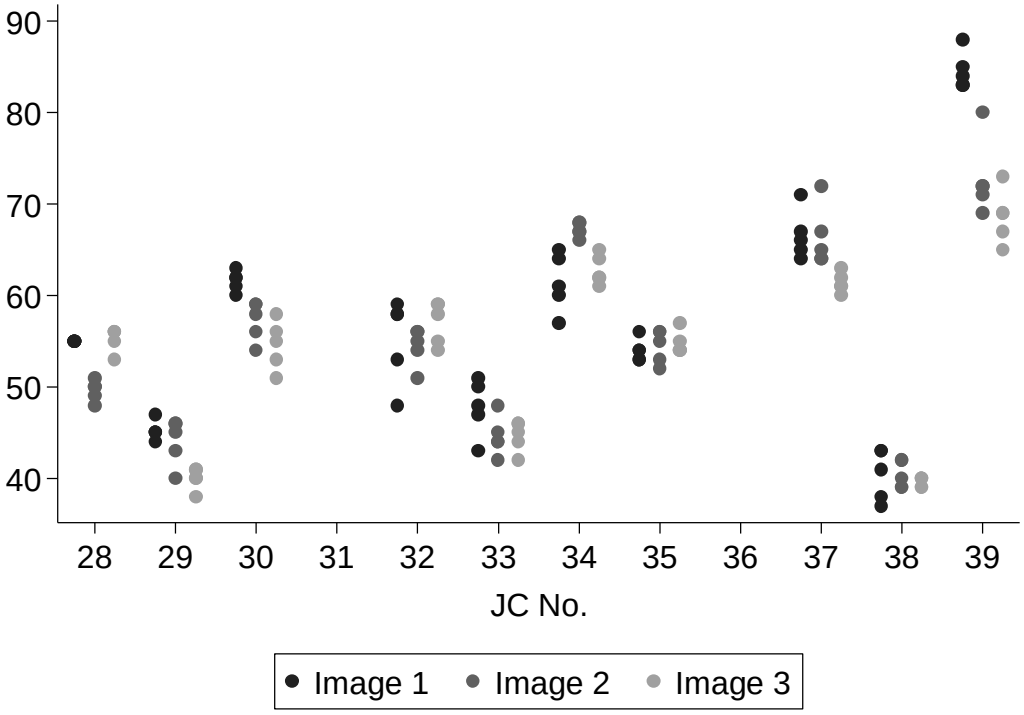

Figure 7- Internal left ventricular area, within and between subject variation

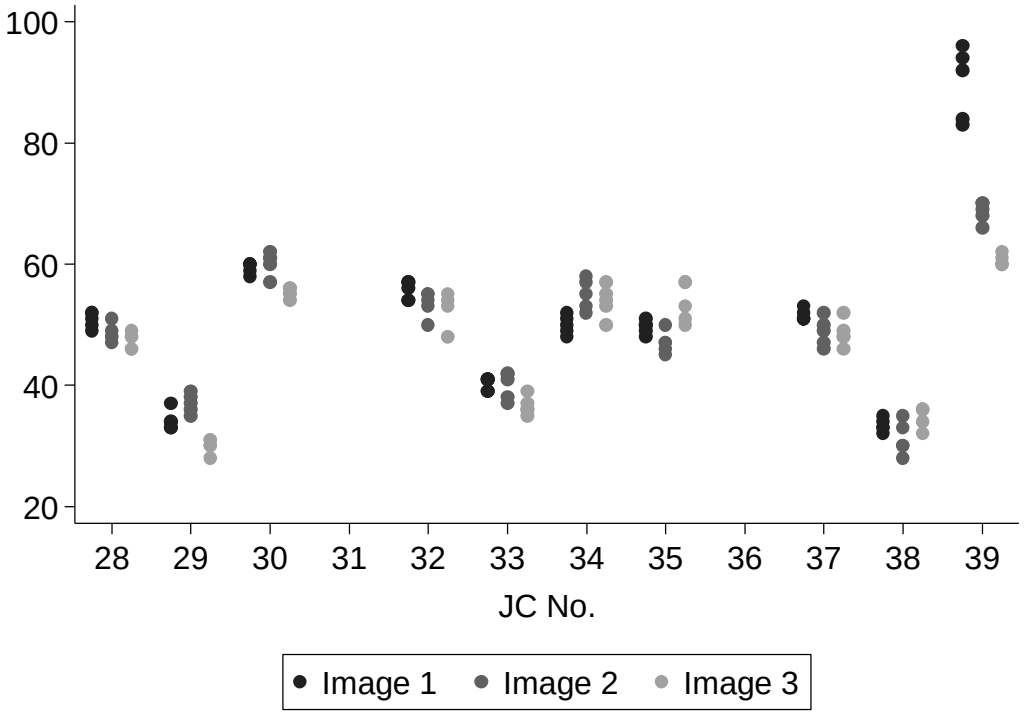

Figure 8- Internal right ventricular area, within and between subject variation

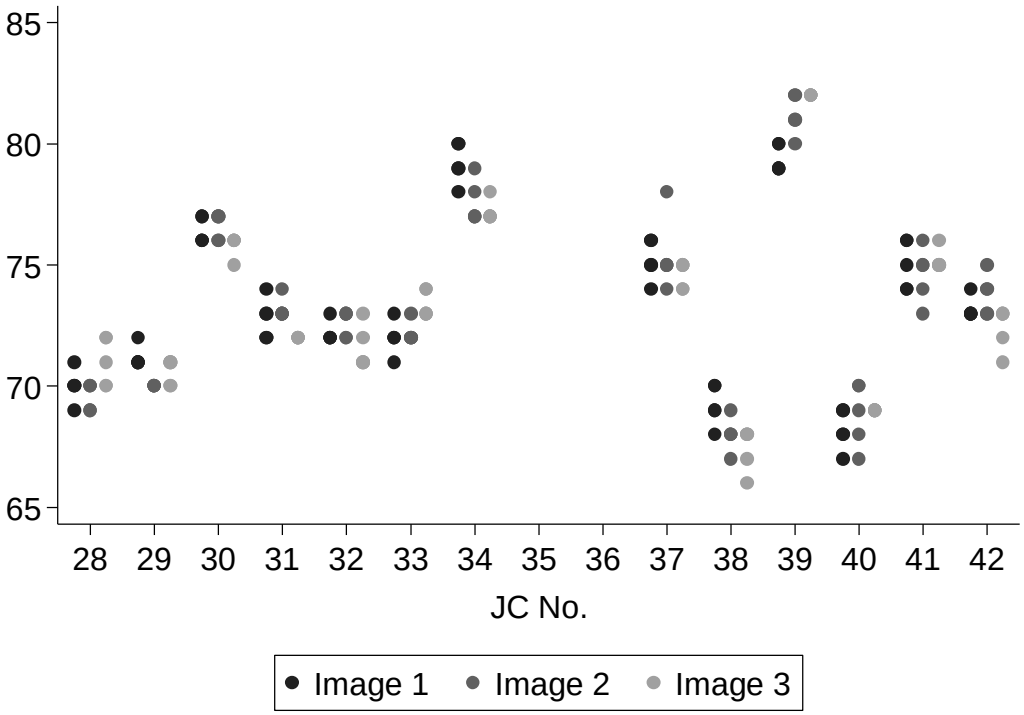

Figure 9- Total cardiac circumference, within and between subject variation

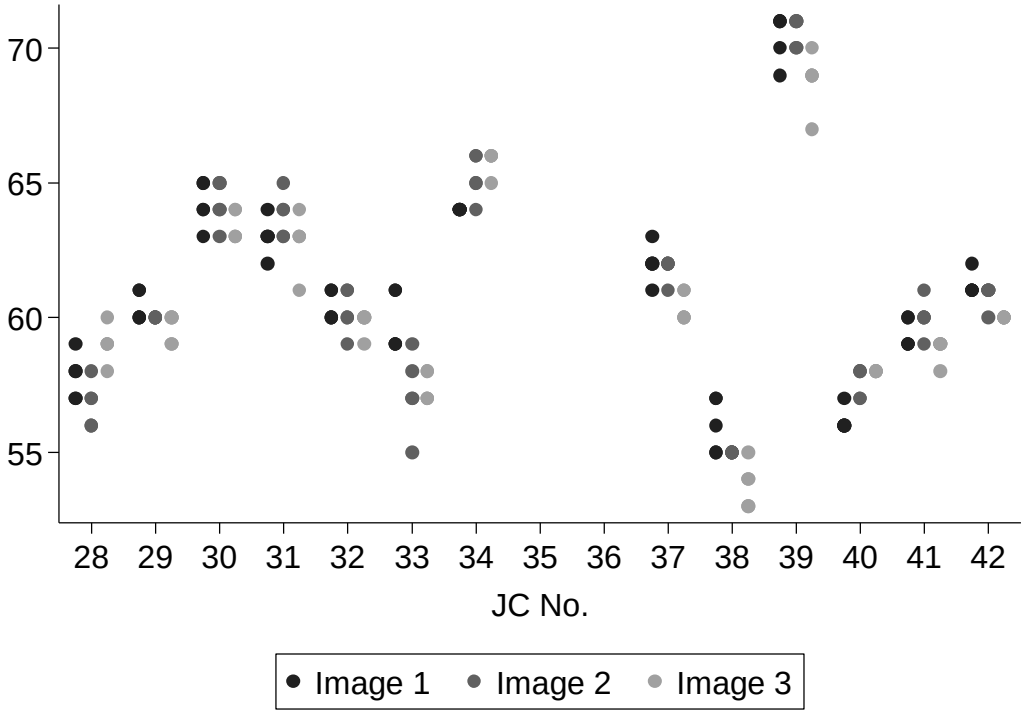

Figure 10- Total ventricular circumference, within and between subject variation

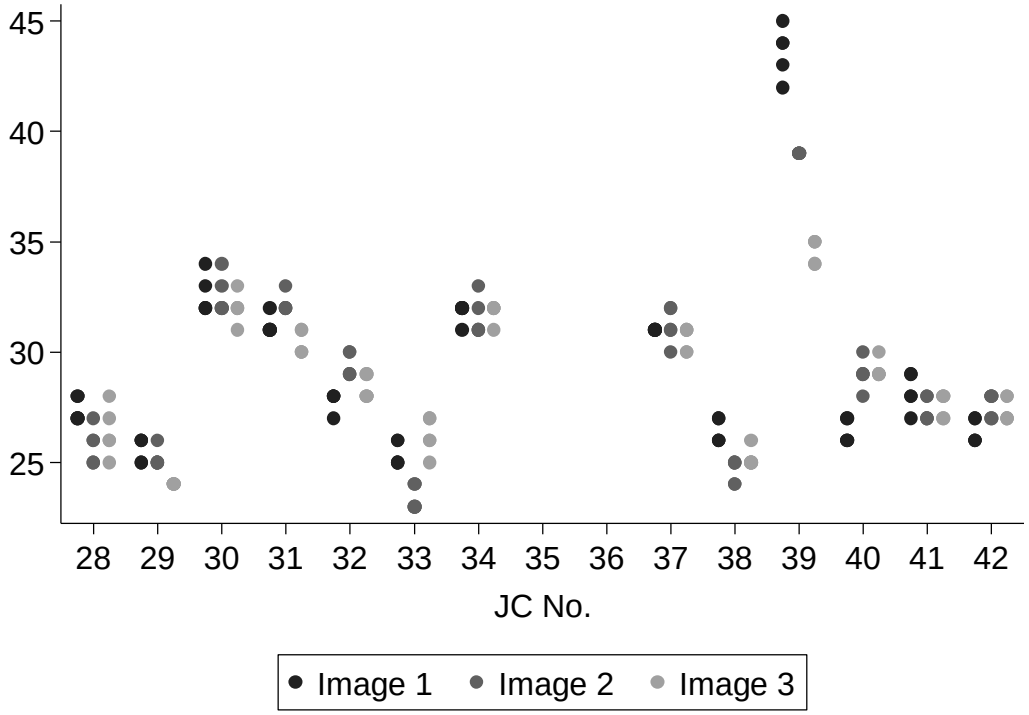

Figure 11- Internal right ventricular circumference, within and between subject variation

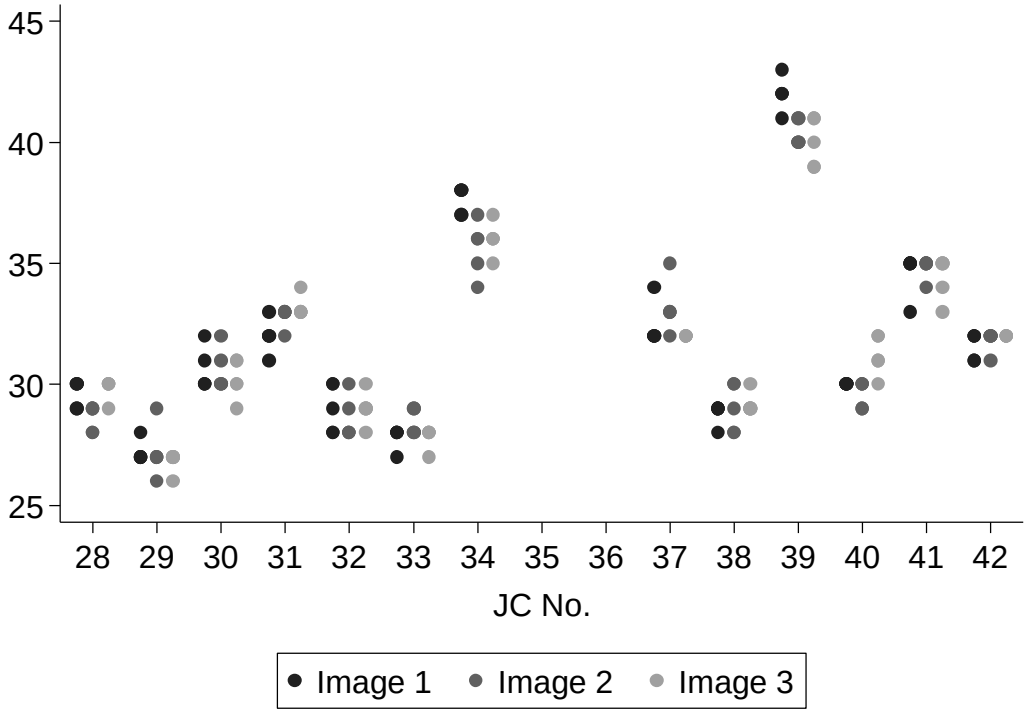

Figure 12- Internal left ventricular circumference, within and between subject variation

These graphs demonstrate a tight clustering of measurements within each subject between the three images showing the measurements to be reproducible. The exception to this is subject 39 particularly with the internal right ventricular measurements. For both area and circumference the five measurements of the third image were much smaller than the first two images. On reviewing the images to try to explain this, the third image did appear to be at a slightly different point of the cardiac cycle so that the papillary muscles within the right ventricle were still closely approximated to the ventricular wall and were not included in the measurement as they should have been. It was apparent that the image chosen was not quite at end-diastole and that the image three frames further on was when the ventricles were at their fullest point, just before the opening of the aortic and pulmonary valves. On measuring this image the measurements were closer to those obtained in the first two images. It was also noted from the position of the ribs that the entire sequence was slightly oblique.

Having established the reproducibility of the measurement technique further analysis was performed to establish sources of variance, defined as the square of the standard deviation, as summarised in the following table.

Variable	**Variance due to subject (mm^2)**	***95% CI of variance due to subject***	**Variance due to cycle (mm^2)**	***95% CI of variance due to cycle***	**Variance due to image (mm^2)**	***95% CI of variance due to image***
TCC	13.21	*5.87 to 29.73*	0.43	*0.21 to 0.84*	0.54	*0.43 to 0.67*
TVC	14.97	*6.64 to 33.75*	0.63	*0.34 to 1.18*	0.49	*0.40 to 0.62*
ILVC	14.43	*6.44 to 32.34*	0.25	*0.11 to 0.53*	0.49	*0.39 to 0.61*
IRVC	14.79	*6.38 to 34.30*	**2.17**	*1.23 to 3.81*	0.40	*0.32 to 0.50*
TCA	2375.37	*929.59 to 6069.76*	96.57	*47.82 to 194.99*	63.97	*49.67 to 82.38*
TVA	1422.33	*551.09 to 3670.97*	103.58	*53.22 to 201.56*	38.20	*29.66 to 49.20*
ILVA	112.31	*43.10 to 292.66*	11.19	*5.68 to 22.03*	5.19	*4.03 to 6.68*
IRVA	133.54	*49.63 to 359.34*	**27.25**	*14.36 to 51.73*	4.61	*3.58 to 5.94*

Table 2 - Sources of variance in Cardiac Chamber Measurements

In order for the measurements to provide any meaningful results when applied to a larger cohort there needs to be a detectable biological difference between different subjects. In our group there was a wide variance between the subjects, although some of this may be explained by gestational age. This indicates that it should be possible to detect variations in measurements in relation to different clinical situations.

The variance due to cycle was the variation in the different measurements between the three separate images, represented by the difference between the horizontal plots in the graphs above. If image reproducibility and selection were good then this variance would be low with narrow confidence intervals. For the circumference measurements this was indeed the case, although for the internal right ventricular circumference measurements the variance was higher, mostly attributable to subject 39 as discussed above.

The variance due to image was the difference between the five measurements of each of the four parameters performed in each image. This provides a measure of how reliable and accurate the measurement technique is. If the measurement technique is good then again the variance should be low with narrow confidence intervals as is the case in the table above, particularly when measuring circumference rather than area.

As selecting three images and measuring each of the four variables five times each was a very time consuming task, taking an average of 45 minutes we also calculated the correlation with a hypothetical ‘true’ measurement depending on the number of different images measured across a number of cardiac cycles and the number of measurements performed on each image.

Variable	Correlation with one image five cycles	Correlation with two images five cycles	Correlation with three images five cycles	Correlation with three images four cycles	Correlation with three images three cycles	Correlation with three images two cycles	Correlation with three images one cycle
TCC	0.9804	0.9901	0.9934	0.9930	0.9925	0.9914	0.9881
TVC	0.9765	0.9881	0.9920	0.9917	0.9913	0.9904	0.9877
ILVC	0.9882	0.9941	0.9960	0.9958	0.9953	0.9944	0.9916
IRVC	0.9317	0.9640	0.9756	0.9754	0.9750	0.9743	0.9723
TCA	0.9777	0.9887	0.9924	0.9922	0.9918	0.9911	0.9889
TVA	0.9631	0.9810	0.9872	0.9870	0.9866	0.9859	0.9838
ILVA	0.9497	0.9739	0.9823	0.9820	0.9814	0.9802	0.9766
LRVA	0.9087	0.9511	0.9666	0.9663	0.9659	0.9650	0.9625

Table 2- Correlation of cardiac chamber measurements based on number of images and repeat measurements

The above table demonstrates that whilst the best correlation is to measure three separate images, selected from different points in the cineloop sequence but at the same point in the cardiac cycle, five times each, there is very little reduction in correlation by measuring three different images once as outlined in the last column with correlations above 0.96 for all variables. The small reduction in correlation would certainly be outweighed by the benefits of reduced measuring times.

3.3 Cardiac Function

If cardiac structural differences were detectable then we would aim to determine if this translated to an alteration in cardiac function. Various methods have been used to assess fetal cardiac function by ultrasound.

Determination of left, right and combined cardiac output using Doppler waveforms was performed in 222 fetuses from 13 -41 weeks (Mielke and Benda 2001). This was done by measurement of internal diameters of the aortic and pulmonary valve annulus and mid-point of ductus arteriosus at 90°, Doppler waveforms of the aorta from the 5-chamber view and Doppler waveforms of the pulmonary artery and aorta in the sagittal view. The Doppler angle was less than 10° and 5 consecutive waveforms were obtained. Normal ranges were determined for left, right and combined cardiac output, which increased with gestational age. This technique was limited in the first trimester with only 50% of measurements obtained at 13 weeks and highly dependent on the skill of the operator.

An alternative technique to assess fetal cardiac function is the myocardial performance index, a Doppler-derived index, which is non-invasive, easily measured and independent of both ventricular geometry and heart rate. It is defined as the sum of the isovolumetric contraction time (ICT) and isovolumetric relaxation time (IRT) divided by the ejection time (ET), providing a global measure of myocardial performance, incorporating systolic and diastolic function. This can be performed in both the left and right ventricles to assess each ventricle independently and determine differences in systolic and diastolic function. Pulsed Doppler is used with the gate placed just below the mitral or tricuspid valve in the four-chamber view, obtaining inflow and outflow signals throughout the cardiac cycle. The resulting waveform pattern can be used to identify the ICT, IRT and ET. The opening and closing of the valves may also be identified by a peak or 'valve click' which is a useful landmark for measurement. This technique is modified in the right ventricle in the second trimester where the increasing

size of the chamber and angle of the outflow tract make it very difficult to obtain both inflow and outflow signals simultaneously. These waveforms are therefore obtained separately, meaning that the ICT and IRT are not measurable individually.

As this measurement has been reported to be easily obtainable in the first trimester we reasoned that it could be a valuable technique in possibly identifying early signs of fetal adaptation if a difference could be determined between right and left ventricular function. Valuable information may also be gained by correlating the myocardial performance index at 13 and 20 weeks with placental volume at 13 and 20 weeks, fetal biometry and maternal body composition.

3.3.1.1 Measuring the Myocardial Performance Index

The machine is set to the fetal cardiac setting and the four-chamber view zoomed as large as possible to identify where to place the cursors. The ideal fetal position is either with the heart pointing straight up or straight down, but the waveform can be obtained in most positions. The cursor should be placed within the ventricle just below the a-v valve. When a succession of satisfactory waveforms has been obtained the image is frozen and the time between the valve clicks measured. If the valve clicks are not identifiable, then the landmarks are from the end of one a-wave (atrial contraction) to the beginning of the next e-wave (passive filling of the atria). This time (a) is equivalent to ICT+ET+IRT. These components can also be measured individually. The time between the start and end of ventricular ejection (b) is then measured (ET). This is repeated for three measurements and the average taken. The MPI index can then be calculated as a-b/b, equivalent to ICT+IRT/ET.

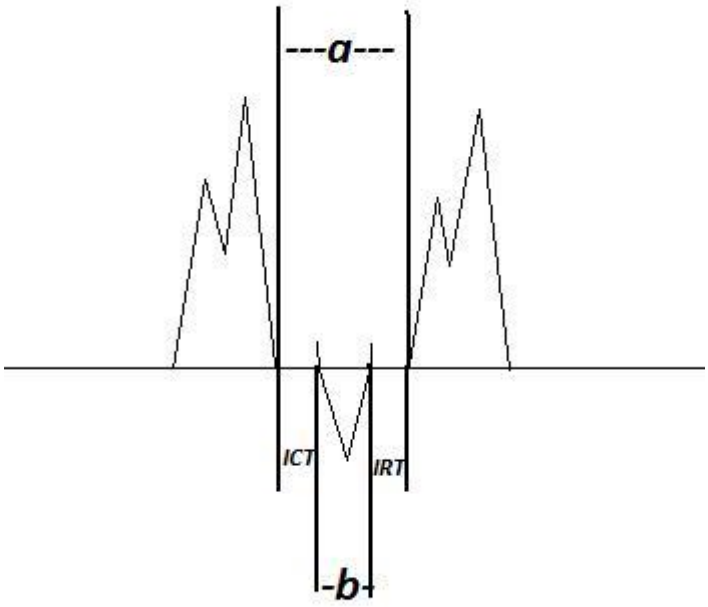

Figure 13- Measurement of the components of the myocardial performance index

We have performed this measurement on 127 fetuses, 89 at 11-14 weeks and 55 at 19-21 weeks. Our ultrasound machine was a Siemens Sequoia using a 5-8MHz curvilinear probe in the cardiac setting where we were able to maintain a live image of the scan picture in order to readily identify correct Doppler gate placement. We found this to be particularly valuable in the first trimester where minimal movement of the fetus could result in the gate being shifted from one side of the heart to the other which was easily identified with the image on screen at all times whilst placing the callipers and watching the waveforms. It was also possible to adjust the image to try to optimise the waveform, with the 'cleanest' waveforms obtained when the apex of the heart was pointing either directly up or directly down. The sweep speed was maintained at the factory default setting of 5cm/s as increasing this to the next possible speed of 10cm/s resulted in difficulty identifying optimum waveforms due to the speed of passage across the screen. The gain was

individually adjusted for each subject to reduce artefact as much as possible whilst maintaining the valve clicks as a landmark for calliper placement.

In each subject at least 3 waveforms were measured, the myocardial performance index calculated as above and then averaged. In a number of subjects we did repeated triplets of measurements on separate images in order to assess the variability between waveforms and between images as well as the between subject variability. Twenty-one repeat sets of measurements were obtained in each group.

The graphs below illustrate the MPI measurement on 3 waveforms in each of image 1, 2 and 3 plotted vertically against each subject along the x-axis. If there were two measurements the same on different waveforms in the same image the dots will be superimposed on each other.

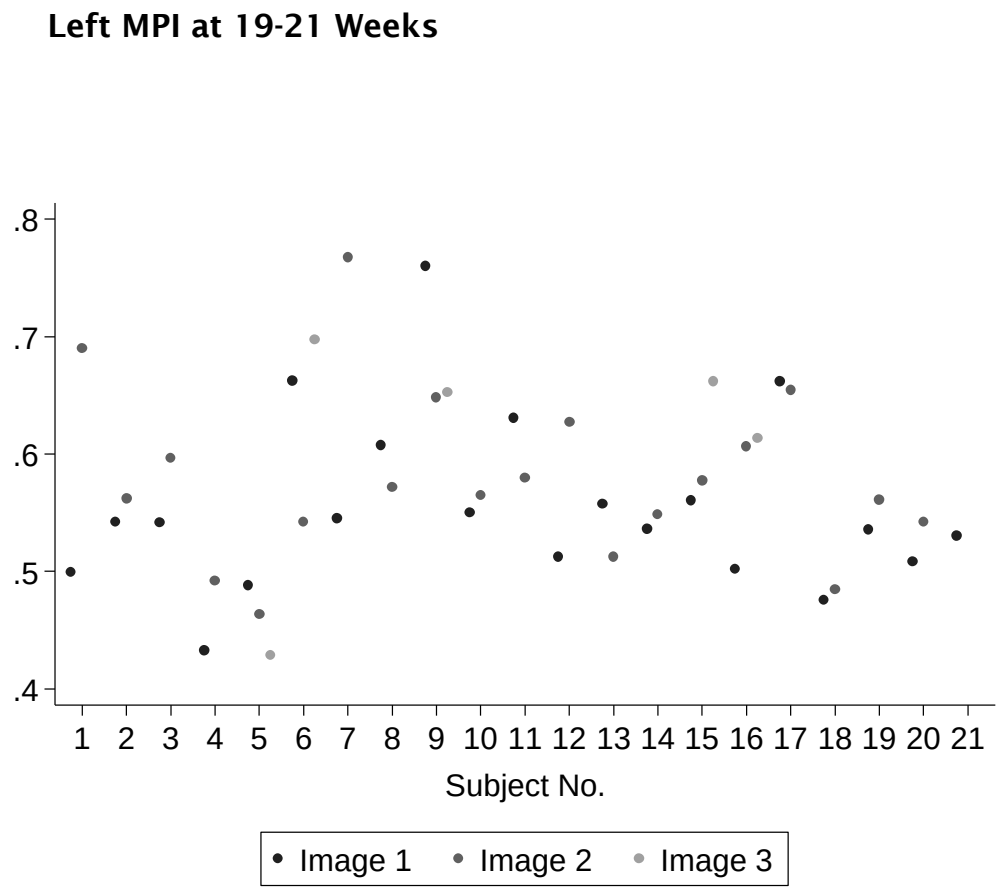

Figure 14- Second trimester left myocardial performance index, variation between different waveforms, different images and subjects

Subject variability	0.0032
Image variability	0.0012
Waveform variability	0.0090

This graph illustrates the measurements obtained from the left ventricle at 19-20 weeks. Each triplet of measurements, i.e. 3 waveforms from the same image, is recorded vertically, while the repeated image measurements are recorded horizontally. For this data the variability between subjects was 0.0032, between images was 0.0012 and between waveforms on a single image 0.0090, shown by a wide scattering of results for both image and waveform. Whilst there was a wide variance due to measurement this was not as wide as the variance between subject with the least variability being found

between the 3 waveforms captured and measured on a single image and the largest variability being between subjects. Our measurements may therefore be reliable and reproducible enough to detect a true biological variation in the LVMPI at 19-21 weeks.

The same analysis was performed on the RVMPI at 19-21 weeks and the LVMPI and RVMPI at 11-14 weeks with different results.

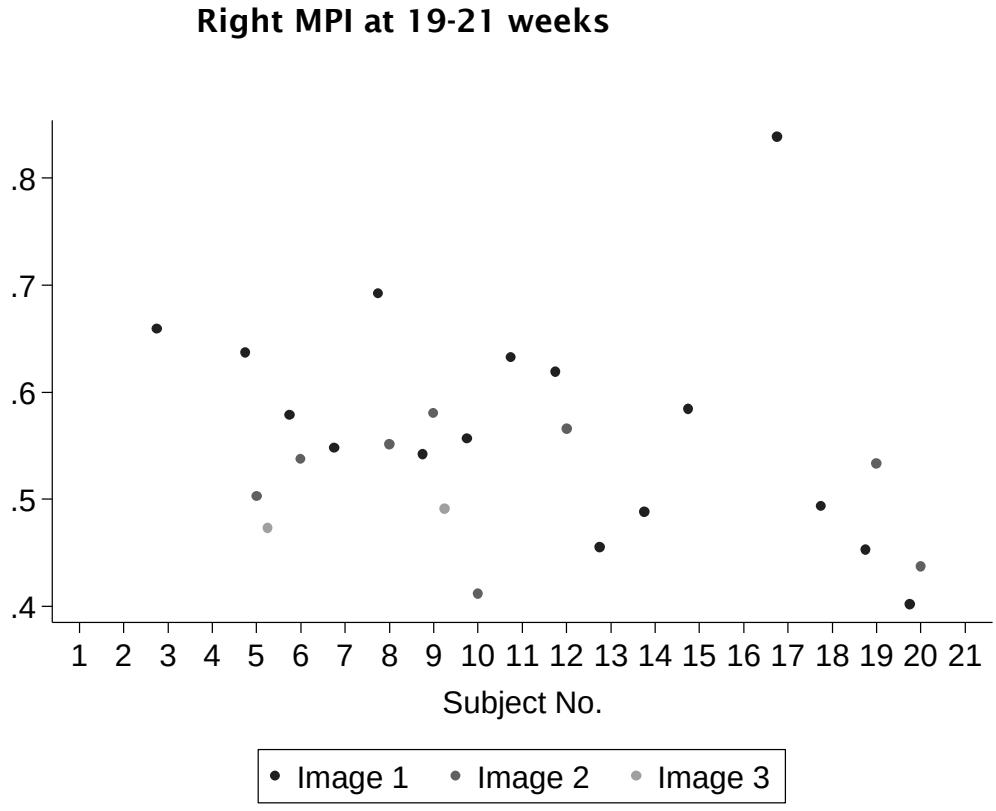

Figure 15- Second trimester right myocardial performance index, variation between different waveforms, different images and subjects

Subject variability	0.0068
Image variability	0.0071
Waveform variability	0.0083

There were a reduced number of repeated observations on the right as this is the more difficult waveform to obtain, particularly at 20 weeks for the reasons outlined above. In this graph the subject variability was even wider than the measurements from the left ventricle while the variability between waveforms and images was also wide, indicating that this measurement was not reproducible and would be unlikely to detect any differences in the true, underlying value.

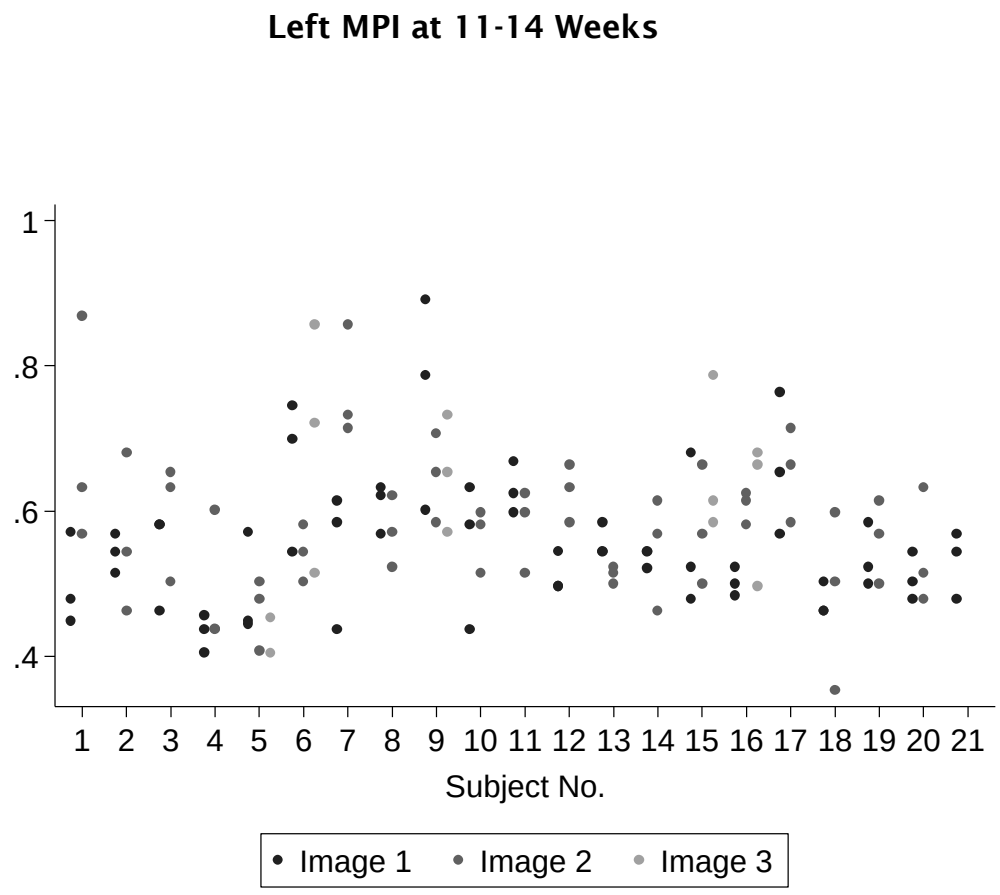

Figure 16-First trimester left myocardial performance index, variation between different waveforms, different images and subjects

Subject variability	0.0023
Image variability	0.0016

Waveform variability	0.0061

In the first trimester the measurement of the LVMPI shows wide variation between waveforms within a single image but less variability due to different images, which is less than the variability between subjects. The measurement error may again be too much to be able to reliably detect any true underlying biological difference.

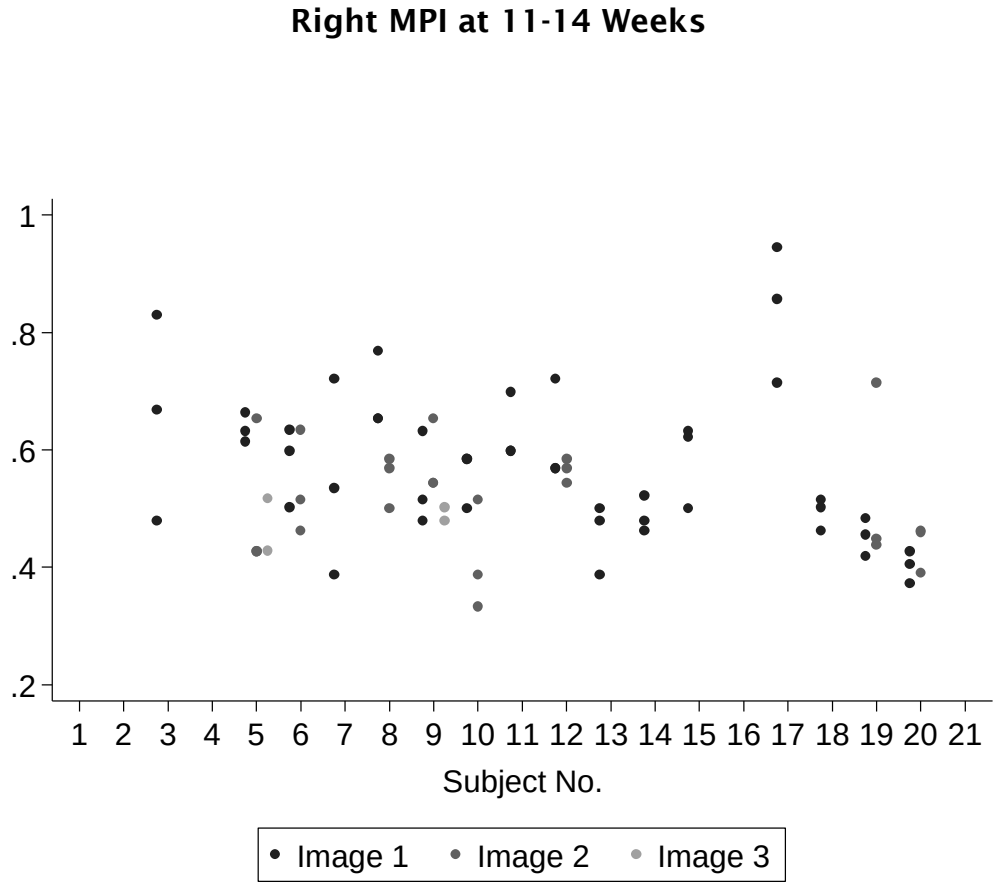

Figure 17- First trimester right myocardial performance index, variation between different waveforms, different images and subjects

Subject variability	0.0048
Image variability	0.0027

Waveform variability	0.0073

There were again a small number of repeat measurements on the right ventricle in the first trimester, but the variability due to measurement between both waveforms and images was large compared to subject variability, making it likely that this would not detect any true differences.

Previously reported series have found the MPI to be easily measured in fetuses. (Tsutsumi, Ishii et al. 1999) measured the MPI in 50 normal fetuses, 35 growth restricted fetuses and 30 fetuses of diabetic mothers between 18 and 40 weeks. They found that the MPI was independent of heart rate and decreased throughout gestation with a normal range of 0.62 +/-0.07 on the left and 0.62 +/-0.06 on the right at 18-20 weeks and 0.43+/-0.03 and 0.49 +/-0.05 beyond 34 weeks. Other observers have noted different ranges and not seen any change associated with gestation. (Mori, Rice et al. 2001) studied 70 normal fetuses between 20-39 weeks gestation in order to compare them to a group who had been prescribed indomethacin and therefore had potential constriction of the ductus arteriosus. They reported a normal range of 0.35+/-0.03 for the LVMPI and 0.35+/-0.07 for the RVMPI. Intra- and inter- observer variability studies on 10 traces showed good correlation with r=0.94 and 0.87 on the right and left respectively between observers and r=0.97 and 0.90 for intra-observer variability. However all these measurements were performed retrospectively on stored Doppler images, inevitably introducing selection bias as only those images with all the necessary waveforms to calculate the MPI could be included. Another retrospective study (Eidem, Edwards et al. 2001), studying stored images from 125 normal fetuses between 20 and 40 weeks gave normal ranges of 0.32+/-0.03 for the RVMPI and 0.35+/-0.03 for the left. They also reported on their reproducibility based on 20 repeat measurements and found a mean percentage error of 4% for inter-observer variability and 3.2 % for intra-observer variability with an average of 5 waveforms measured for each fetus. Whilst they also found that the MPI was independent of heart rate they did not find an association with gestation.

In prospective studies (Friedman, Buyon et al. 2003) reported a normal range for the LVMPI of 0.53 +/-0.13, based on 74 normal fetuses between 20 and 40 weeks, while the normal range in the first trimester has been reported as 0.5+/-0.1 for both the right and left, based on 28 fetuses examined between 12 and 15 weeks (Russell, McAuliffe et al. Oral Communication, BMFMS, 2007).

In 2005 a new technique was described to measure the MPI in the left ventricle (Hernandez-Andrade, Lopez-Tenorio et al. 2005) which involved placing the Doppler sample gate in the internal leaflet of the mitral valve with the heart in an apical projection in order to obtain high signal 'valve clicks' from both the mitral and aortic valve as the valves opened and closed. This is feasible in the left ventricle due to the proximity of the two valves, but would not be applicable to the right side due to the different anatomical relationship between the tricuspid and pulmonary valves. They reported much improved intra- and inter-observer variability using this technique. The same group published gestational age adjusted reference values for this modified MPI measurement in the left ventricle (Hernandez-Andrade, Figueroa-Diesel et al. 2007), reporting a slight increase in the modified MPI with gestation, from 0.35+/-0.027 at 19 weeks to 0.37+/-0.029 at 39 weeks gestation. They recommended setting the sweep speed as fast as possible to delineate the valve clicks as clearly as possible and reducing the gain to eliminate lower velocity signals. In this study there was no effect on the MPI with changes in heart rate. In the 557 fetuses scanned they found that the ICT, IRT and ET all decreased by 13-15% with an increase in fetal heart rate from 130 to 160 beats per minute.

Whilst the reproducibility of the MPI, where it has been reported, appears to be good, there are differences between observers on the normal ranges and relationship to gestation. Reasons for our variability results may be technical, such as the sensitivity of the ultrasound measuring controls when placing the callipers or poor quality of images, which can be related to fetal activity or position or maternal body habitus. There may also be operator error,

particularly early on in the project at the start of the learning curve, or incorrect identification of landmarks for measuring. This may be particularly true in identifying the ICT, which is often poorly distinguished. The measurement technique on the right ventricle at 19-21 weeks may also be a source of error if the heart rate is significantly different between identification of the inflow and outflow waveforms as this is done separately.

3.3.1.2 Exploring measurement error in the Myocardial Performance Index

In order to address some of these issues we analysed the reported variabilities in the sample of patients used above where there were most repeat measurements in chronological order to see if the variability had improved over time as evidence for a learning curve effect. This was done by calculating the standard deviation of the measurements for each subject to see if this narrowed over time, giving evidence for an improvement in measurement technique as experience was gained.

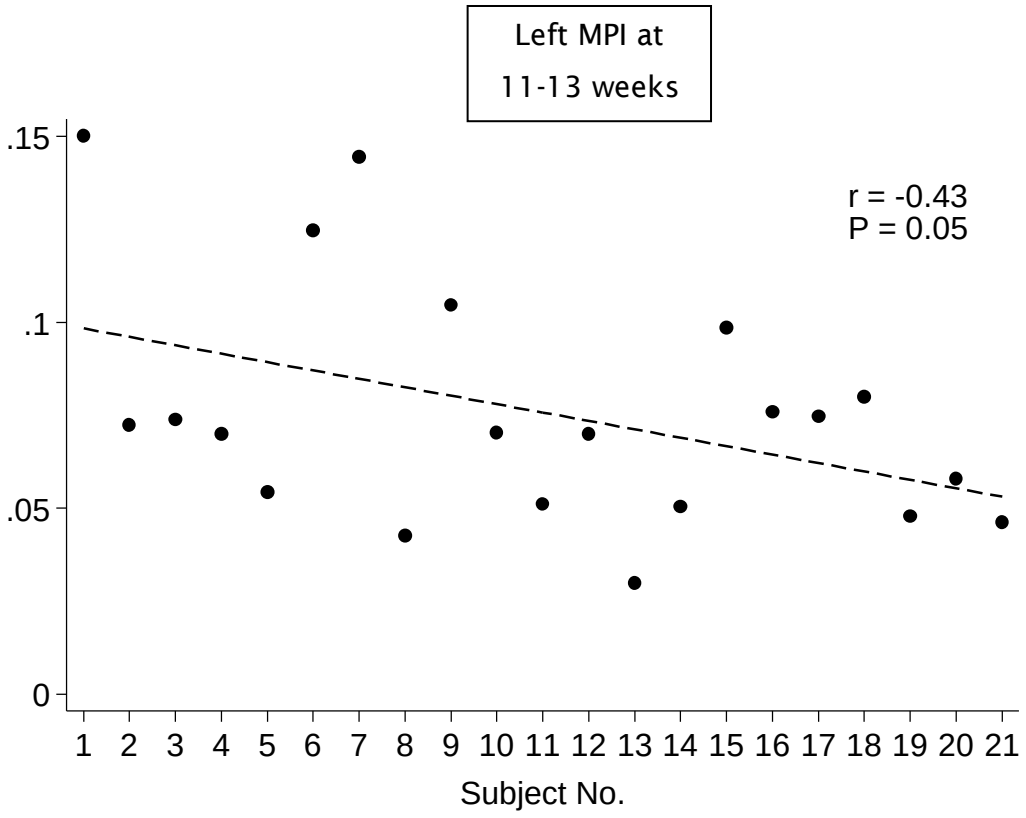

Figure 18- First trimester left myocardial performance index, standard deviation of measurements by subject over time

At 11-13 weeks, the standard deviation in the measurement of the MPI in the left ventricle became narrower over time, reaching statistical significance with a P value of 0.05. This suggests that the variability improved over time as operator experience increased.

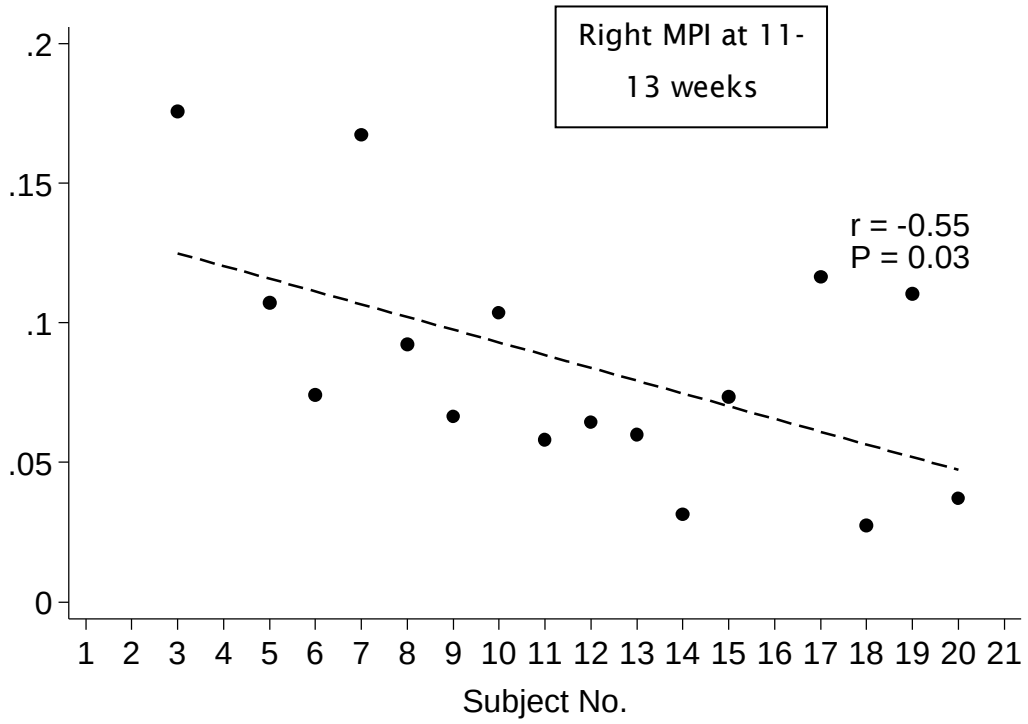

Figure 12- First trimester right myocardial performance index, standard deviation of measurements by subject over time

The findings in the right ventricle demonstrated in the graph above were similar, with a narrowing of the SD over time, again reaching statistical significance.

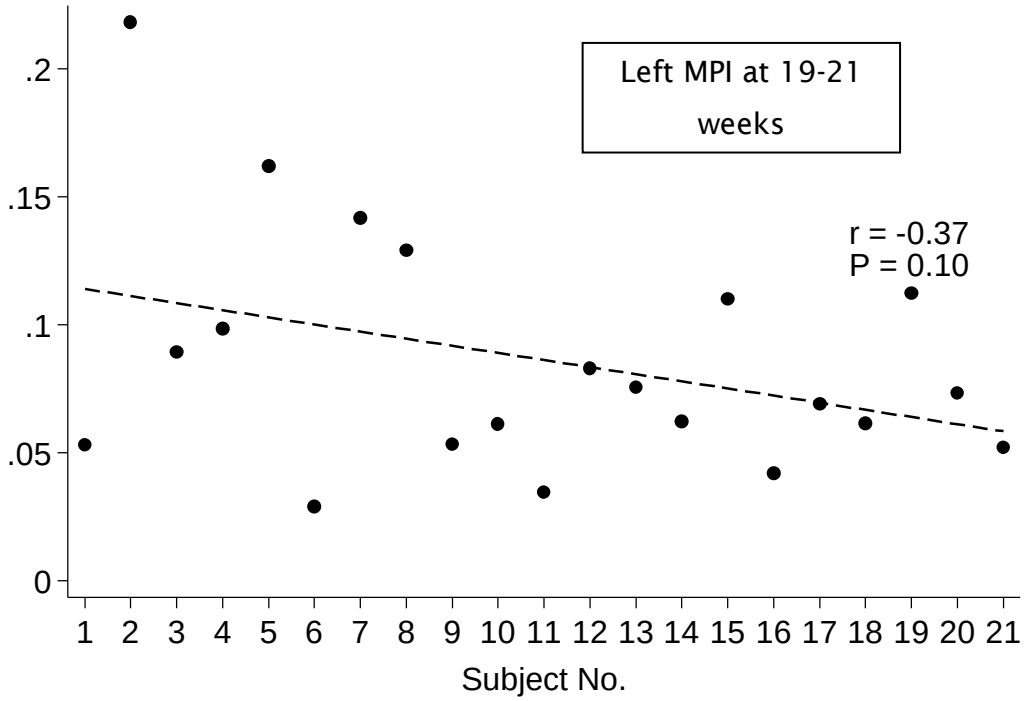

Figure 20- Second trimester left myocardial performance index, standard deviation of measurements by subject over time

At 19-21 weeks there was again evidence of a learning curve effect in the measurements of the left ventricle MPI with a narrowing in the range of the standard deviation, although this did not reach statistical significance.

In the right ventricle at 19-21 weeks, where there was a reduced number of repeat measurements in keeping with the difficulties of obtaining the measurement on this side. There was no evidence of an improvement in measurement technique over time. The standard deviations appeared to slightly worsen over time although this was not statistically significant.

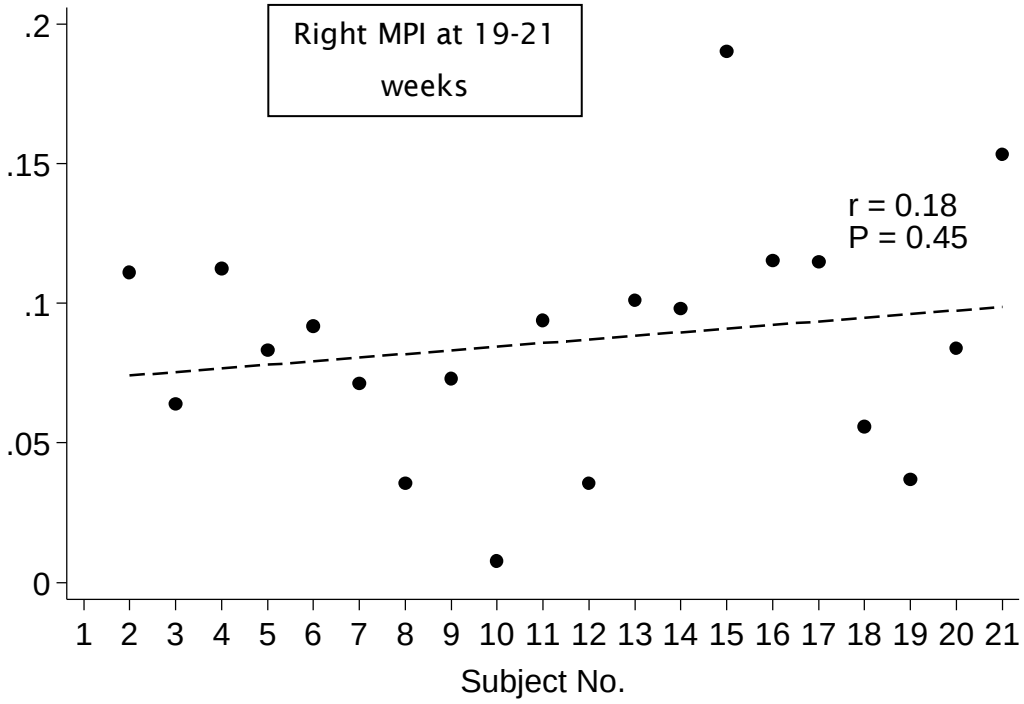

Figure 13- Second trimester right myocardial performance index, standard deviation of measurements by subject over time

Given that there was significant evidence of a learning effect in the first trimester we considered whether it was worth repeating a number of measurements performed earlier in the study. In order to identify up to which time-point it would be logical to do this we compared the slope of the line for the best fit between the standard deviations before and after 5, 7, 10 and 12 patients. If there were a large difference in the slope of the line before and after a particular subject this would identify the point at which the learning curve hit a plateau.

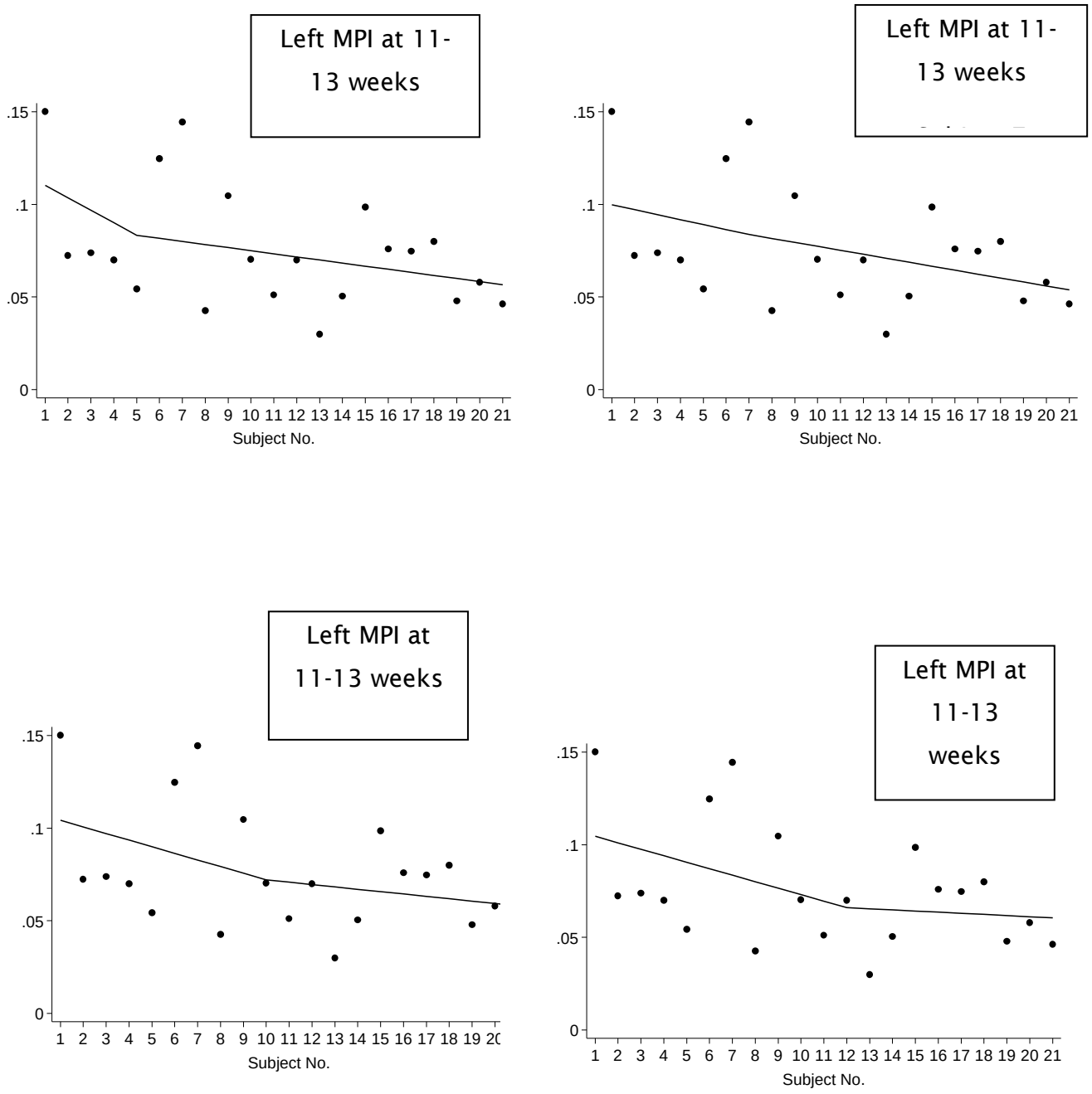

Figure 14- First trimester left myocardial performance index, identification of learning curve plateau

For the left ventricle in the first trimester there was some flattening of the slope over time, but no identifiable point at which repeating the earlier measurements would be likely to have a significant effect.

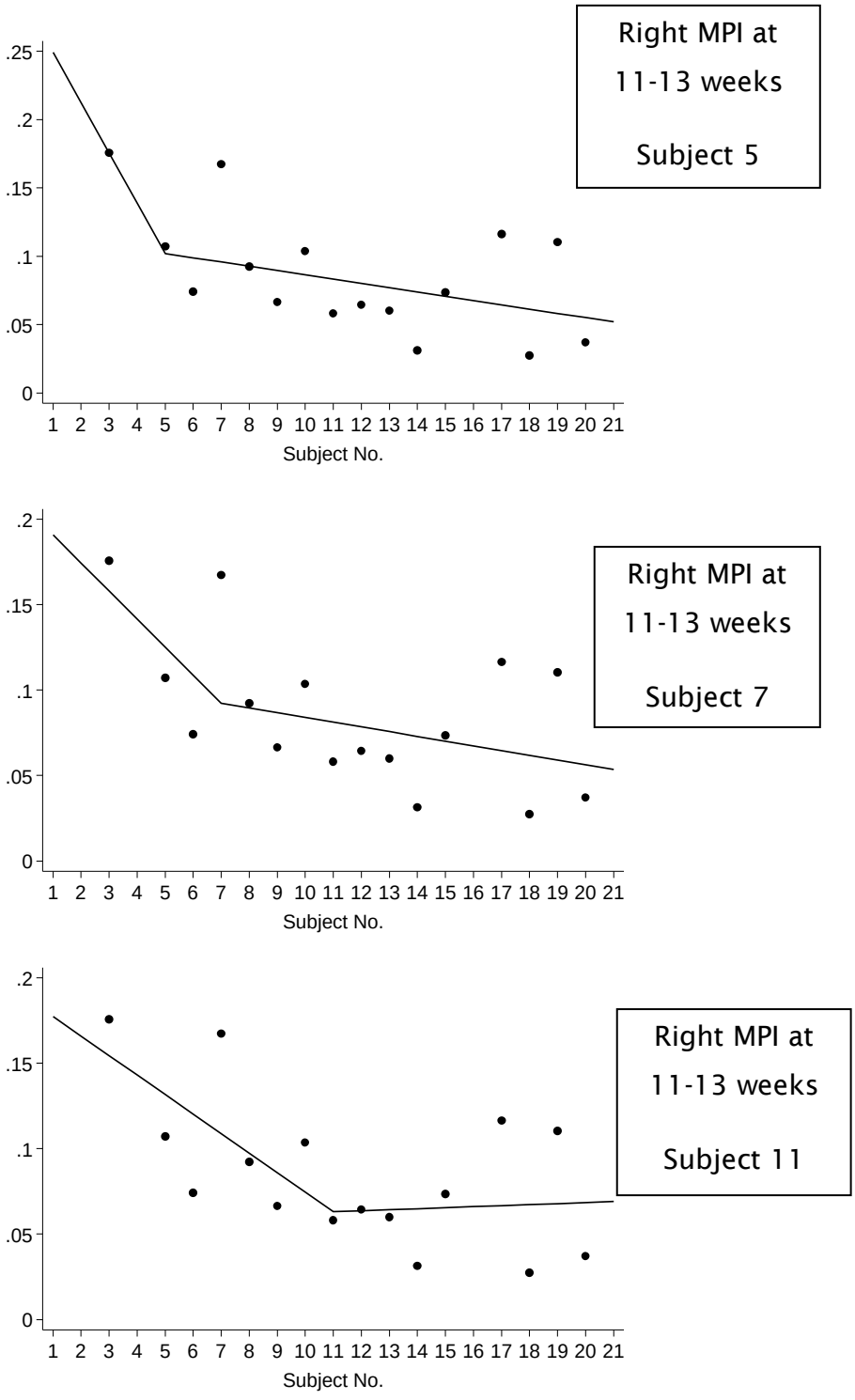

Figure 15- First trimester right myocardial performance index, identification of learning curve plateau

For the right ventricle at 11-13 weeks there were less measurements obtained and so only three separate analyses were performed, before and after 5, 7 and 11 patients. In this example there was a greater difference in the slope of the line between the earlier and later subjects suggesting greater reliability in the measurements performed after subject 5.

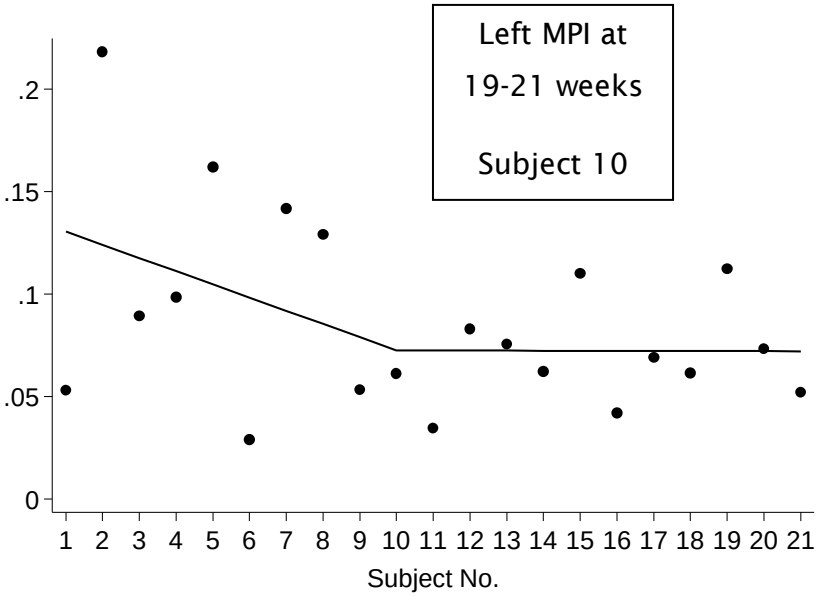

Figure 16- Second trimester left myocardial performance index, identification of learning curve plateau

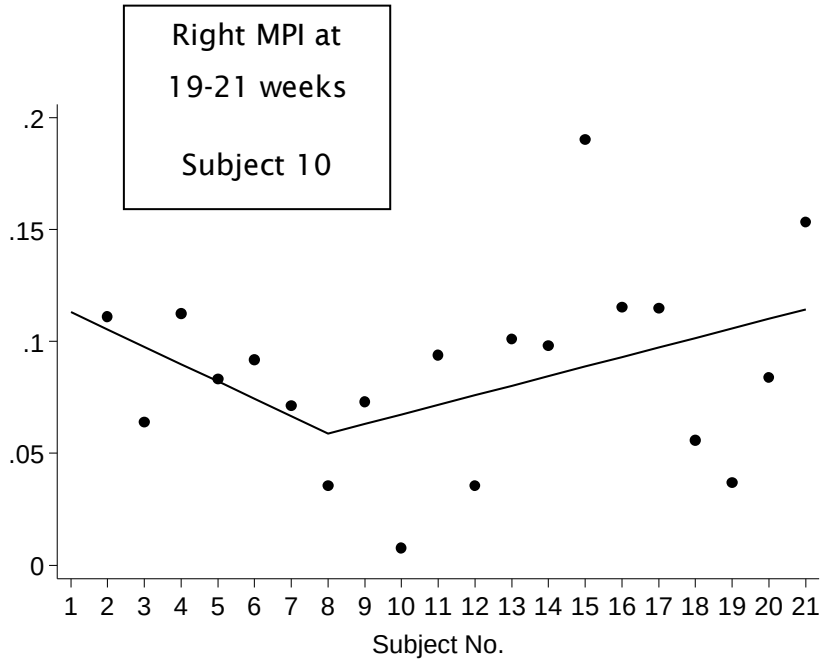

Figure 17- Second trimester right myocardial performance index, identification of learning curve plateau

At 19-21 weeks, where there was no evidence of a statistically significant difference in the measurements performed earlier or later in the study, there was again some suggestion of a plateau demonstrating a narrowing in the SD of measurements performed after subject 10. However for

the right ventricle at 19-21 weeks there appeared to be some initial improvement, but then worsening of the SD range over subsequent subjects which is difficult to explain.

Another potential source of error in the calculation of the MPI is a difference in heart rate when the right MPI is measured at 19-21 weeks. As the inflow and outflow are measured at different time-points the duration of the different components of the calculation may be altered. We therefore adjusted the MPI for heart rate by assuming that the heart rate when 'a' was measured was the correct heart rate and altering the measurement of 'b' where there was a difference in heart rate by the same proportion as the difference in the heart rates in order to recalculate the MPI. In some cases the heart rate at the point in time when 'a' was measured was exactly the same as the point in time when 'b' was measured, but where a difference occurred this could alter the MPI significantly as demonstrated in the table below, with the highlighted results being those where the heart rate was different at the two measurement time points of a and b.

This analysis assumes that an alteration in heart rate alters the duration of the different components of the MPI calculation, a and b, by the same proportion as reported in the study by Hernandez-Andrade (Hernandez-Andrade, Figueroa-Diesel et al. 2007). In our ongoing analysis we adjusted the right ventricular MPI at 19-21 weeks for heart rate.

RV average 'a'	Heart rate at time 'a'	RV average 'b'	Heart rate at time 'b'	RV MPI	RV ave 'b' adjusted for heart rate	RV MPI adjusted for heart rate
251	150	171	150	0.46	171	0.46
242	138	187	138	0.30	187	0.30
253	138	164	138	0.54	164	0.54
220	153	167	153	0.32	167	0.32
242	167	160	167	0.51	160	0.51
267	155	169	155	0.58	169	0.58
260	150	171	150	0.52	171	0.52
262	145	171	153	0.53	162	0.62
262	136	173	150	0.52	157	0.67
264	150	180	141	0.47	191	0.38
265	153	162	141	0.63	176	0.50
247	136	165	132	0.50	170	0.45
258	150	160	148	0.61	162	0.59
251	143	158	150	0.59	150	0.67
251	153	160	148	0.57	165	0.52
258	143	155	155	0.66	143	0.80
251	136	178	138	0.41	175	0.44
267	150	171	150	0.56	171	0.56
240	155	155	153	0.55	157	0.53
260	145	173	153	0.50	164	0.59
260	148	160	153	0.63	155	0.68

RV average 'a'	Heart rate at time 'a'	RV average 'b'	Heart rate at time 'b'	RV MPI	RV ave 'b' adjusted for heart rate	RV MPI adjusted for heart rate
262	148	147	153	0.79	142	0.85
260	155	169	145	0.54	181	0.44
245	148	145	150	0.69	143	0.71
271	143	175	143	0.55	175	0.55
271	145	164	141	0.65	169	0.60
245	150	145	145	0.69	150	0.63
249	150	158	145	0.58	163	0.53
247	153	147	148	0.68	152	0.62
262	143	162	143	0.62	162	0.62
249	141	158	148	0.58	150	0.66
251	141	158	145	0.59	153	0.64
242	145	162	145	0.49	162	0.49
265	147	145	143	0.83	149	0.78
253	150	165	145	0.54	170	0.49

Table 3- Right ventricular myocardial performance index in the second trimester adjusted for heart rate

After adjusting for heart rate the right ventricular MPI was altered in 24 out of the 35 subjects as highlighted in the table. We therefore used this adjusted MPI for the right ventricle in the second trimester in our ongoing analysis.

3.4 Conclusions

We were able to demonstrate that the techniques we developed for measuring placental volume in the first and second trimester and cardiac chamber size in the second trimester were reproducible. For placental volume our results were similar to previous work in the field in terms of the volumes obtained.

With regard to the MPI measurement we had less success in demonstrating the reproducibility of this measurement which is in keeping with previous literature in terms of the wide variation of normal ranges reported for this parameter. In spite of this use of the MPI is still being refined and explored in the context of predicting outcomes for growth restricted fetuses and exploring the mechanism of the influence of the intra-uterine environment on risk of disease in adult life as we will discuss in section 5.1.

4. Chapter 4- Relationships Between Fetal Cardiac Function And Structure, Placental Volume And Fetal And Maternal Anthropometry

In this chapter we will explore the relationship between the measurements of placental volume, cardiac structure and cardiac function. In order to address our hypothesis that fetuses who are adapting to a sub-optimal intrauterine environment may have altered cardiac structure and function we also analysed the relationship of these parameters to standard fetal biometry, maternal body composition and pregnancy outcomes.

Measurements obtained

4.1.1 Myocardial Performance Index

72 out of 80 women had the myocardial performance index measured in the left heart in the first trimester. All women had at least one set of three waveforms measured and averaged to give the MPI value. Fourteen had two sets of three waveforms measured and twelve women had three sets. Where multiple values were obtained they were averaged to give the final value. In the eight women who had no measurement obtained the commonest reason was fetal activity making it difficult to obtain three consecutive waveforms or fetal position particularly where the fetus was steeply cephalic with the spine anterior. The right MPI was obtained in 62 out of the 80 women scanned in the first trimester, with 48 having one set of three waveforms measured, eight having two and six having three. Again the ability to obtain the measurement was affected by fetal activity and position and also influenced by time restrictions more than the left as the tendency was to measure the left first. Increased maternal body mass index also affected the ability to measure the MPI and one woman had multiple fibroids persistently shadowing the fetal heart.

In the second trimester 46 of the 49 women scanned had the left MPI measured, with more success in obtaining multiple sets of measurements. Fifteen women had a single set of three waveforms measured, while 21 had two and ten had three3. The right MPI was also obtained in 46 women with multiple sets of three waveforms obtained in thirteen women.

4.1.2 Placental Volume

The placental volume was obtained in all 112 women at the first trimester scan. In the second trimester five of the 112 women did not have a scan as increased recruitment to the SiPS trial meant women who were not participating in SiPS could not be accommodated as we will explain. Of the 107 who were scanned the placental volume was obtained in 105, 79 by 3D measurements and 26 by 2D measurements.

4.1.3 Cardiac chamber measurements

Cineloops of the fetal heart were obtained in 93 of the 107 women who had a second trimester scan. Fetal position and maternal body habitus were the commonest reasons why the cineloop could not be acquired. Of the cineloops analysed there were two where measurements could not be performed. In one the gain setting was very high, obscuring the details of the heart valves so that it was difficult to confidently identify the point in the cardiac cycle at which to perform the measurement and in another the image was oblique so that the heart valves could not be identified on both sides of the heart.

4.2 Pregnancy outcomes

Pregnancy outcomes were available for all but 3 women who did not deliver in our unit and were lost to follow-up. Of the 109 women for whom outcomes were available 64 were primigravida and 45 were multiparous. The average gestation at delivery was 39.67 weeks (25.71-42.0 weeks) and the average birth weight was 3346g (870-4840g). 19 women underwent induction of labour. For 9 (47%) of these the indication was post-maturity. Other

indications included pre-labour rupture of membranes, intra-uterine growth restriction and maternal complications such as pre-eclampsia.

74 women (68%) had a normal vaginal delivery, 17 women (16%) had an operative vaginal delivery, 3 women (2.75%) had an elective Caesarean section and 15 women (13.76%) had an emergency Caesarean section. There were no perinatal deaths. 3 babies were admitted to the neonatal unit immediately after delivery, including one baby born after spontaneous pre-term labour at 25 weeks and 5 days gestation, weighing 870g and one baby who was born weighing 1900g at 37 weeks and 4 days. Thirteen women were reported as having ante-natal complications, including three women who went into spontaneous pre-term labour, Five women who developed pre-eclampsia, one of whom had a growth restricted fetus, one woman with a growth restricted fetus with no evidence of pre-eclampsia and one woman who had a confirmed varicella infection at 12 weeks gestation and had a growth restricted fetus at term weighing 2372g, but with no other stigmata of infection. There was also one woman who developed gestational diabetes and one woman who had a significant ante-partum haemorrhage requiring induction of labour at term.

We explored the relationships between fetal cardiac function and structure, placental volume and growth rate and maternal characteristics and birth weight and placental weight. Placental weights were measured for the women participating in the SiPS in a laboratory setting (n=28) and for the remainder on the Labour ward. However this data was not available for all women as placental weight is no longer routinely done after birth.

4.3 Analysis of measurements obtained

In order to explore our hypothesis we began by establishing the gestation at the time of the scans performed and establishing if our measurements of placental volume, cardiac function and cardiac structure were associated with gestation by performing a linear regression analysis and adjusting our results accordingly.

Chapter 4- Relationships Between Fetal Cardiac Function And Structure, Placental Volume And Fetal And Maternal Anthropometry

We then went on to examine the relationships between the myocardial performance indexes on either side of the heart at each time point. We also established if the measurements of placental volume and myocardial performance index in the first trimester could predict the measurement in the second trimester using a regression analysis and then looked at the relationships of the cardiac chamber measurements to each other.

If our hypothesis is correct we would hope to see a relationship between the placental volume and the cardiac function and structure and also a relationship between the function and the structure and we examined these relationships by a regression analysis to see if placental volume was related to function and/or structure and if function was related to structure. We also devised a ratio comparing the size of the left side of the heart to the right side to see if a difference in this ratio was associated with either placental volume or cardiac structure.

We were also interested in the relationship between placental growth and cardiac parameters to determine if the growth velocity of the placenta had an influence on cardiac function or structure.

We went on to explore the relationship between placental volume measurements, cardiac function and structure and fetal growth, performing a regression analysis to see if any of our parameters were related to fetal biometry.

Maternal anthropometry was also used as a predictor in a regression analysis to see if there was any influence over the outcomes of placental volume, growth rate, cardiac structure and function and fetal growth.

We then looked at pregnancy outcomes to see if any of our measurements of cardiac structure or function were related to birth weight or placental weight.

We then separated our measurement techniques for measuring placental volume in the second trimester and reanalysed our data using the 3D measurements alone.

Finally we analysed the results of a study within our department examining the relationship between fetal liver blood flow and ductus venosus shunting and maternal characteristics including maternal stress.

The statistical analysis was performed using STATA, version 10-12 with the assistance of S.C., a statistician within our department. We deemed a *p* value of less than 0.05 to be of statistical significance.

4.3.1 Gestation at time of scan

Gestation	n	P50	P25	P75	min	max
1st trimester	112	12.7	12.3	13.1	11	16.4
2nd trimester	106	19.9	19.6	20.1	18.4	21

Table 4- Summary statistics- gestation at time of scan

One woman was found to be further advanced in her pregnancy than expected at the time of her fist scan, attending at 16 weeks and 3 days by fetal head circumference. She was scanned by JC at that scan and the myocardial performance index measured. We decided not to exclude her as all other parameters- i.e. placental volumes, cardiac chamber sizes and fetal biometry were adjusted for gestation.

4.3.2 Summary statistics- Placental Volume, Myocardial Performance Index and Cardiac Structure

	N	mean	SD	min	max
Placental Volume 1st Trim (Pvol1 in cm^3)	112	58.6	14.3	26.5	99.9
Placental Volume 2nd Trim (Pvol2 in cm^3)	104	190.8	41.3	114.7	324.4
Left MPI 1st Trim (MPI1l)	74	0.56	0.08	0.36	0.76
Right MPI 1st Trim (MPI1r)	62	0.56	0.09	0.41	0.78
Left MPI 2nd Trim (MPI2l)	47	0.59	0.08	0.46	0.79
Right MPI 2nd Trim (MPI2r)	45	0.53	0.14	0.15	0.80
Total Cardiac Circ (TCC in mm)	93	72	4.1	63.4	81.7
Total Vent Circ (TVC in mm)	93	59.9	3.5	52.7	70.2
Internal Right Vent Circ (IRVC in mm)	93	29.4	2.6	24.9	37
Internal Left Vent Circ (ILVC in mm)	93	33.6	3.4	27	42.7

Table 5- Summary statistics for placental volume, myocardial performance index and cardiac chambers

4.3.3 Associations with gestation

4.3.3.1 First trimester (10^{+0}-13^{+6})

	t	p	95% confidence interval
Placental volume (PVOL1)	8.1	0.000*	11.09 to18.28
Left MPI (MPI1l)	-0.31	0.76	-0.27to 0.02
Right MPI(MPI1r)	1.38	0.17	-0.01to 0.05

Table 6- Associations with gestation in the first trimester

In the first trimester the there was a strong association between gestation and placental volume but none with MPI in either the right or left heart.

4.3.3.2 Second Trimester (18^{+0}-20^{+6})

	t	p	95% confidence interval
Placental volume (PVOL2)	1.46	0.15	-6.1to 39.85
Left MPI (MPI2l)	-0.24	0.81	-0.77to 0.06
Right MPI (MPI2r)	0.84	0.41	-0.72to 0.17
Total Cardiac Circumference (TCC)	4.23	0.000*	2.71to 7.49
Total Ventricular Circumference (TVC)	4.49	0.000*	2.56 to 6.64
Internal Right Ventricular Circumference (IRVC)	4.76	0.000*	2.1to 5.11
Internal Left Ventricular Circumference (ILVC)	3.93	0.000*	1.97to 5.99

Table 7- Association with gestation in the second trimester

In the second trimester there was no significant association between placental volume, or MPI and gestation. All the cardiac circumferences were strongly associated with gestation and, in view of this, the cardiac chamber measurements were adjusted for gestation and further analysis carried out with the adjusted figures.

It was surprising that the placental volume was not associated with gestation in the second trimester, but this may be explained by the different measuring techniques used as we will go on to explore. As the first trimester

placental volume was strongly associated with gestation we decided to adjust the placental volume in the second trimester for gestation as well.

4.3.4 Relationship between fetal cardiac function on the left and right side of the heart

	n	*t* value	*p* value	95% confidence interval
MPI1R v MPI1L	58	3.19	0.002*	0.14 to 0.59
MPI2R v MPI2L	42	1.74	0.08	0.05 to 0.45

Table 8- Association between the left and right MPI in the first and second trimester

In the first trimester there was a strong relationship between the myocardial performance index on the left side and on the right side of the heart, but this was not true in the second trimester once the MPI on the right had been adjusted for heart rate as previously described.

4.3.5 First trimester v. Second trimester

	n	*t* value	*p* value	95% confidence
PVOL1 v PVOL2	104	1.8	0.08	-0.01 to 0.13
MPI1L v MPI2L	13	-0.48	0.64	-0.73 to 0.47
MPI1R v MPI2R	14	1.37	0.196	-0.22 to 0.77

Table 9- Relationship between placental volume in the first and second trimester and MPI in the first and second trimester

We examined the relationship between the scan parameters that were measured in both the first and second trimester using a regression analysis to establish if the first trimester measurement predicted the second trimester value. For the small number of women who had a myocardial performance measurement performed in both the first and second trimester there was no apparent relationship between the measurement which was performed in the first trimester and the measurement performed in the second trimester on either side of the heart. This analysis is very limited due to the small numbers involved.

The placental volume measured in the first trimester was not strongly related to the placental volume in the second trimester, which was surprising. In the first trimester all the placental volumes were measured on stored 3D volumes by a single operator (JC) using the technique described above. In the second trimester there were two different techniques used. For the posterior placentas, where it was feasible to get the whole placenta within the volume box, the placental volume was measured in slices of the 3D volume, again all by a single operator. For some of the anterior placentas in the upper part of the scan range it was not always possible to fit the whole placenta within the volume box to obtain a 3D measurement and so these placentas were

measured at the time of the scan in 2D using a technique developed during a previous study within the department, which will be described later.

4.3.6 Relationships between cardiac circumferences

	n	*t* value	*p* value	95% confidence interval
TCC v TVC	93	18.68	0.000*	0.94 to 1.16
TCC v IRVC	93	7.97	0.000*	0.77 to 1.27
TCC v ILVC	93	7.29	0.000*	0.53 to 0.92
TVC v IRVC	93	6.54	0.000*	0.53 to 0.99
TVC V ILVC	93	7.55	0.000*	0.46 to 0.79
IRVC V ILVC	93	7.94	0.000*	0.36 to 0.6

Table 10- Associations between cardiac cirumferences

All the cardiac circumferences measured as previously described were strongly related to each other. These measurements also had a high reproducibility as previously described.

4.3.7 Relationships between MPI and placental volume

	n	*t* value	*p* value	95% confidence interval
MPI 1L v PVOL1	74	0.73	0.47	-0.001to 0.002
MPI1R v PVOL1	62	0.2	0.84	-0.001to 0.002
MPI2L v PVOL2	47	0.3	0.76	-0.0004 to 0.001
MPI2R v PVOL2	46	0.33	0.74	-0.001to 0.001

Table 11- Association between placental volume and cardiac function

We used a regression analysis with the placental volume as predictor and cardiac function as the outcome. In our women there did not appear to be any relationship between the size of the placenta and the cardiac function of the fetus as measured by the myocardial performance on either side of the heart in either the first or second trimester.

4.3.8 Relationships between cardiac circumferences and placental volume

	n	*t* value	*p* value	95% confidence interval
TCC v PVOL2	92	-0.84	0.4	-0.03-0.01
TVC v PVOL2	92	-0.48	0.63	-0.02-0.01
IRVC v PVOL2	92	0.06	0.96	-0.12-0.01
ILVC v PVOL2	92	-0.41	0.68	-0.02-0.01

Table 12- Association between placental volume in the second trimester and cardiac circumferences

Using the same analysis, with the placental volume in the second trimester as the predictor, there was also no discernible relationship between the cardiac chamber measurements and the placental volume.

4.3.9 Relationships between cardiac circumferences and MPI

	n	*t* value	*p* value	95% confidence interval
TCC v MPI2L	44	0.31	0.76	-15.05 to 20.62
TVC v MPI2L	44	0.67	0.5	-9.97 to 19.96
IRVC v MPI2L	44	0.11	0.91	-10.22 to 11.39
ILVC v MPI2L	44	-0.67	0.5	-18.93 to 9.47
TCC v MPI2R	41	-0.1	0.9	-12.77 to 12.98
TVC v MPI2R	41	0.14	0.886	-9.35 to 11.36
IRVC v MPI2R	41	-0.86	0.396	-10.33 to 4.18
ILVC v MPI2R	41	-1.06	0.295	-14.84 to 3.98

Table 13- Relationship between cardiac function and structure

There was no relationship identified between the cardiac chamber size and cardiac function in the second trimester. This was disappointing as we had hypothesised that altered flow through the heart would affect structural formation, with different effects evident on the left and right. It may have been more logical to investigate whether cardiac chamber sizes were altered with variations in uterine or umbilical blood flow. This data was not available in our cohort but this may certainly be worth investigating in a future study.

4.3.10 Ratio of left to right internal ventricular circumferences

The ratio of the internal left ventricular circumference to the internal right ventricular circumference (ILRRATIO) was calculated as it was noted during the measuring process that while usually the left ventricle was bigger than the right, in some fetal hearts the two ventricles had very similar circumferences. All of these hearts were judged anatomically normal and so the question was raised as to whether there was a measurable difference in function in hearts at either end of this spectrum. If function was different then flow may be altered, leading to a different architecture of the heart and possibly altered disease susceptibility. The ratio of the internal circumferences of the left and right ventricles was therefore calculated and the relationship between this ratio, cardiac function and placental volume in both the first and second trimesters was explored using a correlation analysis.

	n	*r* value	*p value*
ILRRATIO v PVOL1	93	-0.08	0.46
ILRRATIO v PVOL2	92	-0.06	0.56
ILRRATIO v MPI1L	57	-0.02	0.88
ILRRATIO v MPI1R	50	0.04	0.79
ILRRATIO v MPI2L	44	-0.19	0.22
ILRRATIO v MPI2R	43	-0.09	0.57

Table 14- Associations between the ratio of ventricular circumferences, placental volume and cardiac function in the first and second trimesters

In our small cohort there was no statistical relationship between the ratio of the internal left and right ventricular circumferences and either the placental volume or the cardiac function.

4.3.11 Relationship between rate of placental growth and cardiac structure and function

The rate of placental growth between the first and second trimesters was calculated by creating a Z-score (Zpvoldiff) to see if there was a difference in fetal cardiac structure or function in pregnancies where the rate of placental growth differed. This was done by calculating the correlation co-efficient (*r*).

	n	*r* value	*p* value
Zpvoldiff v MPI1L	66	0.2	0.1
Zpvoldiff v MPI1R	58	-0.002	0.98
Zpvoldiff v MPI2L	47	0.03	0.83
Zpvoldiff v MPI2R	46	0.04	0.79
Zpvoldiff v TCC	92	-0.1	0.36
Zpvoldiff v TVC	92	-0.05	0.64
Zpvoldiff v IVRC	92	0.01	0.91
Zpvoldiff v ILVC	92	-0.02	0.86
Zpvoldiff v ILRRATIO	92	-0.04	0.74

Table 15- Relationship between placental growth rate and cardiac function in the first and second trimester and cardiac structure

The rate of growth of the placenta did not correlate with either the size of the cardiac chambers or the cardiac function as measured by the myocardial performance index in the first or second trimester.

4.4 Fetal biometry on ultrasound related to cardiac structure and function

The summary statistics of the fetal biometry measurements obtained as previously described are shown in the table below.

	N	MEAN	SD	MIN	MAX
crl1ag	108	4.28	0.21	3.42	5.05
nt1ag	31	0.11	0.04	0.04	0.23
bpd2ag	103	4.28	0.21	3.82	4.93
hc2ag	103	15.87	0.58	14.64	17.42
ac2ag	103	13.82	0.62	12.15	15.06
fl2ag	103	2.92	0.14	2.58	3.41
bpd3ag	37	8.73	0.40	7.54	9.67
hc3ag	37	32.0	0.98	30.55	35.50
ac3ag	37	30.47	1.43	27.39	33.54
fl3ag	37	6.48	0.25	5.65	6.97
tc3ag	37	24.76	1.09	22.09	27.44

Table 16- Summary statistics of fetal biometry in the first, second and third trimesters

crl1ag- crown rump length 1st trimester adjusted for gestation

nt1ag- nuchal translucency 1st trimester adjusted for gestation

bpd2ag- biparietal diameter 2nd trimester adjusted for gestation

hc2ag- head circumference 2nd trimester adjusted for gestation

ac2ag- abdominal circumference 2nd trimester adjusted for gestation

Chapter 4- Relationships Between Fetal Cardiac Function And Structure, Placental Volume And Fetal And Maternal Anthropometry

fl2ag- femur length 2nd trimester adjusted for gestation

bpd3ag- biparietal diameter 3rd trimester adjusted for gestation

hc3ag- head circumference 3rd trimester adjusted for gestation

ac3ag- abdominal circumference 3rd trimester adjusted for gestation

fl3ag- femur length 3rd trimester adjusted for gestation

tc3ag- thoracic circumference 3rd trimester adjusted for gestation

All measurements are in centimetres. All the fetal biometry was normally distributed and strongly associated with gestation as expected and we therefore adjusted for gestation using a linear regression analysis.

4.4.1 Relationship between placental volume and fetal biometry

	Pvol1ag	Pvol2ag	zpvoldiff
crl1ag	n=108 t=1.22 p= 0.23	n=101 t=-0.78 p=0.437	n=101 t=-1.06 p=0.293
nt1ag	n=31 t=0.29 p=0.78	n=28 t=0.28 p=0.783	n=28 t=0.25 p=0.806
bpd2ag	n=103 t=0.35 p=0.73	n=101 t=1.62 p=0.109	n=101 t=1.40 p=0.165
hc2ag	n=103 t=-0.24 p=0.81	n=101 t=0.61 p=0.544	n=101 t=0.62 p=0.538
ac2ag	n=103 t=1.89 p=0.06	n=101 t=1.43 p=0.157	n=101 t=0.83 p=0.410
fl2ag	n=103 t=-0.36 p=0.719	n=101 t=0.75 p=0.455	n=101 t=0.89 p=0.375
bpd3ag	n=37 t=0.97 p=0.340	n=36 t=0.30 p=0.767	n=36 t=-0.02 p=0.988
hc3ag	n=37 t=2.02 p=0.051	n=36 t=1.10 p=0.281	n=36 t=0.42 p=0.679
ac3ag	n=37 t=1.13 p=0.266	n=36 t=2.43 p=0.02*	n=36 t=1.82 p=0.078
fl3ag	n=37 t=1.24 p=0.222	n=36 t=4.16 p=0.000*	n=36 t=3.15 p=0.003*

Table 17- Association between placental volume and fetal biometry

We performed a regression analysis using the placental volume as the predictor and the fetal size as the outcome. Surprisingly there was no significant relationship between the placental volume adjusted for gestation in the first or second trimester or the rate of placental growth between the first and second trimester and the size of the fetus. It is only in the third trimester, where there were a much smaller number of women scanned, that there appears to be any relationship between the size of the placenta earlier on in the pregnancy and the size of the fetus.

It is possible that some of these unexpected results can be explained by the different techniques used to measure placental volume in the second trimester and this will be explored later.

4.4.2 Ratio of fetal to placental size in first trimester related to cardiac function and structure

As altered placental size relative to the birth weight has been associated with increased risk of disease in adult life part of our hypothesis was that in fetuses destined to show recognised signs of adaptation to a suboptimal intrauterine environment there will be evidence early in the pregnancy of alterations in placental volume to fetal size, which may be associated with changes in cardiac function and size. In order to investigate this we developed a simple ratio of fetal size to placental volume in the first trimester by dividing the crown- rump length (crl1ag) by the placental volume (pvol1ag), both parameters having been adjusted for gestation. We used a regression analysis to explore if a difference in this ratio could predict alterations in cardiac function and structure.

	n	t	p	95% Confidence intervals
Fetal:placental ratio v MPI1R	58	-0.26	0.79	-1.33 to 1.02
Fetal:placental ratio v MPI1L	70	-0.46	0.65	-1.26 to 0.79
Fetal:placental ratio v MPI2R	39	-0.26	0.8	-3.08 to 2.38
Fetal:placental ratio v MPI2L	43	-0.57	0.57	-1.75 to 0.97
Fetal:placental ratio v TCC	88	-0.21	0.84	-50.37 to 40.84
Fetal:placental ratio v TVC	88	0.28	0.78	-34.41 to 45.8
Fetal:placental ratio v IRVC	88	0.04	0.97	-29.39 to 30.68
Fetal:placental ratio v ILVC	88	0.54	0.59	-28.2 to 49.2
Fetal:placental ratio v PVol2ag	73	-2.67	0.01*	-919.49 to -133.83
Fetal:placental ratio v zpvoldiff	73	0.63	0.53	-7.38 to 14.24

Table 18- Relationship between fetal:placental ratio and cardiac function, structure and placental volume

Differences in relative fetal to placental size in the first trimester were not associated with any differences in either cardiac function or structure in either the first or second trimester. There was a significant relationship with the placental volume in the second trimester, whereby the bigger the fetus relative to the placenta in the first trimester, the smaller the placenta in the second trimester. However there was no association with placental growth rate, suggesting that placentas that are already smaller in the first trimester remain small but do no display a difference in their growth pattern between the first and second trimester.

4.4.3 Relationship between fetal biometry and cardiac function

	Mpi1l	Mpi1r	Mpi2l	Mpi2r
crl1ag	n=72 t=-0.10 p=0.923	n=60 t=0.85 p=0.398	n=45 t=0.12 p=0.909	n=41 t=0.02 p=0.985
nt1ag	n=22 t=-0.07 p=0.944	n=19 t=0.88 p=0.391	n=10 t=0.04 p=0.969	n=10 t=-0.67 p=0.524
bpd2ag	n=67 t=-1.26 p=0.214	n=57 t=-0.73 p=0.470	n= 44 t=2.56 p=0.01*	n=41 t=0.09 p=0.93
hc2ag	n=67 t=-0.69 p=0.491	n=57 t=0.53 p=0.599	n=44 t=1.91 p=0.062	n=41 t=0.23 p=0.822
ac2ag	n=67 t=1.15 p=0.253	n=57 t=1.06 p=0.293	n=44 t=2.71 p=0.01*	n=41 t=0.47 p=0.64
fl2ag	n=67 t=-1.58 p=0.119	n=57 t=-0.13 p=0.893	n=44 t=0.80 p=0.426	n=41 t=1.12 p=0.269
bpd3ag	n=22 t= 2.07 p=0.05*	n=18 t=-0.17 p=0.865	n=17 t=0.21 p=0.834	n=16 t=-0.4 p=0.698
hc3ag	n=22 t=2.34 p=0.03*	n=18 t= -0.24 p=0.813	n=17 t=-0.44 p=0.664	n=16 t=0.18 p=0.856
ac3ag	n=22 t=1.40 p=0.176	n=18 t=3.3 p=0.01*	n=17 t=0.24 p=0.814	n=16 t=0.28 p=0.781
fl3ag	n=22 t=1.57 p=0.133	n=18 t=2.06 p=0.056	n=17 t=-1.87 p=0.081	n=16 t=1.5 p=0.155
tc3ag	n= 22 t=2.27 p=0.04*	n=18 t=1.46 p=0.164	n=17 t=-0.06 p=0.954	n=16 t=1.56 p=0.142

Table 19- Association between fetal biometry and cardiac function in the first and second trimester

Whilst there did not appear to be a relationship between the myocardial performance index and the size of the fetus in the first trimester, in the second trimester the left MPI was positively associated with the biparietal diameter and abdominal circumference and the association between left MPI and head circumference was borderline statistically significant. It is difficult to draw conclusions in view of the number of observations.

In the third trimester, where the number of women scanned was even smaller, there was a positive association between the left MPI measured in the first trimester and the head and thoracic circumference, whilst the right MPI in the first trimester was positively associated with the abdominal circumference in the third trimester. This is a very interesting observation as it is in keeping with the distribution of the fetal circulation from each side of the heart and raises the possibility of fetal cardiac function in the first trimester having some predictive value regarding fetal growth, although again any conclusions are potentially confounded by the small number of women scanned in the third trimester.

4.4.4 Relationship between fetal biometry and cardiac chamber size

	TCC	TVC	IRVC	ILVC	ILRRATIO
crl1ag	n=90 t=1.15 p=0.252	n=90 t=1.41 p=0.161	n=90 t=-0.66 p=0.509	n=90 t= 0.54 p=0.592	n=90 t=1.29 p=0.200
nt1ag	n=25 t=0.31 p=0.756	n=25 t=1.08 p=0.292	n=25 t=1.26 p=0.222	n=25 t=0.36 p=0.723	n=25 t=-0.60 p=0.552
bpd2ag	n=90 t=1.43 p=0.156	n=90 t=1.74 p=0.085	n=90 t=2.36 p=0.021*	n=90 t=1.18 p=0.241	n=90 t=-1.04 p=0.300
hc2ag	n=90 t=2.9 p=0.01*	n=90 t=3.88 p=0.000*	n=90 t=2.9 p=0.01*	n=90 t=1.99 p=0.05*	n=90 t=-0.60 p=0.551
ac2ag	n=90 t=2.99 p=0.004*	n=90 t=3.34 p=0.001*	n=90 t=1.45 p=0.149	n=90 t=1.43 p=0.156	n=90 t=0.14 p=0.887
fl2ag	n=90 t=1.69 p=0.095	n=90 t=2.08 p=0.04*	n=90 t=-0.22 p=0.825	n=90 t=1.21 p=0.229	n=90 t=1.78 p=0.079
bpd3ag	n=32 t=1.63 p=0.113	n=32 t=1.71 p=0.097	n=32 t=0.90 p=0.374	n=32 t=0.99 p=0.330	n=32 t=0.11 p=0.912
hc3ag	n=32 t=0.71 p=0.486	n=32 t=1.11 p=0.277	n=32 t=0.27 p=0.793	n=32 t=0.54 p=0.595	n=32 t=0.41 p=0.684
ac3ag	n=32 t=1.08 p=0.289	n=32 t=1.41 p=0.169	n=32 t=-0.39 p=0.701	n=32 t=0.02 p=0.987	n=32 t=0.59 p=0.557
fl3ag	n=32 t=-0.63 p=0.535	n=32 t=-0.97 p=0.340	n=32 t=-1.37 p=0.180	n=32 t=-0.36 p=0.720	n=32 t=1.37 p=0.180
tc3ag	n=32 t=1.71 p=0.097	n=32 t=1.94 p=0.061	n=32 t=1.02 p=0.318	n=32 t=1.54 p=0.133	n=32 t=0.69 p=0.495

Table 20- Associations between fetal size and cardiac chamber size

All of the cardiac chamber sizes measured in the second trimester were positively associated with fetal biometry, particularly the head circumference. The associations between the total cardiac circumference and total ventricular circumference were stronger than those for the internal ventricular circumferences on either the right or left. These associations would be anticipated, i.e. the bigger the fetus, the bigger its overall heart size, but we

were unable to establish a difference between the two sides of the heart in terms of the ratio of the left to the right ventricle related to fetal size.

There is no apparent association between the size of the fetal heart in the second trimester and the size of the fetus in the third trimester. Again it is difficult to draw conclusions due to the small number of scans performed in the third trimester.

4.5 Maternal characteristics

Maternal characteristics were recorded in both the first and second trimester. These included maternal age, height, weight, body mass index and the percentage of body fat as measured by the Tanita MC180 body composition analyser. The summary statistics are represented below.

1st trimester	n	mean	Std dev	min	max
Age	110	27.8	5.2	18	38
Height	110	164.9	6.4	150.2	182.7
Weight	110	67.1	15.3	43.2	135.2
BMI	110	24.6	5.7	16.83	55.8
% body fat	110	31.2	6.6	16.4	49.3

Table 20- Maternal characteristics in the first trimester

2nd trimester	n	mean	Std dev	min	max
Age	104	27.8	5.1	18	38
Height	104	164.9	6.4	150.2	182.7
Weight	104	69.5	14.1	46.6	117.9
BMI	104	25.5	4.9	18.7	43.3
% body fat	104	32.6	6.0	21.6	50.3

Table 21- Maternal characteristics in the second trimester

Maternal weight and body mass index were not normally distributed in our cohort, but skewed due to a small number of women with a high weight at

the start of the pregnancy. In view of this both the weight and BMI were log transformed and standardised to give a Z-score (zlmwt and zlmbmi) which was used for ongoing analysis, so that the reported relationship is between unit change in standard deviation rather than unit change in weight.

4.5.1 Relationship between fetal anthropometry and maternal characteristics

	Maternal age	Maternal height	Maternal percentage body fat	Maternal weight	Maternal BMI
crl1ag	n=107 t=-3.04 p=0.003*	n=107 t=-0.63 p=0.528	n=107 t=-1.75 p=0.083	n=107 t= -1.65 p=0.101	n=107 t=-1.46 p=0.148
nt1ag	n=31 t=1.16 p=0.256	n=31 t=0.89 p=0.382	n=31 t=0.49 p=0.625	n=31 t=0.47 p=0.642	n=31 t=0.20 p=0.845
bpd2ag	n=102 t=-0.65 p=0.519	n=102 t=0.77 p=0.443	n=102 t=0.14 p=0.889	n=102 t=0.14 p=0.890	n=102 t=-0.16 0.875
hc2ag	n=102 t=-1.30 p=0.197	n=102 t=0.34 p=0.734	n=102 t=-0.12 p=0.906	n=102 t=0.38 p=0.707	n=102 t= 0.26 p=0.799
ac2ag	n=102 t=1.14 p=0.259	n=102 t=1.27 p=0.208	n=102 t=1.99 p=0.05*	n=102 t=2.71 p=0.01*	n=102 t=2.28 p=0.03*
fl2ag	n=102 t=-0.04 p=0.968	n=102 t=0.75 p=0.456	n=102 t=1.43 p=0.156	n=102 t=1.80 p=0.074	n=102 t=1.58 p=0.118
bpd3ag	n=37 t=0.67 p=0.509	n=37 t=0.95 p=0.350	n=37 t=1.40 p=0.170	n=37 t=2.08 p=0.05*	n=37 t=1.88 p=0.069
hc3ag	n=37 t=1.21 p=0.233	n=37 t=1.25 p=0.219	n=37 t=1.25 p=0.218	n=37 t=2.33 p=0.03*	n=37 t=1.98 p=0.055
ac3ag	n=37 t=0.85 p=0.399	n=37 t=1.16 p=0.253	n=37 t=0.87 p=0.390	n=37 t=1.97 p=0.057	n=37 t=1.64 p=0.109
fl3ag	n=37 t=0.37 p=0.717	n=37 t=-0.20 p=0.84	n=37 t=-0.36 p=0.718	n=37 t=0.49 p=0.629	n=37 t=0.65 p=0.523
tc3ag	n=37 t=-0.21 p=0.832	n=37 t=-0.62 p=0.542	n=37 t=0.99 p=0.331	n=37 t=1.27 p=0.213	n=37 t=1.75 p=0.089

Table 22- Associations between maternal anthropometry and fetal size

In the second trimester there were positive associations between maternal percentage body fat, weight and body mass index, as measured in the first

trimester, and the abdominal circumference of the fetus as we would expect. There was no association between maternal characteristics and fetal size in the first trimester.

In the third trimester there was a positive association between maternal weight and fetal head size, which was not noted in the second trimester, and other associations which came close to statistical significance in terms of relating fetal size to maternal body fat, weight and body mass index.

4.5.2 Relationship between placental volume and maternal characteristics

First trimester	n	t value	p value	95% confidence interval
Pvol1 v mage1	110	0.21	0.8	-0.46 to 0.57
Pvol1 v mht1	110	-1.07	0.29	-0.65 to 0.19
Pvol1 v mpfat1	110	-0.02	0.98	-0.42 to 0.41
Pvol1 v zlmwt1	110	0.26	0.79	-2.3 to 3.1
Pvol1 v zlmbmi1	110	0.68	0.5	-1.8 to 3.6
Second trimester				
Pvol2 v mage2	102	1.5	0.14	-0.38 to 2.8
Pvol2 v mht2	102	0.71	0.48	-0.82 to 1.74
Pvol2 v mpfat2	102	-0.51	0.61	-1.72 to 1.01
Pvol2 v zlmwt2	102	-0.04	0.97	-8.4 to 8.04
Pvol2 v zlmbmi2	102	-0.34	0.74	-9.6 to 3.6

Table 23- Association between maternal characteristics and placental voluume in the first and second trimesters

We performed a regression analysis with placental volume as the outcome and did not detect any relationship between the placental volume and the maternal characteristics age, height, weight, body mass index and percentage body fat in either the first or second trimester.

	n	t value	p value	95% confidence interval
Zpvoldiff v mage1	76	0.53	0.6	-0.03 to 0.06
Zpvoldiff v mht1	76	1.27	0.21	-0.01 to 0.06
Zpvoldiff v mpfat1	76	-0.06	0.95	-0.03 to 0.03
Zpvoldiff v zlmwt1	76	0.51	0.61	-0.17 to 0.28
Zpvoldiff v zlmbmi1	76	0.05	0.96	-0.22 to 0.23

Table 24- Relationship between placental growth rate and maternal characteristics

There was also no relationship between maternal body composition at the start of the pregnancy and the rate of placental growth using the same analysis with placental growth rate as the outcome.

4.5.3 Relationship between fetal cardiac function and maternal characteristics in the first trimester

	n	t value	p value	95% confidence interval
MPI1L v mage1	72	-0.45	0.66	-0.04 to 0.003
MPI1L v mht1	72	1.14	0.26	-0.001 to 0.004
MPI1L v mpfat1	72	-0.53	0.6	-0.004 to 0.002
MPI1L v zlmwt1	72	0.94	0.35	-0.01 to 0.002
MPI1L v zlmbmi1	72	0.51	0.61	-0.01 to 0.02
MPI1R v mage1	60	-1.09	0.23	-0.01 to 0.002
MPI1R v mht1	60	0.74	0.46	-0.002 to 0.005
MPI1R v mpfat1	60	0.02	0.97	-0.004 to 0.004
MPI1R v zlmwt1	60	0.69	0.49	-0.02 to 0.03
MPI1R v zlmbmi1	60	0.42	0.68	-0.02 to 0.03

Table 25- Association between maternal characteristics and fetal cardiac functon in the first trimester

In the first trimester the fetal cardiac function on either the right or left side of the heart as measured by the myocardial performance index was not altered according to maternal age, height, percentage body fat, weight or body mass index.

4.5.4 Relationship between fetal cardiac function and maternal characteristics in the second trimester

	n	t value	p value	95% confidence interval
MPI2L v mage2	47	-1.1	0.3	-0.01 to 0.002
MPI2L v mht2	47	0.09	0.93	-0.003 to 0.004
MPI2L v mpfat2	47	-1.25	0.22	-0.006 to 0.001
MPI2L v zlmwt2	47	-0.61	0.54	-0.03 to 0.02
MPI2L v zlmbmi2	47	-0.67	0.5	-0.03 to 0.02
MPI2R v mage2	46	-1.6	0.12	-0.01 to 0.001
MPI2R v mht2	46	-0.38	0.7	-0.01 to 0.005
MPI2R v mpfat2	46	-0.8	0.43	-0.009 to 0.004
MPI2R v zlmwt2	46	0.03	0.98	-0.04 to 0.04
MPI2R v zlmbmi2	46	0.21	0.84	-0.04 to 0.04

Table 26- Association between maternal characteristics and fetal cardiac functon in the second trimester

Similarly in the second trimester there was no discernible relationship between the maternal characteristics and fetal cardiac function.

4.5.5 Relationship between cardiac chamber size and maternal parameters

Total Cardiac Circumference (TCC)	n	t value	p value	95% confidence interval
TCC v mage2	91	-0.44	0.66	-0.2 to 0.13
TCC v mht2	91	0.76	0.45	-0.08 to 0.18
TCC v mpfat2	91	1.86	0.07	-0.01 to 0.3
TCC v zlmwt2	91	1.85	0.07	-0.06 to 1.76
TCC v zlmbmi2	91	1.61	0.11	-0.17 to 1.68

Table 27- Association between total cardiac circumference and maternal characteristics

Total Ventricular Circumference (TVC)	n	t value	p value	95% confidence interval
TVC v mage2	91	-0.93	0.35	-0.2 to 0.07
TVC v mht2	91	0.68	0.5	-0.07 to 0.15
TVC v mpfat2	91	1.64	0.11	-0.02 to 0.23
TVC v zlmwt2	91	1.92	0.06	-0.02 to 1.46
TVC v zlmbmi2	91	1.73	0.09	-0.1 to 1.41

Table 28- Association between total ventricular circumference and maternal characteristics

Internal Right Ventricular Circumference (IRVC)	n	t value	p value	95% confidence interval
IRVC v mage2	91	-0.29	0.77	-0.09 to 0.12
IRVC v mht2	91	-0.54	0.59	-0.11 to 0.06
IRVC v mpfat2	91	0.73	0.46	-0.06 to 1.76
IRVC v zlmwt2	91	0.41	0.68	-0.47 to 0.71
IRVC v zlmbmi2	91	0.7	0.49	-0.39 to 0.8

Table 29- Association between internal right ventricular circumference and maternal characteristics

Internal Left Ventricular Circumference (ILVC)	n	t value	p value	95% confidence interval
ILVC v mage2	91	-0.38	0.71	-0.17 to 0.11
ILVC v mht2	91	0.35	0.73	-0.09 to 0.13
ILVC v mpfat2	91	1.61	0.11	-0.02 to 0.24
ILVC v zlmwt2	91	1.08	0.28	-0.35 to 1.2
ILVC v zlmbmi2	91	1.00	0.32	-0.39 to 1.18

Table 30- Association between internal left ventricular circumference and maternal characteristics

Whilst there was no statistically significant relationship identified between the cardiac chamber measurements and maternal characteristics, there did seem to be a trend towards a significant relationship between both the total cardiac circumference (TCC) and total ventricular circumference (TCC) and maternal weight with mothers that weighed more having fetuses with larger hearts. This possible relationship was not observed when considering the internal circumferences of the right and left ventricle (IRVC and ILVC). It is likely that heavier mums have bigger babies with larger hearts proportionate to their bigger birth weights and our small numbers were not enough to confirm this.

4.5.6 Relationship between the ratio of the left to right ventricular circumferences and maternal characteristics

	n	r	p value
ILRRATIO v mage	93	0.1	0.32
ILRRATIO v mpfat	93	0.09	0.4
ILRRATIO v zlmwt	93	0.09	0.4
ILRRATIO v zlmbmi	93	0.05*	0.65

Table 31- Correlation between the ratio of the left to right ventricular circumferences and maternal characteristics

We analysed the relationship between the ratio of the internal circumferences of the left to right ventricle and maternal characteristics to see if there was a difference in this ratio influenced by any of these maternal parameters. This was done by a devising a correlation coefficient (r), which showed that there was no direct relationship between maternal body composition and the relative size of the left ventricle compared to the right.

4.5.7 Summary Statistics- Birth weight and placental weight

	N	mean	SD	min	max
Birth Weight (kg)	108	3.35	0.56	0.87	4.84
Placental weight (g)	57	585.7	133.2	347	877

Table 32- Summary statistics for birth weight and placental weight

Both the birth weight and placental weight were normally distributed and associated with gestation and were therefore adjusted for gestation by linear regression for the ongoing analysis.

4.5.8 Relationship between fetal cardiac function and birth outcomes

	BIRTH WEIGHT (birthwtag)	PLACENTAL WEIGHT (placwtag)
L MPI 1st trimester (MPI1L)	n=72 t=1.2 p=0.23	n=42 t=0.70 p=0.49
R MPI 1st trimester (MPIR1)	n= 60 t= 1.23 p=0.23	n=36 t=0.92 p=0.36
L MPI 2nd trimester (MPIL2)	n= 45 t= 0.26 p=0.79	n= 22 t= -0.10 p=0.92
R MPI 2nd trimester (MPIR2)	n=43 t=0.72 p=0.48	n= 19 t=-0.65 p=0.53

Table 33- Association between fetal cardiac function in the first and second trimester and pregnancy outcomes

Using a regression analysis there was no identifiable relationship between fetal cardiac function measured by the myocardial performance index on either side of the heart in either the first or second trimester and either birth weight or placental weight at delivery. We will go on to compare this with the findings of other groups.

4.5.9 Relationship between fetal cardiac structure and birth outcomes

	BIRTH WEIGHT (birthwtag)	PLACENTAL WEIGHT (placwtag)
TOTAL CARDIAC CIRC (TCC)	n=89 t= 1.40 p=0.166	n=45 t= 0.19 p=0.85
TOTAL VENT CIRC (TVC)	n= 89 t= 2.24 p=0.03*	n=45 t= 0.77 p=0.45
INTERNAL RIGHT VENT CIRC (IRVC)	n=89 t=0.17 p=0.87	n= 45 t=0.71 p=0.48
INTERNAL LEFT VENT CIRC (ILVC)	n=89 t=0.74 p=0.46	n=45 t=0.31 p=0.76
RATIO OF ILVC:IRVC (ILRRATIO)	n=89 t=0.76 p=0.45	n= 45 t= -0.43 p=0.67

Table 34- Association between cardiac circumferences and pregnancy outcomes

There was a positive association between the total ventricular circumference and birth weight as might be expected but surprisingly no association between total cardiac circumference and birth weight. We would expect that bigger babies would have bigger hearts.

In terms of the ventricular circumferences and their size relative to each other we could not discern a relationship with birth weight or placental weight. If there are differences in cardiac structure related to relative under

nutrition in utero, even within the normal range of birth weights, then it is likely that the differences would be very subtle, requiring a much larger cohort to distinguish any relationship.

4.6 Birth Outcomes

4.6.1 Relationship between placental volume and birth outcomes

	BIRTH WEIGHT (birthwtag)	PLACENTAL WEIGHT (placwtag)
PLACENTAL VOL 1st trim (pvol1)	n=108 t= 2.05 p=0.04*	n= 57 t= 3.78 p=0.00*
PLACENTAL VOL 2ND trim (pvol2)	n=100 t=4.24 p=0.00*	n= 53 t=2.03 p=0.047
PLACENTAL GROWTH RATE (zpvoldiff)	n= 100 t= 3.39 p=0.00*	n=53 t= 1.01 p=0.319

Table 35- Association between placental volume and pregnancy outcome

As expected the placental volume in both the first and second trimester positively correlated with both the birth weight and placental weight. Whilst the birth weight was strongly associated with the rate of placental growth the placental weight was not and the associations between second trimester placental volume and placental weight were not as strong as expected, only just reaching statistical significance. As previously described the placental volume in the second trimester was measured using two different techniques and we will go on to explore this further. It may also suggest that the final placental weight is determined earlier in the pregnancy, such that placentas which are going to weigh less at birth already weigh less by 12 weeks, are still small at 20 weeks, but have the same growth velocity between those time points.

4.6.2 Relationship between fetal:placental ratio and pregnancy outcomes

To explore this further we also looked at the relationship between the previously described fetal:placental ratio and pregnancy outcomes.

	n	t	p	95% confidence interval
Fetal:placental ratio v birth weight	103	-1.31	0.19	-9.21 to 1.89
Fetal:placental ratio v placental weight	55	-3.25	0.002*	-4494.7 to -1064.64

Table 36- Association between fetal:placental ratio and pregnancy outcomes

This showed no relationship between the size of the fetus in the first trimester relative to the placenta and the birth weight of the baby, but a similar significant relationship between the ratio and the placental weight as previously noted between the ratio and placental volume in the second trimester.

4.6.3 Relationship between maternal characteristics and birth outcomes

	BIRTH WEIGHT (birthwtag)	PLACENTAL WEIGHT (placwtag)
MATERNAL AGE 1st trimester (mage1)	n=106 t= 0.02 p=0.99	n= 57 t= 0.04 p=0.97
MATERNAL HEIGHT 1st trimester (mht1)	n=106 t=1.8 p=0.07	n=57 t= 1.44 p=0.16
MATERNAL WEIGHT 1st trimester (zlmwt1)	n= 106 t=1.2 p=0.23	n= 57 t=0.86 p=0.39
MATERNAL BMI 1st trimester (zlbmi1)	n=106 t=0.54 p=0.59	n=57 t= 0.35 p=0.73
MATERNAL % BODY FAT 1st trimester (mpfat1)	n=106 t=0.31 p=0.76	n= 57 t=-0.21 p=0.84

Table 37- Association between maternal characteristics and pregnancy outcomes

We analysed the relationship between the mother's basic anthropometry and the birth weight and placental weight and did not find any strong correlations. We would expect to find a relationship, particularly with the mother's weight, body mass index and percentage body fat, but as previously discussed this relationship is a complex one as there is a recognised association between maternal body mass index and both macrosomia and intra-uterine growth restriction. Given this, with the number of subjects we had it may not be possible to discern a relationship between the maternal characteristics and pregnancy outcomes.

4.7 Measurement of placental volume in the second trimester

In previous studies within our department (Inskip, Godfrey et al. 2006) (Holroyd, Harvey et al. 2012) prior to the use of 3D scanning, placental volume was calculated using 2D measurements.

The measurements were performed by the sonographers in the University department (either PM or CN). The sonographer measured the placenta in two planes, initially along the longest edge of attachment to the uterine wall (length) and then at 90˚ to this, so that the image plane bisected the longitudinal axis (breadth). Each measurement was performed in triplicate and the mean value used for analysis. Placental volume was later calculated using the two-dimensional ultrasound measurements. To estimate the volume of the placenta it was assumed that it was an ellipsoid. The two measured section perimeters and two areas were expressed as functions of the three ellipsoid radii. Estimates of the radii, obtained by least squares, were then combined to estimate the volume as expressed in the formula below.

The following equation was shared via personal communication (February 2011) from Clive Osmond, the statistician who devised it.

4.7.1 Placental volume from projected areas and circumferences

Figure 26- Diagram of an ellipsoid with a, b and c planes

Assume that the placenta is an ellipsoid with diameters a, b and c.

We are given the area and circumference of projections of the placenta onto the (a, b) and (b, c) planes.

The area in the (a, b) plane is $\pi ab/4$, and the area in the (b,c) plane is $\pi bc/4$.

Define constants $p=2-\sqrt{2}$ and $q=2+\sqrt{2}$, then using my Simpson's rule approximation (see my 1994 correspondence with Doug Altman), the circumference in the (a, b) plane is

$$\pi.\{a+b+\sqrt{[2(a^2+b^2)]}+2\sqrt{[p.a^2+q.b^2]}+2\sqrt{[q.a^2+p.b^2]}\}$$

And the circumference in the (b, c) plane is

$$\pi.\{b+c+\sqrt{[2(b^2+c^2)]}+2\sqrt{[p.b^2+q.c^2]}+2\sqrt{[q.b^2+p.c^2]}\}$$

So we have four equations in three unknowns. The program identifies the combination (a, b, c) that minimises the sum of squared errors between the

observed and fitted areas and circumferences. The placental volume is then given by $\pi.a.b.c/6$

Example (units are = cm, cm^2 and cm^3 ??)

Given data: area(a, b)=27.18; area(b, c)=28.24; circumf(a, b)=30.01; circumf(b, c)=28.02

Fitted values a=13.966, b=2.546, c=13.739

Fitted volume=255.823

Fitted data: area(a, b)=27.93; area(b, c)=27.47; circumf(a, b)=29.24; circumf(b, c)=28.80

No other combination of (a, b, c) will get so close.

The advantage of being able to measure the placenta in 2D in the second trimester is that these measurements are easily obtainable regardless of placental site. To take a 3D measurement the entire organ must fit within the volume box and in anterior placentas in the upper, narrower part of the scan sector this is not always possible.

In our cohort the posterior placentas were measured using the 3D technique previously described, while the anterior placentas were measured using the 2D method above. This gave us the opportunity to compare the 2 techniques.

Technique	n	mean	Std error	Std dev	95% CI
3D	79	185.15	4.27	37.94	176.7-193.6
2D	26	207.85	9.35	47.66	188.6-227.1

Table 37- Summary statistics of second trimester placental volume measureuments according to technique

Performing a regression analysis (mean 3D- mean 2D) on the two techniques by comparing the means (mean 3D- mean 2D) shows a significant difference between the 2 techniques.

	Mean 2D
Mean 3D	n=105 t=-2.478 p=0.01*

Table 38- Relationship between second trimester placental volume measurements by different techniques

As might be expected with the smaller numbers measured using the 2D technique, the standard error is larger with wider confidence intervals and the values not normally distributed.

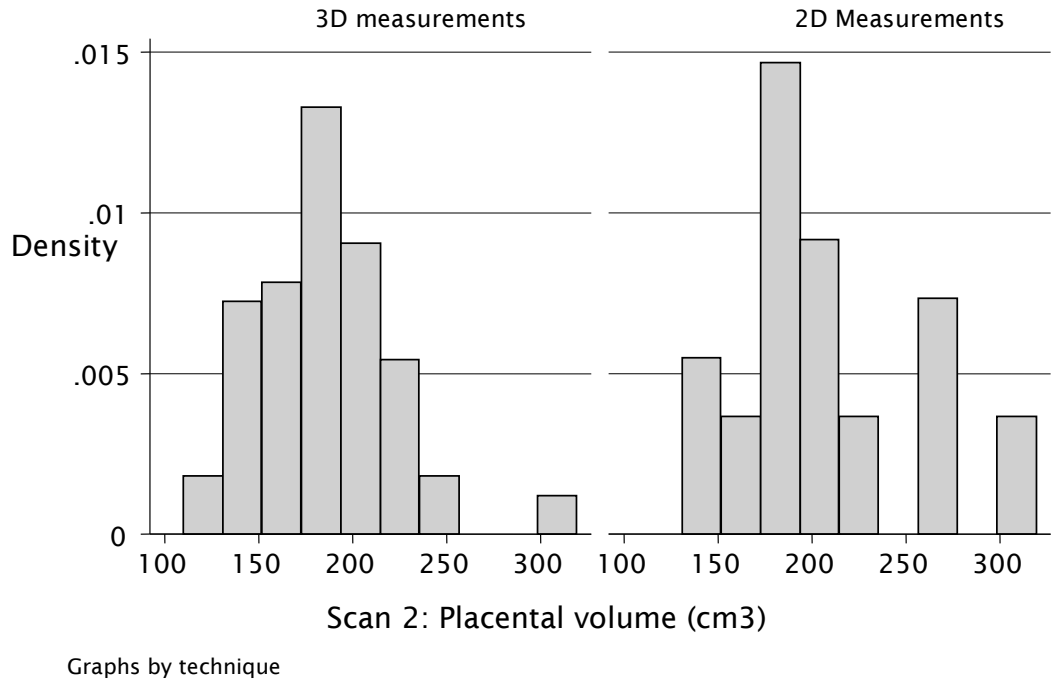

Figure 27- Differences in second trimester placental volume measurements by 2D or 3D scanning

Given the differences between the two techniques we discarded the measurements obtained by the 2D technique and reanalysed our results using only the 3D measurements. We performed a regression analysis using placental volume as the predictor.

Pvol2agjc N=79	n	t	p
MPI1L	48	1.6	0.117
MPI1R	43	0.72	0.475
MPI2L	37	-0.55	0.587
MPI2R	34	1.08	0.288
PVOL1	78	2.72	0.008
TCC	68	-1.47	0.148
TVC	68	-1.01	0.315
IVRC	68	-1.04	0.301
IVLC	68	-1.1	0.274
birthwtag	74	4.26	0.000*
placwtag	40	2.46	0.019*
Crl1ag	75	0.26	0.796
Nt1ag	21	-1.56	0.136
Bpd2ag	75	0.77	0.445
Hc2ag	75	-0.09	0.931
Ac2ag	75	0.55	0.587
Fl2ag	75	0.75	0.458

Table 38- Associations between second trimester placental volume measured by 3D ultrasound and fetal cardiac function, cardiac structure and size

On this analysis there was a stronger association between the placental volume measured using the 3D technique and placental volume in the first trimester,

birth weight and placental weight. This is reassuring as we would expect these variables to be strongly associated, suggesting our 3D technique is a valid one. However there was still no association with any of the fetal biometry in either the first or second trimester which is surprising as generally we would expect bigger fetuses to be associated with bigger placentas, i.e. a positive association. There was also no association between the placental volume in the second trimester and either the cardiac function or structure.

5. Chapter 5- Discussion

We had two principal aims in our study- firstly to establish the feasibility of measuring cardiac function in the first and second trimester and establishing the reliability and reproducibility of 2D measurements of cardiac structure in the second trimester and, secondly, relating these measurements along with fetal biometry and placental volume to basic maternal anthropometry. We reasoned that the well recognised increased risk of cardiovascular disease in later life related to lower birth weight may be due in part to altered cardiac function and structure in-utero influenced by placental function and maternal nutritional status.

Our results demonstrated difficulties with reliably measuring fetal cardiac function by means of the myocardial performance index, particularly in the first trimester and on the right side of the heart, but that 2D measurements of the cardiac chamber sizes appeared reliable and reproducible. We were also able to refine our technique for measuring placental volume in 3D making this a less time consuming, but equally reliable process and had an opportunity to compare this 3D technique with a method of measuring the placenta in 2D developed within and previously employed by our department. There were some interesting findings relating fetal cardiac function and structure measurements to each other and to maternal characteristics, although our ability to detect such relationships was potentially limited by our sample size as we will discuss.

In addition we were able to perform an analysis on measurements of blood flow in the ductus venosus and umbilical vein which had been previously taken by a single ultrasonographer within our department in order to calculate the degree of ‘liver-shunting’. Relating this to a wide-range of maternal parameters suggested that maternal reported stress levels may influence fetal circulatory adaptations which we postulated may be via hormonal axes.

5.1 Measurement of Fetal Cardiac Function

The myocardial performance index continues to be used as a tool in fetal medicine research in a number of scenarios. As we discussed previously there is a wide range in reported normal values, suggesting that the measurement of this parameter is dependent on individual technique and that the reproducibility may therefore be limited.

This was recently addressed by the group in Barcelona who noted that alterations in sweep speed and gain affected the calculation of the MPI value and its reproducibility (Lobmaier, 2012 #95). They found that decreasing the sweep speed from 8cm/s to 5cm/s altered the absolute value of the MPI measurement as well as decreasing the interclass co-efficient (ICC) of agreement. They also found that lowering the gain affected the value of the MPI obtained and reduced the ICC making the measurement less reproducible.

As previously mentioned we used a sweep speed of 5cm/s as we found it difficult to identify optimal waveforms due to the speed of passage across the screen with higher sweep speeds. However this meant that the individual waveforms were narrower possibly reducing the accuracy of placing the callipers to measure time in comparison to the more spread out wave-forms obtained at the higher sweep speeds. With a sweep speed of 5cm/s the smallest possible movement used in positioning the calliper would alter the time measurement by 7ms. Given that a typical measurement for ‘a’ would be 240ms and for ‘b’ 147ms, it can be seen that if there is a potential error of 7ms each time a calliper is placed to measure these times then this would have a large influence over the final value after the calculation a-b/b has been performed.

Of all the landmarks used for calliper placement it was most difficult to define the isovolumetric contraction time (ICT), the time at the end of the E/A wave, prior to the ejection time when the ventricle is beginning its contraction,

but there is no flow through the mitral or tricuspid valves. This should appear as a gap in the optimal waveform, the beginning of which would define the point of calliper placement to begin measuring ‘a’ and the end of which defines the start of the ejection time, i.e. where the calliper is placed to begin measuring ‘b’. This gap tended to be easier to identify in the first trimester when the Doppler gate covered more of the valve and the valve clicks were more pronounced. In the second trimester it was often more difficult to identify the gap and distinguish the valve click from underlying artefact even when turning the gain down. This could be somewhat improved by moving the Doppler gate more centrally within the valve, away from the interventricular septum. Our overall tendency was to reduce the gain to try to reduce the artefact which, as discussed above, has subsequently been shown to reduce the reproducibility of the measurement.

We noted other limitations in obtaining the MPI. Fetal activity, particularly in the first trimester made it difficult to obtain consistent wave-forms in some instances. Although it was possible to obtain a measurable waveform in most fetal positions, if the inter-ventricular septum was perpendicular to the ultrasound beam it was very difficult to get adequate, identifiable E/A waves. As with any ultrasonic examination we were also sometimes limited by raised maternal body mass index.

In our cohort the MPI measured on the right side of the heart was strongly associated with the MPI measured on the left side of the heart in both the first and the second trimester. When we attempted to establish whether the MPI in the first trimester was associated, or could even predict the MPI in the second trimester for either the right or left side, we could not identify any relationship between the values in the first trimester compared to those in the second, although the numbers, particularly on the right side were very small and likely underpowered to identify any such association. We were also unable to establish any relationship between the MPI on either side in any trimester and the placental volume. Given subsequent findings from other groups

discussed below about the alteration of the MPI in growth restricted fetuses we could potentially have hoped to see an inverse relationship between the MPI and placental volume. We did however find some weak relationships between the MPI and some of the fetal biometry on ultrasound, but not with actual birth weight in our low-risk group. Again further larger studies would be required to investigate this.

We are also unable to identify a relationship between the MPI and maternal anthropometry in either trimester. Our group has previously identified fetal circulatory adaptations related to maternal characteristics with fetuses of slimmer mothers with lower body fat stores demonstrating decreased shunting through the ductus venosus into the inferior vena cava and towards the right atrium. In our cohort we could not establish a link between maternal body fat stores and alteration in cardiac function, but our ability to detect this was limited by the number of women scanned.

The potential use of the myocardial performance index in both clinical and research settings continues to be explored. It has been recognised that the left MPI is altered in fetuses with intra-uterine growth restriction in a study by Crispi et al (Crispi, Hernandez-Andrade et al. 2008) They compared a group of appropriately grown fetuses both at term and pre-term and compared them with a group of growth-restricted fetuses, defined as having an estimated fetal weight below the 10th percentile with umbilical artery pulsatility index (PI) more than 2 standard deviations above the mean. They were further classified as stage 1, 2 or 3 dependent on whether end-diastolic flow was present, absent or reversed. The MPI was significantly raised in all stages of growth restriction, rising progressively across the stages, with all the time periods used in the calculation of the MPI showing a significant difference. There was also a significant increase in the MPI in those fetuses that died compared with survivors. The same group went on to assess the potential predictive value of the MPI in predicting fetal death and calculated an odds ratio (OR) for fetal demise of 1.6 with an MPI value above the 95th centile (Cruz-Lemini, Crispi et

al. 2012). However this was much less than the OR for fetal demise related to gestational age below 28 weeks, absent or reversed ductus venosus atrial flow, PI in the middle cerebral artery below the 5th centile or absent or reversed end diastolic flow in the umbilical artery suggesting these parameters are likely to be more useful in management and timing of delivery in these difficult pregnancies. They also assessed the timing of changes in the MPI and ductus venosus flow in fetuses with early onset growth restriction and umbilical artery Dopplers above the 95th centile and noted that the MPI tended to increase to a level above the 95th centile around 3 weeks earlier than changes in the ductus venosus PI, suggesting there may be a role monitoring these at-risk fetuses (Cruz-Martinez, Figueras et al. 2011).

Having established alterations in the MPI in growth restricted fetuses the same group went on to examine a group of children who had been identified in utero as being growth-restricted and had had MPI measurements performed in utero to identify evidence of in-utero cardiovascular programming extending into childhood (Cruz Lemini, 2012 #94). They defined post-natal cardiovascular adverse outcomes as having a diastolic blood pressure above the 95th centile with aortic intima-media thickness above the 95th centile. Children that had an increased MPI in-utero had an odds ratio of 3.1 (CI 1.6-6.1) for a postnatal cardiovascular adverse outcome. The findings of this study suggest that the MPI may be a useful tool for detection of fetuses undergoing cardiovascular programming which may increase their risk of developing cardiovascular disease in later life.

In our cohort we had difficulty in measuring the myocardial performance index in order to gain consistent, reliable and reproducible measurements and the possible reasons for this have been outlined above. Other groups have acknowledged similar difficulties and demonstrated that use of the valve clicks, higher sweep speeds and higher gain may all help with refining the technique to give a more meaningful result. Given the findings above in papers published

after we had completed scanning our cohort with regard to changes in the MPI in growth-restricted fetuses and in fetuses subsequently found to have cardiovascular changes in childhood this technique certainly seems likely to find a place both in the clinical care of high risk pregnancies and in the ongoing elucidation of the mechanism of developmental origins of increased cardiovascular risk in later life.

5.2 Measurement of Cardiac Architecture using 2D Ultrasound

Part of our hypothesis postulated that altered cardiac flow would result in altered cardiac structure due to influence of flow and shear pressures on the developing myocardium. We hypothesised that this would have different effects on the right and left side of the heart due to their different curvature. In order to investigate this we explored the feasibility of measuring the cardiac chamber sizes and relating these to each other, cardiac function, placental volume, fetal biometry and maternal anthropometry.

We chose to measure the circumferences of the whole heart, the ventricular portion of the heart and the internal circumferences of each ventricle as outlined above. We found these measurements to be easily obtainable and highly reproducible. We also found that there was enough variation between subjects to suggest a true underlying biological variation, meaning that these measurements may have an application in detecting differences in cardiac structure in different scenarios, possibly related to altered blood flow.

The measurements were all strongly associated with each other as we would expect and there were associations with fetal biometry in the second trimester. We attempted to identify differences in cardiac structure by calculating a simple ratio between the internal ventricular circumferences on the left and right side reasoning that in altered flow due to altered placental

function or maternal nutritional status there may be a detectable difference between the two sides. It was also noticeable during measurement of these circumferences that in most fetal hearts measured the left ventricle circumference was larger than the right, but in some cases they were more equal and in a small number the right was larger, suggesting a difference in architecture. In our cohort we were unable to determine a relationship between the ratio and cardiac function, placental volume or fetal biometry in either the first or second trimester. There was also no apparent relationship between the ratio of the left to right ventricular circumference and maternal anthropometry.

In order to investigate this further larger cohorts would be required to distinguish more subtle relationships and it may also be worth considering relating the cardiac measurements in other ways, e.g. the ratio of the total ventricular circumference to the total cardiac circumference. Since we completed scanning our cohort further work has been published on using two dimensional cardiac measurements to identify cardiac remodelling in children and fetuses (Crispi, Bijnens et al. 2010).

Based on the previous observations that growth restricted fetuses developed changes in utero similar to a dilated cardiomyopathy the investigators calculated the sphericity index of the heart based on measuring the long axis of each ventricle from the valve to the internal apex in the apical view at end-diastole. They also measured the basal diameter of each ventricle by measuring across the valves from the interventricular septum to the valve insert on the internal edge of the lateral ventricular wall. The sphericity index was calculated by dividing the long axis of the ventricle by the basal diameter and this measurement was found to be reproducible with good intra- and interclass correlation coefficients and interobserver agreement (Cruz Lemini, 2012 #93). The same group performed these measurements on children who had been identified as being growth-restricted in utero at a mean age of 5 years. They found that compared to controls the right and left sphericity indices were significantly decreased in children who had been either mildly or severely growth restricted as fetuses, indicating that these children had differently shaped, more globular hearts. The same children also had

significantly higher diastolic blood pressure and carotid artery intima media thickness indicating cardio-vascular remodelling, possibly as a result of their in-utero environment, and an ongoing increased risk of cardiovascular disease (Crispi, 2010 #81). When the same group measured the sphericity index in fetuses (Cruz Lemini, 2012 #92) they found a significant reduction in the right sphericity index, i.e. more globular hearts, in fetuses that were classified as either early or late onset growth restricted and controls.

Their findings confirm that there is a role for two-dimensional cardiac measurements in assessment of clinically growth restricted fetuses and fetuses that are undergoing fetal programming as a result of a sub-optimal intrauterine environment but who exhibit growth in the normal range. Whilst our measurements were easily obtainable and reproducible they may not be measuring the correct parameters to identify these fetuses as the ventricular circumferences may be influenced in the same way by alterations in intrauterine environment and they do not take into account different geometry of the ventricle itself. A further area to explore may be the relationship between each ventricle relative to the size of the whole heart (the TCC), rather than relative to each other which may uncover differences in the structure of the heart secondary to altered flow pressures in adapting fetuses.

5.3 Placental Volume

In order to investigate our hypothesis we measured placental volume by both three dimensional and two-dimensional ultrasound. As we have previously discussed the role of the placenta in fetal programming is a complex one, reflecting the many roles the placenta has, not just as an organ for the transfer of oxygen and nutrients from the mother, but also as an important metabolic and endocrine organ. Evaluating the placenta in terms of volume alone only allows a crude insight into the relationship between the placenta and fetal growth. Variable associations have previously reported between the two although it is recognised that growth-restricted fetuses generally have smaller placentas and so we reasoned that it may be possible to relate placental

volume to fetal growth and cardiac function and explore the relationship with maternal characteristics.

We were able to refine and validate our technique of using a single sweep of the placenta along its longest axis and measuring in serial slices, avoiding the use of reconstructed planes and allowing for irregularities in shape of both the placenta and underlying uterine wall. Using thicker slices in the second trimester placental measurements saved considerably on time, making the technique much more feasible while maintaining minimal variability between measurements. The limitation of 3 dimensional scanning of the placenta in the second trimester is that if the placenta is anterior sometimes it was difficult to fit the entire organ into the volume box. In this situation we used a 2 dimensional technique applied in previous studies within our department, but found that the two techniques did not correlate.

In our cohort we found that placental volume was associated with gestation in the first trimester, but not in the second trimester. While the placental volume in the first and second trimester were both strongly associated with birth weight and placental weight as we would expect, there were few associations between placental volume and fetal biometry on ultrasound which may reflect the small magnitude of biological variations in size between fetuses at this stage of gestation. However, the rate of placental growth between the first and second trimester was strongly associated with birth weight, so that where the placenta grew well in the first half of pregnancy the baby's birth weight was greater, suggesting an association between placental volume as measured on ultrasound and fetal growth but only when assessed over time.

It was also not possible to determine a relationship between cardiac chamber sizes and placental volume or rate of placental growth in our cohort. As previously discussed we would expect the size of the heart to be related to

the size of the fetus but were unable to establish this. If altered placental function altered flow through the fetal heart we hypothesised, then we might expect to see changes in both cardiac structure, particularly the ratio of right to left ventricles, and cardiac function, but in the absence of either uterine or umbilical artery Doppler measurements, placental volume alone would be unlikely to detect such a relationship.

As discussed previously, other investigators have noted a relationship between fetal cardiac function as measured by the myocardial performance index and growth restriction in utero. Given these findings we could postulate that a relationship may exist between placental volume and cardiac function, whereby in growth restricted fetuses with smaller placentas there would be an inverse relationship between placental volume and the myocardial performance index. We were unable to demonstrate this in our unselected cohort, with no apparent relationship between placental volume or rate of placental growth and myocardial performance index at either gestation but this may warrant further investigation as we will discuss.

Measurement of the placental volume has yet to find an established role in clinical practice. Other studies have tried to establish if the placental volume measured in pregnancy can be used to predict low birth weight babies, allowing better identification and increased surveillance of these high-risk pregnancies (Hafner, Philipp et al. 1998, Thame, Osmond et al. 2001, Plasencia, Akolekar et al. 2011). Other indices such as biochemical markers of placental function, e.g. serum pregnancy-associated plasma protein (PAPP-A) and free beta-human chorionic gonadotrophin, and uterine artery Doppler in the first trimester (Schuchter, Metzenbauer et al. 2001) have been used in association with placental volume to increase the accuracy of such predictions with some promising results. As techniques for measuring the MPI improve and normal ranges become more established then it is possible that measuring fetal cardiac function in association with placental volume, serum markers and

possibly uterine artery Doppler will further refine our ability to identify at risk fetuses early in pregnancy allowing more intensive monitoring.

We did not find any relationship between the volume of the placenta measured on ultrasound and maternal anthropometry, an association that has been previously reported (Thame, Osmond et al. 2004), and nor was there a relationship between maternal anthropometry and either birth weight or placental weight at birth. As the placenta obviously plays the key role in transfer of nutrients from mother to fetus we reasoned that alterations in maternal nutrition and placental function will alter the nutritional status of the fetus, which the fetus will adapt to. In the context of further investigating the mechanisms behind the increased risk of cardiovascular disease related to intra-uterine environment and maternal nutrition, placental volume alone is unlikely to give any insight, but in combination with other parameters including serum markers and Doppler studies it may be possible to identify those fetuses that are within the normal range for growth but who are adapting to a sub-optimal environment. Calculating a ratio of fetal size to placental size in utero did not bring any further insights in the same way that previous studies of birth weight to placental weight ratios (Godfrey, Redman et al. 1991) have identified an altered ratio in association with maternal nutrition. Earlier identification of these fetuses may allow for dietary intervention in order to improve outcomes.

5.4 Conclusions

The long-term effects of the intra-uterine environment on the risk of disease in adult life are well established, but the mechanisms for this are still being explored. Alterations in the environment the fetus experiences will influence many different pathways including gene expression, growth-factor production, hormonal responses and circulatory responses. Maternal factors including nutritional status and psychological stress may alter these pathways with the placenta, as the interface between the mother and developing fetus, playing a key role in the regulation of fetal growth.

Our hypothesis was that in fetuses destined to show recognised signs of adaptation to a suboptimal intrauterine environment there will be evidence early in the pregnancy of alterations in placental volume to fetal size and changes in cardiac function and structure. This gave rise to two aims. Firstly we aimed to demonstrate that cardiac function and placental volume would be measurable reliably and reproducibly in the first trimester and that the same parameters plus cardiac structure would be measurable in the second trimester. Secondly we aimed to determine the relationships between these measurements, both to each other and with maternal anthropometry in an unselected population to determine the influence of maternal characteristics on fetal cardiac function and structure.

We were able to demonstrate that placental volume could be reliably measured using 3D ultrasound in the first trimester, refining the technique to make it quicker and therefore more feasible. In the second trimester we could also demonstrate that the placenta could be reliably measured on 3D ultrasound and determined the optimum technique to maintain accuracy whilst minimising the time taken to obtain the volume.

The results of our measurements of cardiac function by means of the myocardial performance index were disappointing. We established a normal range similar to previous reported studies and found that when measuring the left MPI at 20 weeks the intra-subject variability was low compared with the inter-subject variability suggesting that true biological variations would be detectable. However when measuring the MPI in the first trimester on the right or left and on the right in the second trimester we found that there was a wide variation in the result obtained in the same subject during the same scan which was only partly attributable to an operator learning curve. As discussed other investigators have noted difficulty in reliably measuring this result and we identified some aspects of our technique which may have made our measurements less reliable, but which could be easily adapted in future studies. The MPI certainly seems a promising tool in both the clinical assessment of growth restricted fetuses and in identification of fetuses undergoing intra-uterine programming due to a sub-optimal environment.

Our two-dimensional measurements of cardiac structure were easily obtainable and reproducible. We based our hypothesis on animal studies showing a difference in cardiomyocyte formation in response to altered flow through the heart and that alterations in flow, which occurs as a fetal adaptation to reduced oxygen or nutrient supply, affect each side of the heart differently due to the different curvature of the walls. It was interesting to note an apparent difference in some fetuses in the ratio of the left ventricular circumference to the right ventricular circumference, but disappointing not to relate this to any other alterations in cardiac function, placental volume or fetal growth. It is possible that alterations in cardiac structure occurring at a cellular level would not be detected by our technique. Other groups have been able to identify alterations in 2D cardiac measurements in growth restricted fetuses looking for dilated cardiomyopathy-like changes by calculating a sphericity index. This demonstrates that 2-dimensional measurements of cardiac structure can be valuable in the assessment of growth-restricted fetuses and may therefore have the potential to detect fetuses whose growth is in the

normal range, but who are adapting to a sub-optimal environment, raising their disease susceptibility in later life.

Our finding of the association between maternal stress and increased shunting of blood through the ductus venosus and reduction of blood flow to the fetal liver demonstrates another factor worthy of exploration in elucidating the mechanisms of the developmental origins of disease in adult life. Alterations in the maternal sympathetic nervous system and hormonal axes in response to stress may also contribute to alterations in fetal growth and development.

In order to explore our hypothesis further it would have been important to assess alterations in fetal blood flow, particularly in the third trimester looking for recognised signs of fetal adaptation to an adverse environment, but few of our women were scanned at this point in pregnancy. This would need to include Doppler measurements of umbilical artery and middle cerebral artery indices and umbilical vein and ductus venosus flow. Relating these back to measures of fetal cardiac function and placental volume in the first trimester may identify fetuses undergoing intra-uterine programming in response to a sub-optimal environment. Performing these measurements in a fully characterised pregnant population with detailed information about health, diet and lifestyle may help identify areas for future interventions aiming to improve the nutritional status and physical and psychological well-being of the mother in order to improve the environment in which the fetus finds itself and reduce the long-term risk of disease in adult life.

Appendices

5.5 Appendix 1- SiPS Consent form for eligibility screening

Appendix 1

VOLUNTEER INITIAL CONSENT FORM

Thank you for reading the information about our research project. If you would like to take part, please read and sign this form.

Title of study: **The effects of oily fish in pregnancy on signs of allergic diseases in infants at high risk of developing allergies**

Local ethical committee submission number……

Chief Investigator: Professor Philip Calder, Tel: 023 8079 5250

Contact details for research team: Dr Liz Miles, Tel: 023 8079 6925 or 023 80 79 5252

*PLEASE **INITIAL** THE BOXES IF YOU AGREE WITH EACH SECTION:*

1. *I am not taking part in any other study (i.e. Southampton Women's Survey).* ☐

2. *I have read the initial information sheet dated 8/05/07 (version 2) for the above study and have been given a copy to keep. I have had the opportunity to ask questions, understand why the research is being done and any possible risks which have been explained to me.* ☐

3. *I understand that my participation in this study is voluntary and that I am free to decline entry into the study, and that during the study I am free to withdraw at any time without giving a reason and that withdrawal will not affect any future medical care.* ☐

4. *Data to be collected. I agree to have my weight, body composition and height measured for research in this project (delete as appropriate).* ☐

5. *I agree to have additional measurements made of the growth and development of my baby and allow a sample of my afterbirth (placenta) to be collected.* ☐

6. *Information to be collected. I agree to complete a short questionnaire on my health and I give permission for someone from the research team to look at my medical notes to get information pertinent to the study. I understand that the information will be kept confidential.* ☐

7. *I agree to take part in the above study* ☐

_____ _____ _____

Name of Patient *Date* *Signature*

_____ _____ _____

Name of Person taking consent *Date* *Signature*

1 for patient, 1 for researcher, 1 to be kept with hospital notes.

Glossary

Fetal programming-	when an intra-uterine stimulus or insult has a lasting effect on the structure or function of a developing organ
Developmental plasticity-	a phase in the development of a system where that system is 'plastic' and can adapt to external influences to which it is sensitive
Phenotype-	the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment
Genotype-	The genetic constitution of an individual organism
Epigenetics-	a single genotype can give rise to a range of phenotypes
Ponderal index-	birth weight/length^3
Body mass index-	weight in kg/height in m^2
Hypoxaemia-	decreased partial pressure of oxygen in blood
Hypoxia-	deficiency in the amount of oxygen reaching the tissues
Ischaemia-	insufficient supply of blood to an organ
Pulsatility index-	a measure of the variability of blood velocity in a vessel, equal to the difference between the peak systolic and minimum diastolic velocities divided by the mean velocity during the cardiac cycle.
Oligohydramnios-	reduced amniotic fluid volume
Impedance-	obstruction or opposition to flow
Angiogenesis-	development of new blood vessels

Haematocrit- the ratio of the volume occupied by packed red blood cells to the volume of the whole blood

Trophoblast- the outermost layer of cells of the blastocyst that serves as a nutritive pathway for the embryo

Syncytiotrophoblast- the outer layer of the trophoblast that actively invades the uterine wall forming the outermost fetal component of the placenta

Caruncle- oval or round thickenings in the uterine mucosa, the only site in the uterus to form attachments with fetal membranes in sheep

Myocardial Performance Index- a global measure of myocardial performance, incorporating systolic and diastolic function

Isovolumetric contraction time- the early phase of systole, in which the myocardial muscle fibres have begun to shorten but have not developed enough pressure in the ventricles to overcome the aortic and pulmonary end-diastolic pressures and open the aortic and pulmonary valves

Isovolumetric relaxation time- part of the cardiac cycle between the time of aortic valve closure and mitral opening, during which the ventricular muscle decreases its tension without lengthening so that ventricular volume remains unaltered

Ejection time- part of the systolic phase of the cardiac cycle where the arterio-ventricular valves are open and there is flow of blood through the valves

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