# Speckle Tracking as a tool for Assessing foetal Cardiac Ventricular Imbalance (STACVI)

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DClinSci

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## ABSTRACT

**Introduction**: Speckle Tracking echocardiography (STE) plays an exciting new role in the assessment of cardiac function. It is now widely used in the adult and paediatric sectors to evaluate the percentage of myocardial deformation, or % strain, with which the contractility of the muscle can be determined. It has proven validity, feasibility, and reproducibility in adult cardiology whilst demonstrating a significant contribution to clinical management, but there is still doubt regarding the clinical use in foetal cardiology due to technical barriers and a vast variation in normative data. There are, however, clinical presentations in foetal cardiology where long-term outcomes need to be counselled with caution. At 20 weeks gestation the foetal heart can be assessed for structural and functional normality, but where this borders on abnormal, there needs to be some discussion about the potential for deterioration. With little diagnostic evidence to support these borderline normal presentations, a functional tool such as speckle tracking could prove useful in predicting outcomes where there is a level of suspicion.

**Methods**: In this study 40 foetal echocardiograms, where there was a discernible size discrepancy between the left and the right ventricles of unknown aetiology, were analysed using Tomtec Cardiac Performance Analysis (CPA) v.1.2 to provide longitudinal strain of left and right ventricular myocardial deformation. In addition, 40 echocardiograms where this imbalance in size was clearly due to a congenital heart defect were also retrospectively analysed for strain quantification. A further 40 foetal echocardiograms of age-related normal anatomy, were also analysed to provide a control group.

**Results**: In total, 118 foetal echocardiograms were successfully analysed using CPA to obtain ventricular size, volume, and functional parameters, including percentage strain, for both the left and right ventricles across 3 groups – 1) foetuses with a foetal cardiac size discrepancy between the ventricles, 2) foetuses with a congenital heart defect and an imbalance between the size of the ventricles and 3) foetuses with normal foetal cardiac presentation. The foetal echocardiograms were retrospectively collected from foetuses between 15-24weeks gestation and results show that there was no significant difference in strain values in a ventricular size imbalance despite significant differences in size and volume of the ventricles. There was, however, a significant difference between the CHD group and control indicating that strain was reduced in the presence of a cardiac heart defect.

**Discussion**: The results of this study show that myocardial deformation is preserved in the foetal heart where there is a ventricular size disproportion at mid-pregnancy. Significantly lowered left ventricular strain values were demonstrated in the CHD group which suggests that myocardial deformation is reduced in the presence of a congenital heart defect. These results support the theory that Speckle Tracking Echo (STE) may be a useful functional assessment tool at mid-gestation, in the presence of a congenital heart defect but not when there is only a ventricular size discrepancy. Further research is required into variations in methods, technique, and reference values to reduce technical variations, and build confidence in using STE to assess foetal cardiac function in congenital heart disease.

## AUTHORS DECLARATION

This research was supported by TomTec Imaging systems GmbH, Unterschleissheim, Germany, with the commercially available software TomTec Image Arena. The Cardiac Performance Analysis (CPA) software was upgraded to the latest platform, version 1.2 for foetal cardiac use on a temporary licence for the duration of the analysis period. No financial arrangements were made to permit the use of the equipment.

No amount of the content in this thesis has been submitted in support of an application for another degree or qualification of MMU or any other university or other institution of learning.

# CONTENTS

ACKNOWLEDGEMENTS ..... 3

ABSTRACT ..... 5

AUTHORS DECLARATION ..... 7

CONTENTS ..... 8

LIST OF FIGURES ..... 11

LIST OF TABLES ..... 14

LIST OF ABBREVIATIONS ..... 16

CHAPTER 1 INTRODUCTION & BACKGROUND ..... 18

1.1 CARDIAC EMBRYOGENESIS ..... 18

1.2 CONGENITAL HEART DISEASE & THE ROLE OF ANTENATAL DIAGNOSIS ..... 23

1.3 FOETAL ECHOCARDIOGRAPHY ..... 35

1.4 SPECKLE TRACKING AND STRAIN ..... 48

1.5 NORMAL STRAIN VALUES ..... 62

1.6 ABNORMAL CARDIAC PRESENTATION ..... 69

1.7 RESEARCH QUESTION, STUDY AIMS AND OBJECTIVES ..... 84

CHAPTER 2 METHODS AND STUDY DESIGN ..... 88

2.1 STUDY DESIGN, SETTING AND POPULATION ..... 88

2.2 INCLUSION/EXCLUSIONS ..... 89

2.3 DATA COLLECTION ..... 90

2.4 PATIENT AND MATERNAL DEMOGRAPHICS ..... 93

2.5 ULTRASOUND IMAGING ..... 93

2.6 OFFLINE IMAGE ANALYSIS TOOL ..... 98

2.7 TOMTEC ANALYSIS ..... 98

2.8 DATA ANALYSIS ..... 107

2.9 ETHICAL ISSUES ..... 113

CHAPTER 3 RESULTS ..... 115

3.1 RECRUITMENT AND DEMOGRAPHICS ..... 115

3.2 FEASIBILITY OF SPECKLE TRACKING ECHOCARDIOGRAPHY ..... 134

3.3 ASSESSMENT OF PEAK LONGITUDINAL STRAIN ..... 135

3.3 REPEATABILITY OF MEASURES ..... 140

3.4 ASSESSMENT OF VENTRICULAR SIZE AND RATIO ..... 145

3.5 ASSOCIATION BETWEEN GLS VALUES AND OUTCOMES ..... 149

CHAPTER 4 DISCUSSION ..... 151

4.1 KEY FINDINGS ..... 155

4.2 MYOCARDIAL DEFORMATION AND STRAIN ANALYSIS ..... 158

4.3 CLINICAL IMPLICATIONS ..... 166

4.4 LIMITATIONS ..... 168

4.5 FUTURE RESEARCH ..... 173

REFERENCES ..... 180

APPENDICES ..... 205

APPENDIX 1 - HSST Modules completed in addition to the C2 research component .....206

APPENDIX 2 - Foetal Echocardiogram Standard Operating Procedure for University Hospitals of Bristol and Weston .....207

APPENDIX 3 - University hospitals of Bristol and Weston NHS Foundation trust, Research and Development Sponsorship Agreement.....208

APPENDIX 4 - Evidence that Organisational Information Document (OID) or a Schedule of Events (SoECAT) is not required as part of HRA application .....209

APPENDIX 5 - Health Research Association (HRA) Study Approval.....210

APPENDIX 6 - Project Proposal .....211

APPENDIX 7 - Manchester Metropolitan University Feedback from Project Proposal .....236

## LIST OF FIGURES

Figure 1 Cardiac Embryology 4-8weeks gestation ..... 18

Figure 2 MRI derived myofibre architecture of 3 myocardial layers ..... 20

Figure 3 Relative size of the foetal heart in pregnancy ..... 21

Figure 4 Regional distribution of successful antenatal diagnosis across UK and Republic of Ireland ..... 27

Figure 5 FASP Cardiac Protocol ..... 30

Figure 6 Foetal echo 4 chamber view demonstrating the left and right sided chambers. ..... 31

Figure 7 Pictorial examples of foetal orientation in relation to maternal obstetric position ..... 32

Figure 8 Orientations of the 4-chamber view depending on foetal position ..... 33

Figure 9 Left ventricular function mechanics. ..... 38

Figure 10 3 Orthogonal planes of myocardial motion. ..... 40

Figure 11 M-Mode recording of left ventricular radial contractility ..... 42

Figure 12 Left ventricular cavity motion over time in a short axis orientation of the foetal heart ..... 43

Figure 13 Manual trace of the LV endocardial border in both diastole and systole ..... 45

Figure 14 Tricuspid Annular Plane of Systolic Excursion (TAPSE) ..... 46

Figure 15 Orientation of the left ventricular (LV) motion during contractility ..... 49

Figure 16 Left ventricular walls and regions relating to coronary artery distribution ..... 51

Figure 17 Left ventricular strain ..... 52

Figure 18 Relative size of the foetal heart at 20 weeks ..... 53

Figure 19 Anatomical regions of the foetal myocardial walls. ..... 59

Figure 20 2D CPA foetal cardiac border contours.....60

Figure 21 Longitudinal strain curves.....63

Figure 22 Synchrotron x-ray phase contrast imaging of human foetal hearts.....72

Figure 23 Relative disproportion between the left and right sides of the foetal heart. 73

Figure 24 Foetal Echo sagittal images of the aortic and ductal arches with and without dynamic advanced flow .....79

Figure 25 Anatomical positions for foetal cardiac dimensions. (Schneider 2005) .....95

Figure 26 Optimisation of the 4-chamber view .....96

Figure 27 2D CPA m-mode timing.....100

Figure 28 Anatomical markers for CPA analysis. .....101

*Figure 29 Process of manually adjusting endocardial border definition* .....102

Figure 30 Graphical representation of the percentage change in strain across 4 cardiac cycles with the automated quantification. .....104

Figure 31 Flipping process and analysis of the LV.....105

Figure 32 Example of age-related reference ranges for foetal cardiac measurements .....108

Figure 33 Bland-Altman Example.....111

Figure 34 Example of a one-way ANOVA with Bonferroni multiple comparison test..112

Figure 35 One-way ANOVA comparing Control, CHD, LVIM + CHD and LVIM + no CHD with foetal cardiac measurements. .....119

Figure 36 Comparison plots .....120

Figure 37 Flow Chart mapping outcomes .....121

Figure 38 GLS across initial 3 groups.....138

*Figure 39. Comparison of LV GLS, RV GLS and RV Free wall* .....139

*Figure 40 Bland-Altman repeatability plots* ..... 143

Figure 41 Plots of GLS correlation ..... 144

*Figure 42 Logistic regression analysis of the RV/LV ratio* ..... 145

*Figure 43 Logistic regression analysis of the MV/TV ratio* ..... 147

*Figure 44 Comparison of LVS, RVS and RV Free wall between subjects classified as normal (n = 40) and those classified as abnormal (n = 77) on their echo* ..... 149

*Figure 45 Comparison of LV GLS, RV GLS and RV Free wall* ..... 150

## LIST OF TABLES

Table 1 Short- and long-term prognosis for the FASP 4 major criteria conditions.....28

Table 2 Summary of the foetal echo protocol .....37

Table 3 Literature review of the left ventricular (LV) and right ventricular (RV) strain values at 20 weeks (%) .....66

Table 4 Summary of the post-processed research components on TomTec .....94

Table 5 Data output obtained from 2D CPA analysis, TomTec Arena. .....105

Table 6 Two-Way Anova for ICC.....109

Table 7 ICC Score and corresponding reliability .....110

Table 8 Repeatability coefficient for reliability.....110

Table 9 Summary of data from each cohort giving mean ± SD for each index .....117

Table 10 Summary of the z-score data Mean +/- S.D. .....122

Table 11 Summary of the LVIM data .....124

Table 12 Pregnancy outcomes for the LVIM cohort .....127

Table 13 Summary of the CHD Cohort data.....129

Table 14 Confirmed post-natal congenital heart defect (CHD) diagnoses where ± indicates additional cardiac defects.....131

Table 15 CHD group sub-divided into those with a significantly small LV (z-score > -2.0) and those with a LV z-score < -2.0 .....132

Table 16 Summary of the Control Data .....133

Table 17 Foetal Cardiac Strain Analysis Interobserver Variability .....140

Table 18 GLS values obtained between operator 1 and 2 with calculated variability .142

Table 19 Summary of logistic regression analysis for RV/LV ratio.....146

Table 20 Summary of logistic regression analysis for MV/TV ratio .....147

Table 21 Recommendations for future STE study design.....175

Table 22 Appendices.....205

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 2D | 2 Dimensional |
| AoV | Aortic Valve |
| AUC | Area Under the Curve |
| AV | Atrio-ventricular |
| AVSD | Atrio-ventricular Septal Defect |
| BMI | Body Mass Index |
| BVR | Bi-Ventricular Repair |
| CHD | Congenital Heart Disease |
| CoA | Coarctation of the Aorta |
| CPA | Cardiac Performance Analysis |
| CW | Continuous Wave |
| DICOM | Digital Imaging and Communication in Medicine |
| ECHO | Echocardiogram |
| EF | Ejection Fraction |
| ET | Ejection Time |
| FAC | Fractional Area Change |
| FASP | Foetal anomaly Screening Programme |
| FGR | Foetal growth Restriction |
| FS | Fractional Shortening |
| HLHS | Hypoplastic Left Heart Syndrome |
| IVCT | Inter-Ventricular Contraction Time |
| IVRT | Inter-Ventricular Relaxation Time |
| IVS | Inter-Ventricular Septum |
| Levo | Levocardia |

| | |
|---|---|
| LV | Left Ventricle |
| M-mode | Motion Mode |
| MAPSE | Mitral Annular Plane Systolic Excursion |
| MPI | Myocardial Performance Index |
| MV | Mitral Valve |
| PA VSD | Pulmonary Atresia with intact ventricular septum |
| PV | Pulmonary Valve |
| PW | Pulsed Wave |
| RoC | Receiver operating Curve |
| RV | Right Ventricle |
| SoP | Standard Operating Procedure |
| STE | Speckle Tracking Echocardiography |
| T13 | Trisomy 13 (Patau's Syndrome) |
| T18 | Trisomy 18 (Edward's Syndrome) |
| T21 | Trisomy 21 (Downs Syndrome) |
| TAPSE | Tricuspid Annular Plane Systolic Excursion |
| TAPVD | Total Anomalous Pulmonary venous drainage |
| TGA | Transposition of the Great Arteries |
| ToF | Tetralogy of Fallot |
| TV | Tricuspid Valve |
| UHBW | University Hospitals of Bristol and Weston NHS Foundation Trust |
| UVR | Uni-ventricular Repair |
| VSD | Ventricular septal defect |
| VVI | Vector Velocity Imaging |
| WMT | Wall Motion Tracking |

# CHAPTER 1 INTRODUCTION & BACKGROUND

## 1.1 CARDIAC EMBRYOGENESIS

Cardiac embryogenesis occurs in the first 6-7 weeks of human development (Trines & Hornberger, 2004) and the foetal heart is completely formed by week 8 of pregnancy. The heart is the first functional organ whose form changes considerably during development and disease (Crispi, Sepulveda-Martinez, & Crovetto, 2020), therefore normal cardiac development relies on interplay between patterns of gene expression and cues from vascular flow (Andres-Delgado & Mercader, 2016). From the very primitive cardiac tube, seen in the very early stages of cardiac embryogenesis, the various components of the cardiac anatomy develop in sequence after cardiac looping of the heart tube has occurred followed by the septation processes to create the left and right components of the heart (figure 1).

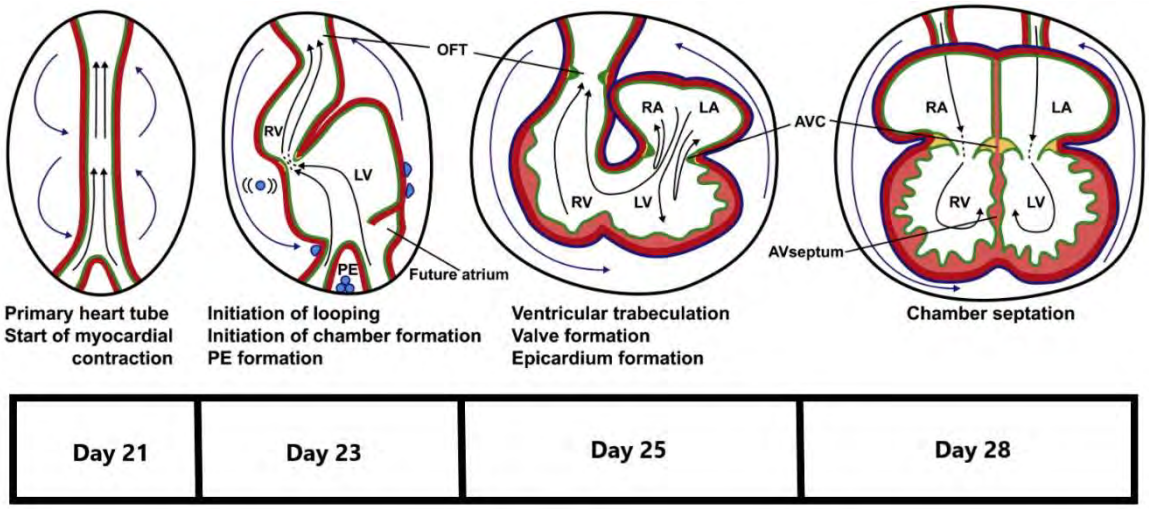



*Figure 1 Cardiac Embryology 4-8weeks gestation*

*(adapted from (Andres-Delgado & Mercader, 2016))*

*<u>At day 21</u> of pregnancy the primitive heart tube provides contractile function to drive the foetal circulation.*

*<u>At day 23</u> segmentation begins to differentiate the 5 primitive components of the foetal heart – Sinus venosus comprising the venous structures, Primitive atria which becomes part of the future atrial wall, Primitive Ventricle which forms the majority of the Left Ventricular (LV) mass, Bulbus Cordis which becomes the main component of the right ventricle and right ventricular outflow (OFT) and the Truncus Arteriosus which septate to become the 2 great arteries (Aorta and Pulmonary Artery).*

*<u>At day 25</u> the looping process has occurred to twist the primitive longitudinal heart tube into a complex C shape moving the atria towards the foetal heart and leftward. Each of the segments develop further structure and bulbar ridges form for the future atrio-ventricular canal (AVC) and valve formation.*

*<u>At day 28</u> the looping process, septation processes and valve formation are completed giving rise to left and right ventricles (LV & RV respectively) and right and left atria (RA & LA) due to the AV septum.*

Ventricular muscle is composed of 3 distinct myofiber layers, endocardium, myocardium, and epicardium (figure 2) which overlap one another in differing orientations to create a complex 3-dimensional mesh to support systemic high blood pressure and volume. The 3 myocardial layers have different characteristics with the inner subendocardial layer orientated along the length of the cavity contributing to longitudinal function. The opposing direction of the subepicardial myofibers contribute towards the rotational twist mechanism. This twist, during systole, acts like a wringing motion which deforms the subendocardial matrix, resulting in the storage of potential energy (Patey, Carvalho, & Thilaganathan, 2020). This energy is then released in the recoil of the twist formation contributing to LV diastolic relaxation and diastolic filling. (Patey, Carvalho, & Thilaganathan, 2020) (Sengupta, Tajik, Chandrasekaran, & Khandheria, 2008)

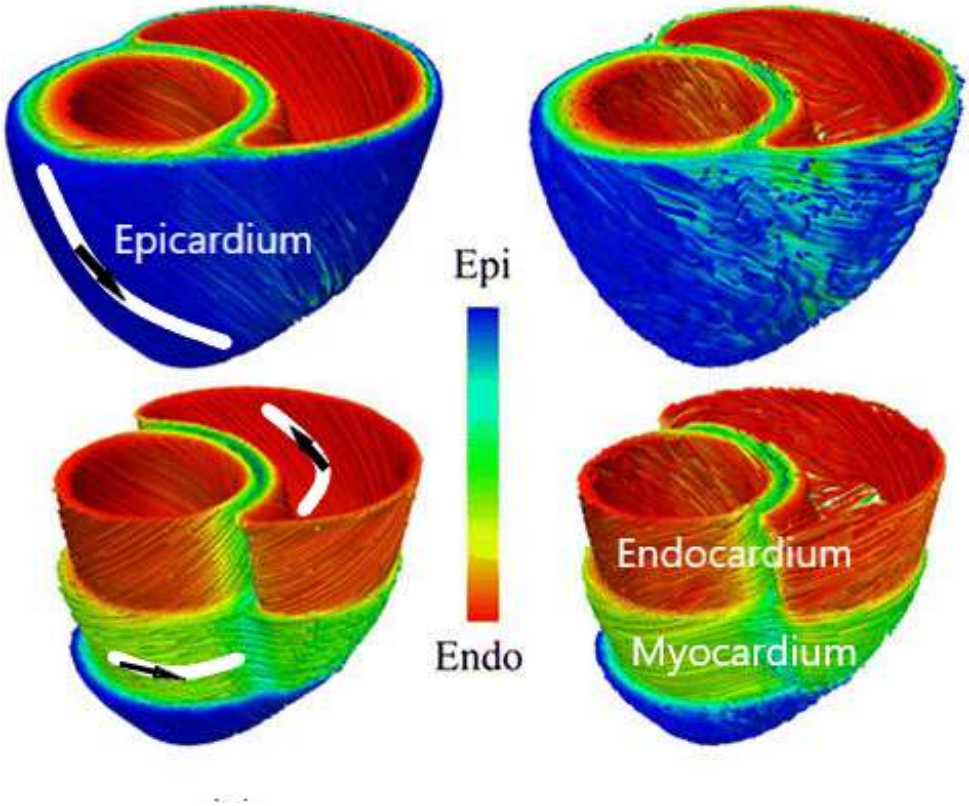



*Figure 2 MRI derived myofibre architecture of 3 myocardial layers*

*(adapted from (Lopez Perez, Sebastian, & Ferrero, 2015))*

The 3 layers of muscle have been colour coded to differentiate between the internal and external layers. The internal layer in red is the endocardium, the mid-layer in green is the myocardium and the outer layer in blue is the epicardial layer. Complex striations can also be seen with differing orientations for understanding twist dynamics of the muscle (Sengupta, Tajik, Chandrasekaran, & Khandheria, 2008).

Where there is a malformation in the structure of the foetal cardiac anatomy or alterations in normal blood flow distribution, this process can be accelerated or delayed by manipulating loading conditions (Sengupta, et al., 2006). The foetal heart remodels, changing in shape and structure to adapt to any insult including congenital heart defects (Crispi, Sepulveda-Martinez, & Crovetto, 2020). The detection of these defects is not usually evident until at least 12 weeks gestation due to the relative size of the foetal heart within the maternal uterus (figure 3). As such, the impact of such a malformation

may not be visually detectable until much later in pregnancy, if at all. The reason for this is due to the varying ability and quality for imaging the foetal heart as well as the ability for the foetal heart to adapt and maintain optimal and efficient function (Crispi, Sepulveda-Martinez, & Crovetto, 2020). Figure 3 provides an overview of the relative size of the foetal heart and the best ultrasound imaging which can potentially be achieved along the pregnancy timeline.

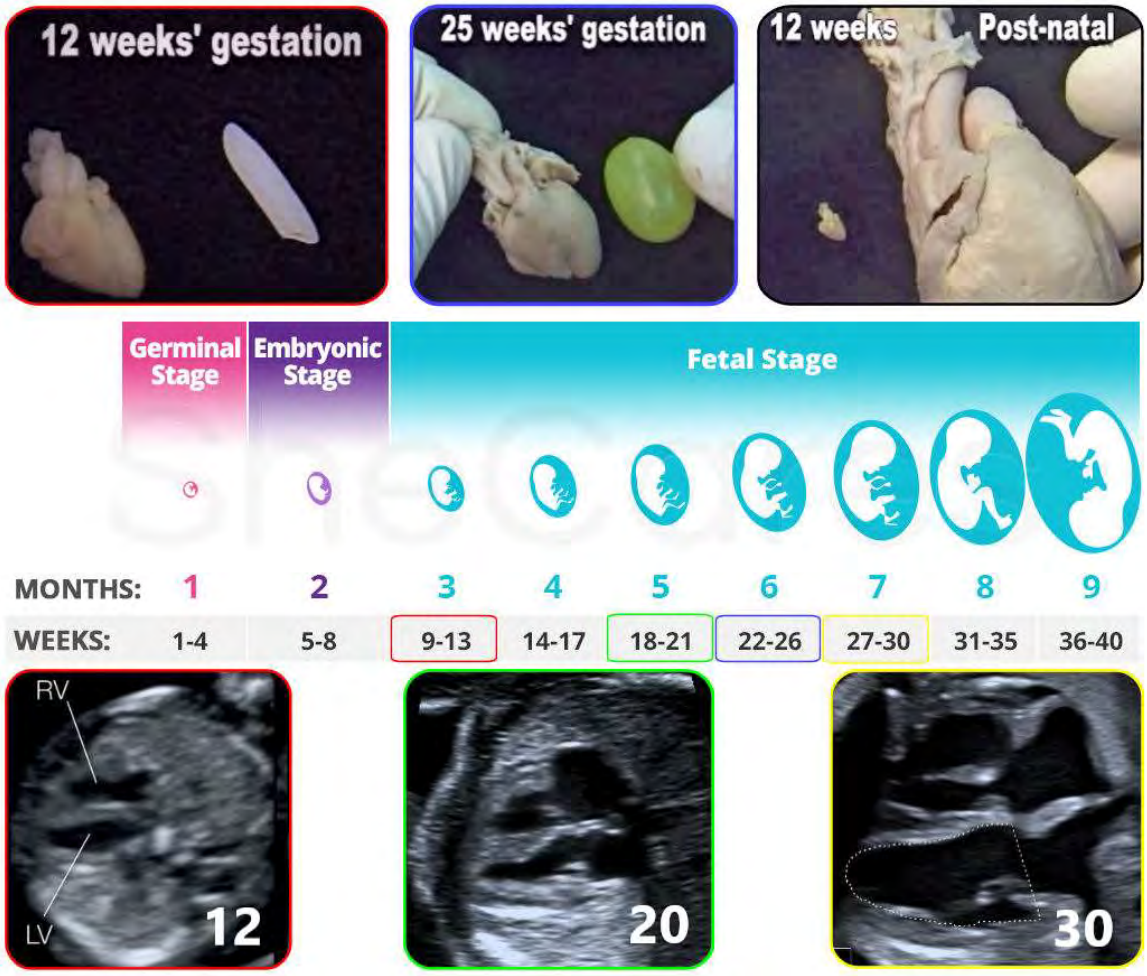



*Figure 3 Relative size of the foetal heart in pregnancy*

*At 12 weeks gestation, foetal development is over a quarter of the way along the pregnancy. The heart is fully formed but is only the size of a grain of rice. With optimal foetal position and maternal habitus, the ventricles can be viewed in a 4-chamber view (12). At mid-gestation, the heart is approximately the size of a small grape but the 4 chambers of the foetal heart can be well visualised with optimal foetal and maternal imaging (20). In later gestation, the anatomical structures are more clearly defined at 30 weeks but at this stage the foetal bones cast shadows over the heart and limit optimal imaging.*

The optimal time for consistent and accurate visualisation of the foetal heart is between $18^{+0}$ – $20^{+6}$ weeks gestation (PHE P. H., 2018).

## 1.2 CONGENITAL HEART DISEASE & THE ROLE OF ANTENATAL DIAGNOSIS

Congenital heart disease (CHD) is defined as a malformation of the heart, aorta, or other large blood vessels that is present at birth (Hoffman & Kaplan, 2002). It remains one of the most common congenital anomalies detected in antenatal screening (Menahem, Sehgal, & Meagher, 2021) and the leading cause of neonatal mortality and morbidity (Chung M, 2010). The incidence has remained constant at approximately 0.8% to 1.2% of live births worldwide (Van Der Linde, et al., 2011), and a prenatal diagnosis and planned delivery in a tertiary neonatal intensive care unit are factors affecting CHD outcomes, especially when defects are 'complex' (Chung M, 2010). Foetal cardiology is a tertiary level, specialist field of medicine which provides diagnostic and enhanced screening for congenital heart disease in the foetus. When a foetal abnormality is detected, referral is made for a foetal cardiac ultrasound examination (foetal echocardiogram) and non-directive counselling is given to families affected by a CHD diagnosis. Depending upon the severity of the lesion, families are given options to consider; these include,

1) observation of the progression of the condition throughout the pregnancy with a view to deliver the baby and intervene medically or surgically where necessary,
2) observation throughout pregnancy with a view to provide palliative care after birth,
3) termination of the pregnancy under section D of the Abortion Act 1967 "where there is a substantial risk that if the child were born it would

> suffer from such physical or mental abnormalities as to be seriously handicapped" (Gov, 1967)

Due to the maternal risks involved with a late gestation medical termination, it is strongly discouraged after 24 weeks and feticide is recommended by the Royal College of Obstetricians for pregnancies over $24^{+6}$ weeks (Peckham C, 2010). This places considerable time restraints and responsibility on foetal cardiology clinicians to ensure that the counselling of a significant cardiac condition pertains to an accurate diagnosis so that patients can make this difficult decision with medically informed choice.

As such, it is imperative that the clinicians have adequate imaging for an accurate diagnosis and evidence-based information for prognosis. This can be very challenging due to the limitations inherent in obstetric scanning (Crispi, et al., 2012) including increased maternal body mass index and suboptimal foetal lie. Whilst there are numerous methods for assessing severity and functionality of heart defects in postnatal transthoracic echocardiography (scanning directly onto the chest wall), the ability to perform these is limited when scanning via the maternal abdomen. Furthermore, many congenital heart lesions are progressively evolving during growth and maturation (Menduina, 2015). In the early stages of an insult, the foetal heart can often adapt and remodel to preserve blood flow, contractility, and myocardial perfusion (Crispi, Sepulveda-Martinez, & Crovetto, 2020). However, what may be considered as a minor cardiac deviation from normal at 20 weeks gestation, may progress into a considerably more significant lesion at birth or/and beyond. In addition, up to 30% of cases of CHD have extracardiac malformations including chromosomal defects which may affect pre- and post-natal outcomes (Allen, 2010). As such, post-natal implications for long term morbidity and mortality can be difficult to predict especially when considerations must

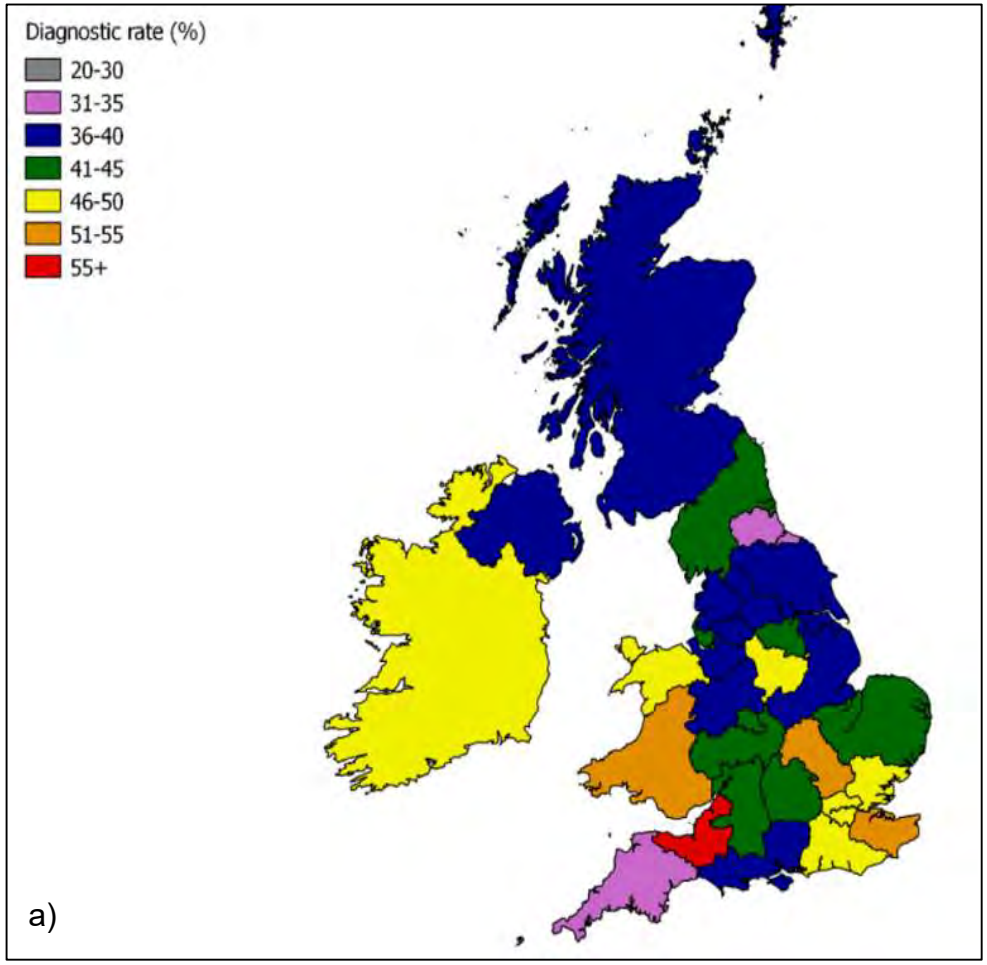
be made for spontaneous intrauterine loss, unanticipated prematurity, unexpected extracardiac malformations and complications of corrective cardiac surgery (Allen, 2010).

This creates an obligation for clinicians to inform patients of the best- and worst-case scenario, for cardiac conditions (and possible extra-cardiac associations) which may have implications for 'termination verses continuation of the pregnancy' (Eckerseley, 2017). Where there is a size discrepancy between the size of the right and left chambers of the foetal heart without an obvious defect to account for this presentation, outcome predictions are not only challenging, but the potential severity spectrum is vast.

A disproportion of the size of the ventricles may normalise during the remainder of the pregnancy or this presentation can be attributed to a simple haemodynamic change that leads to an unbalanced filling of the ventricles, for example where aneurysmal atrial foramen ovale tissue causes flow obstruction into the left ventricle. This physiological anomaly often normalises once the septal tissue is fused to the atrial septum after birth, when the pressure changes occur in the post-natal circulation (Urbinelli, et al., 2015). In these scenarios the post-natal cardiac appearances are unremarkable for any cardiac defect. However, if the ventricle fails to grow at the same rate as the rest of the heart, during the remainder of the pregnancy, then a single ventricle palliative route may be indicated with considerable impact on quality of life and life expectancy. For families who are informed of these findings there is considerable anxiety and uncertainty about the potential outcome of the condition and towards the options to consider in mid-pregnancy (Miranda, et al., 2017).

### 1.2.1 Screening for Congenital Heart Defects

The foetal anomaly screening programme (FASP) was formally introduced in 2001 after years of using biomedical markers only, for antenatal assessment for common syndromic and congenital abnormalities (PHE P. H., 2018). The rapid advance of imaging technology has led to considerable clinical benefit over biomedical markers which were inherently ambiguous (Getz & Kirkengen, 2003). FASP is one of 11 screening programmes in England offering women, who are eligible, access to a series of screening parameters to assess the risk of their unborn baby having a chromosomal or congenital abnormality. Screening provides information and clinical guidance on a range of conditions which may benefit from treatment before or after birth, indications for the most appropriate place of birth and information regarding conditions where the baby may die shortly after birth (PHE P. H., 2018). Congenital heart disease is one of the conditions which is screened between $18^{+0}$ and $21^{+6}$ weeks of pregnancy at the anomaly scan, and antenatal detection retains an important role in reducing mortality related to critical CHD (Eckersley, Sadler, Parry, Finucane, & Gentles, 2014) (Van Velzen, Ket, Van de Ven, Blom, & Haak, 2018). Around half of all cardiac defects are regarded as major, requiring surgery or intervention within the first year of life, but detection rates for congenital heart disease across the UK average 50% with vast geographical variation (Figure 4, NHS, 2016). Foetal cardiac assessment is considered the most challenging component of the anomaly scan, probably due to the rapid movement of the heart in addition to the movement of the fetus (Allen, 2010). Figure 4 demonstrates the regional variation of antenatal detection of congenital heart disease and the change in detection rates between 2014-2017 and 2016-2017 (NICOR, National institute for cardiovascular outcomes, 2017).



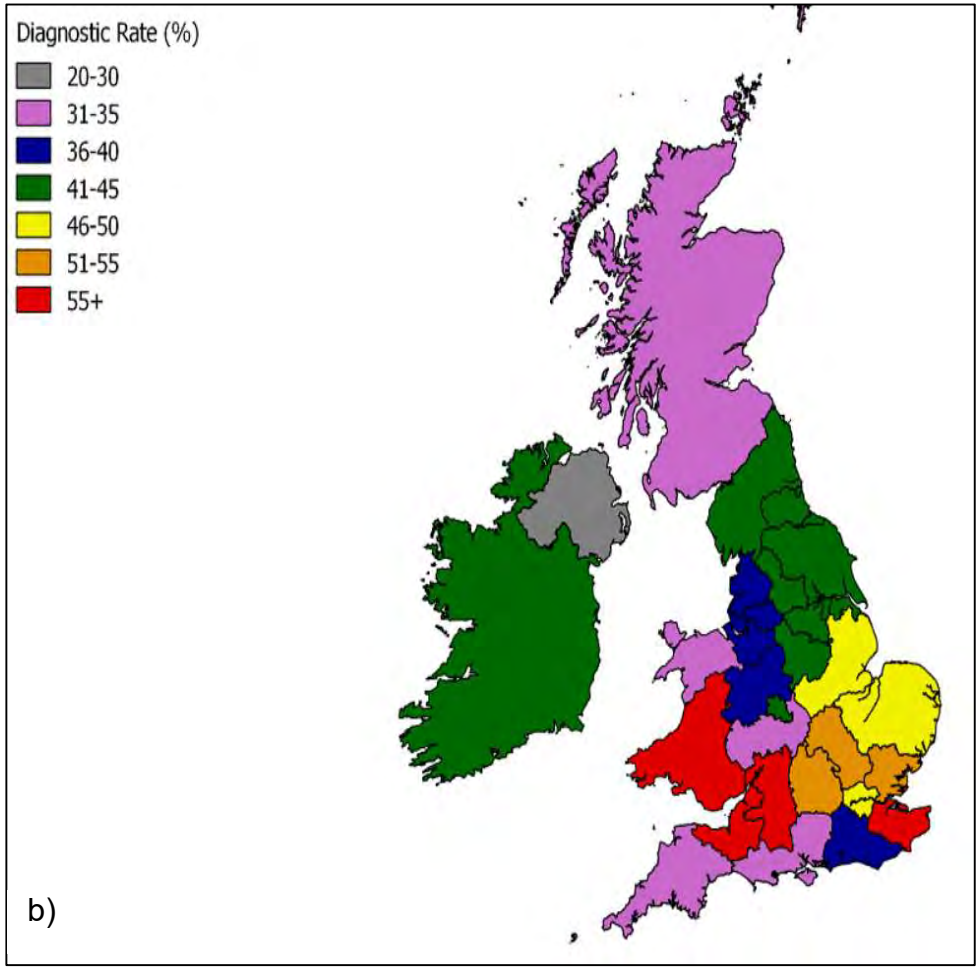




*Figure 4 Regional distribution of successful antenatal diagnosis across UK and Republic of Ireland*

*Each map provides the colour coded average percentage regional antenatal detection rates of congenital heart disease. Map a) demonstrates the diagnostic rates between 2014-2017, whilst map b) shows the change in diagnostic rates in 2016-2017. This demonstrates the variability across the UK in antenatal detection success and where there is inconsistent progression in these rates [image adapted from NICOR report] (NICOR, National institute for cardiovascular outcomes, 2017)*

FASP have focused antenatal detection on 4 of the most common serious heart defects – Hypoplastic Left Heart Syndrome (HLHS), Tetralogy of Fallot (ToF), Atrio-Ventricular Septal Defect (AVSD) and Transposition of the Great Arteries (TGA). These cardiac conditions fall within the major criterion with severe consequences in terms of the impact they have on survival and long-term prognosis (Table 1).

***Table 1 Short- and long-term prognosis for the FASP 4 major criteria conditions***

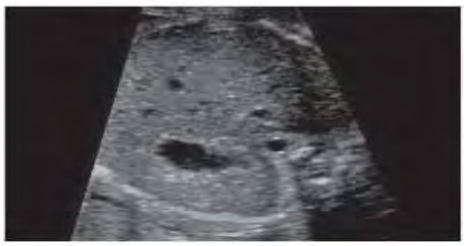
| | No antenatal diagnosis | Post-Surgical outcomes |
|---|---|---|
| **Transposition of the great Arteries (TGA)**<br>**4.7 in 10,000 live births**<br>**3% of all CHD (Szymanski, Moore, & Kritzmire, 2021)** | Untreated, more than 50% of infants will die in the first month of life, 90% in the first year. (The Society of Thoracic Surgeons, 2015-2021) | UK - 30-day post-surgical repair survival 98.5% (NICOR, National institute for cardiovascular outcomes, 2017)<br>90% of patient survive into adulthood (Dominguez-Manzano, et al., 2016) |
| **Tetralogy of Fallot (TOF)**<br>**0.23-0.63 in 1000 live births**<br>**7-10% of all CHD**<br>**(Bhimji, 2018)** | Infants present with cyanosis, murmur, and failure to thrive within the first year of life.<br>Without surgery, TOF can be fatal with long term survival limited to 20 years of age (The Society of Thoracic Surgeons, 2015-2021) | UK 30-day post-surgical repair survival 99.5% (NICOR, National institute for cardiovascular outcomes, 2017)<br>Prognosis has improved significantly after surgical correction with the survival of up to 25 years post-repair (Apitz, Webb, & Redington, 2009) |
| **Hypoplastic Left Heart Syndrome (HLHS)**<br>**1 in 100,000 live births**<br>**7-9% of all CHD (National Organization for Rare Disorders, 2003)** | Left untreated life-threatening complications usually occur within 48hrs (National Organization for Rare Disorders, 2003) | UK 30-day post-surgical survival 89.6% (NICOR, National institute for cardiovascular outcomes, 2017) |
| **Atrioventricular septal defect (AVSD)**<br>**4-5.3 in 10,000 live births**<br>**75% occur in individuals with Down's Syndrome (Calkoen, et al., 2016)** | Around 50% of the patients die during infancy, either due to heart failure or pulmonary infections (Ahmed & Anjum, 2011) | UK 30-day post-surgical repair survival 98.5% (NICOR, National institute for cardiovascular outcomes, 2017) |

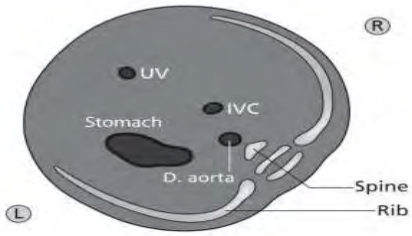
| | | |
|---|---|---|
| NB: The incidence of each condition is based on the UK's National Congenital Anomaly System of live births which is not a true reflection of prevalence. This data excludes pregnancies terminated after diagnosis, and/or foetal demise in pregnancies affected with congenital heart disease **(Gardiner, et al., 2014)** | | |

These 4 conditions have been specifically selected for audit and quality assurance monitoring of the most severe cardiac lesions which can be detected in antenatal screening. However, the presentation of each of these conditions can vary in terms of severity and may be challenging to confirm. In addition, other cardiac defects, such as total anomalous pulmonary venous drainage (TAPVD) (which is also a life-threatening cardiac condition) are extremely difficult to exclude. TAPVD can be seen either in isolation, or as a component to a complex cardiac condition and has a significant impact on prognosis. It is therefore important to recognise the limitations inherent to all antenatal screening and obstetric scanning, in that antenatal detection will never become 100% diagnostic for all congenital abnormalities.

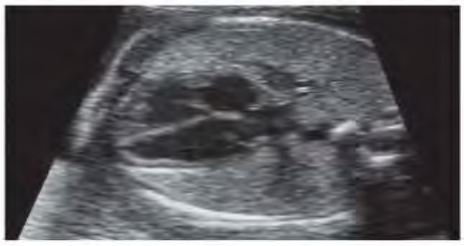
The cardiac screening component of the FASP protocol (Figure 5) involves experienced and Healthcare Professionals Council (HCPC) registered sonographers providing ultrasound images and evaluation of 5 axial views of the foetal cardiac anatomy. This is in addition to the measurements and assessment of the remaining foetal anatomy. 5 standard views form the basis of normal cardiac anatomy screening and, where normal, can exclude most major congenital heart defects. Beyond the structural features of the foetal heart, functional assessment is limited to visual evaluation of apparently normal cardiac contractility and a normal foetal heart rate as observed by non-cardiac specialist sonographers.

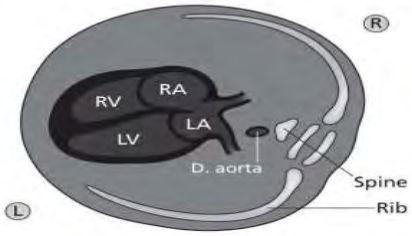
**Visceral situs/laterality**



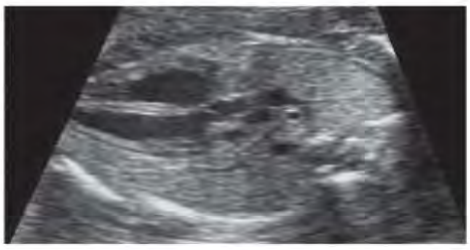


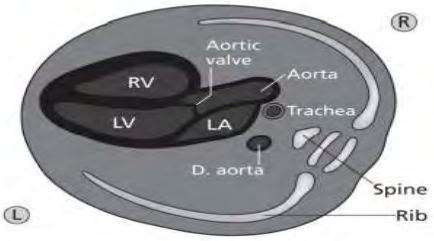

**4 chamber view (4CH)**



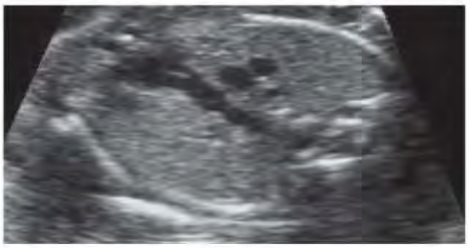


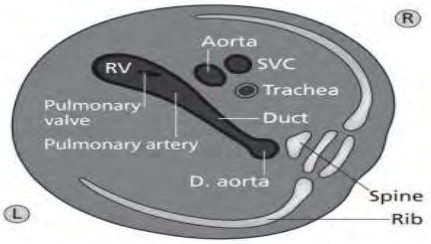

**Aorta (AO)/left ventricular outflow tract**



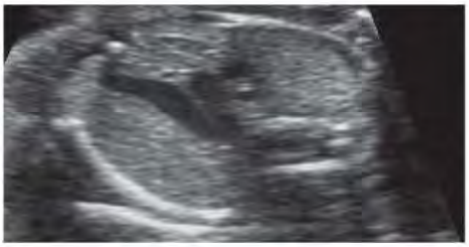



**Pulmonary artery (PA)/right ventricular outflow tract or 3 vessel view (3VV)**






**3 vessel and trachea view (3VT)**



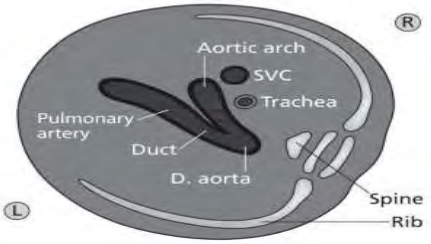



*Figure 5 FASP Cardiac Protocol*

*[figure taken from NHS FASP Programme Handbook] (PHE P. H., 2018)* The ultrasound images here demonstrate the axial plane images which are the minimum requirement for foetal cardiac assessment and pictorial representation of normal cardiac anatomy. This is the basis of the foetal cardiac examination performed during the 20-week anomaly scan.

### 1.2.2 Foetal Cardiac Anatomy & assessment at Mid-Gestation

Foetal cardiac assessment by ultrasound is optimal between $18^{+0}$-$20^{+6}$ weeks due to the relative proportions of the foetal cardiac size to fetus ratio. One of the earliest introduced, and most fundamental components of foetal cardiac assessment is that of the 4-chamber view in an axial thoracic plane (Figure 6). In this view, the left and right sided structures - atria (collecting chambers), ventricles (pumping chambers) and the AV valves can be visualised and evaluated for anatomical normality.

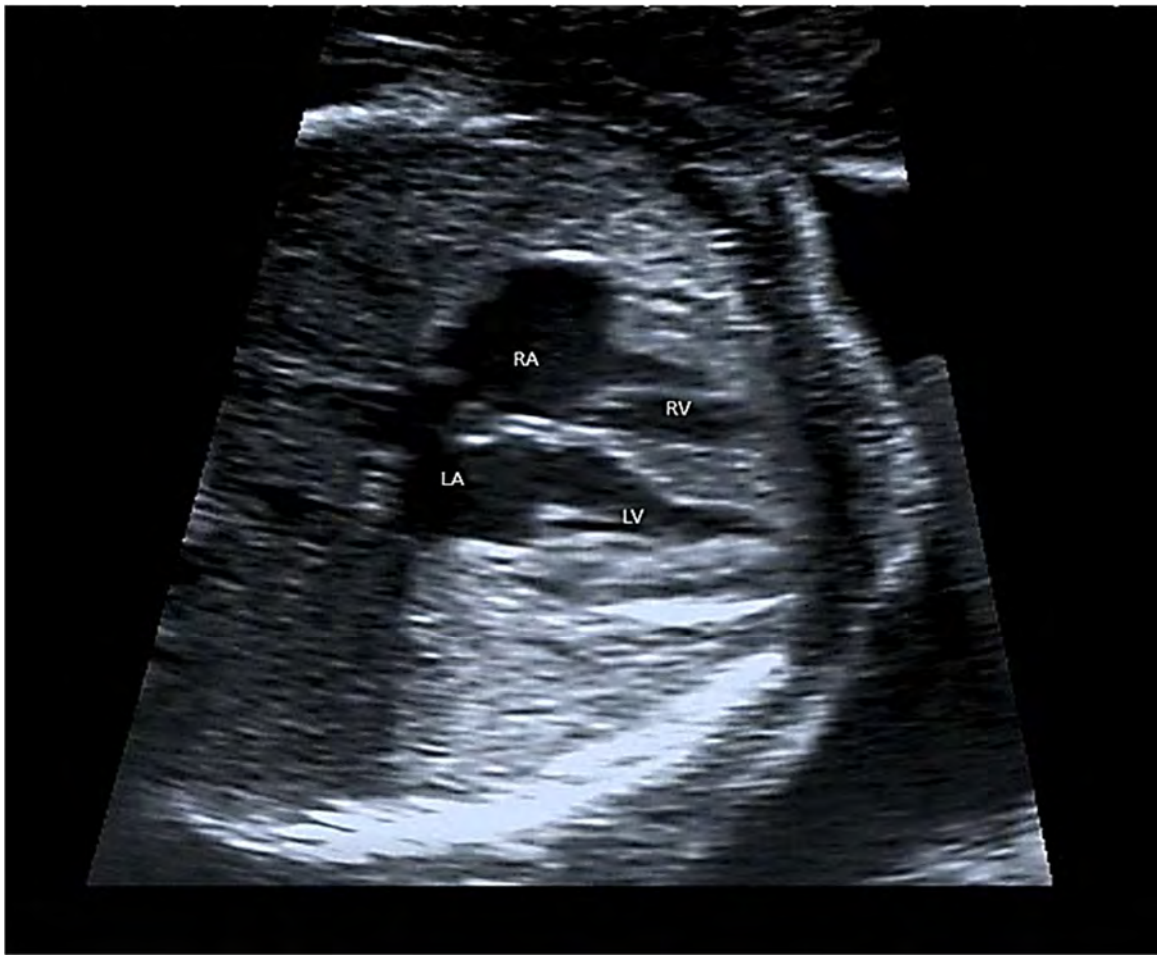



*Figure 6 Foetal echo 4 chamber view demonstrating the left and right sided chambers.*

*Ultrasound image taken at 20 weeks gestation of a transverse view of the foetal thorax including the foetal heart. RA: Right atrium, RV: Right Ventricle, LA; Left atrium, LV; Left Ventricle*

The ventricles are usually of equal size (Gabbay-Benziv, Turan, Harman, & Turan, 2015) at 20 weeks gestation and evaluation of this anatomical presentation is made by experienced sonographers performing the 20-week anomaly scan as a visual observation of balance. In foetal echo, the anatomical dimensions between the valves and the length of the ventricles are studied using age-related z-score comparisons against normal distribution curves to determine the balance of the ventricles. Examination of the foetal heart anatomy, however, can often be compromised by foetal

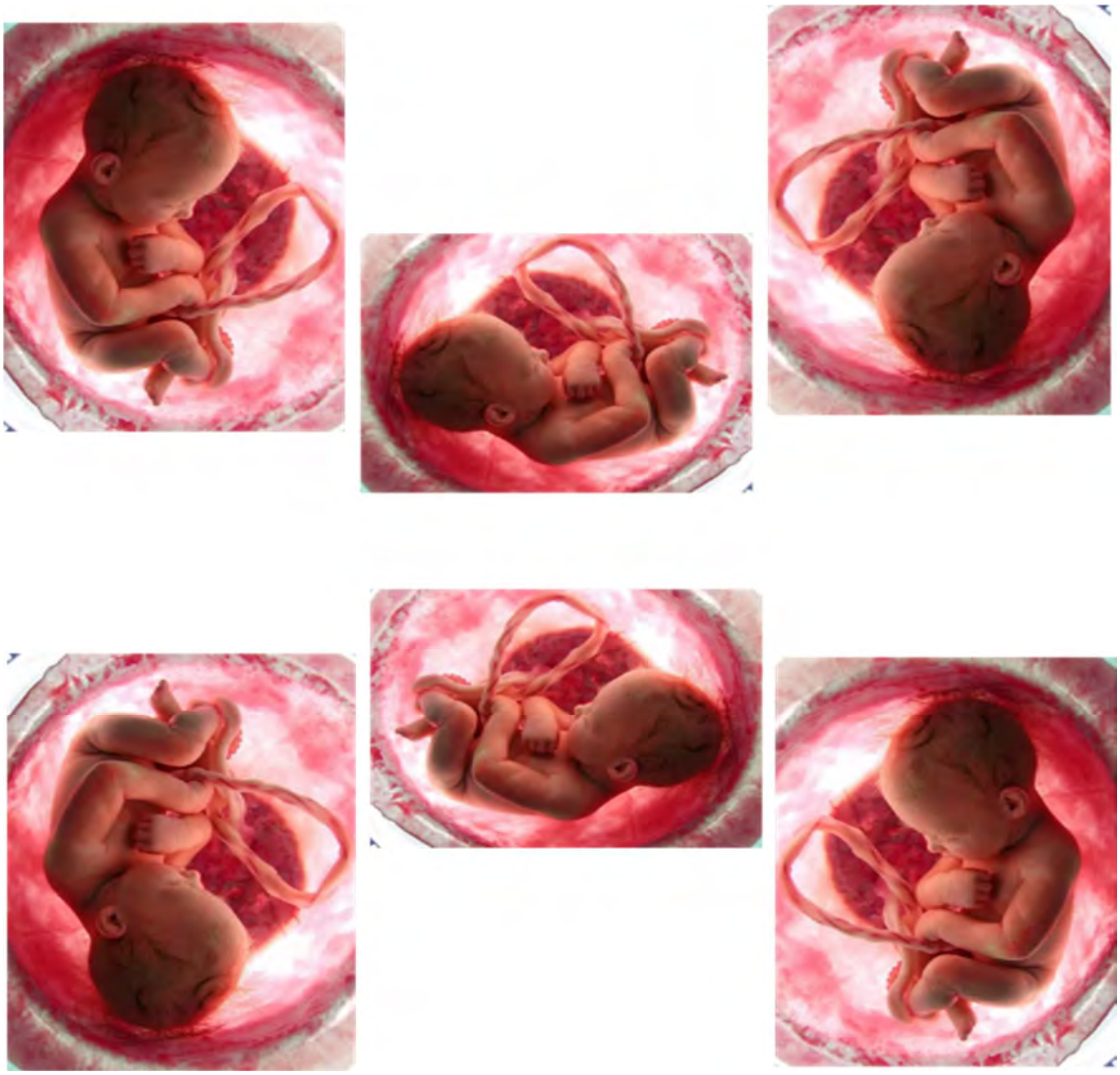


*Figure 7 Pictorial examples of foetal orientation in relation to maternal obstetric position*

*[images adapted from (DeVore GR P. B., 2016)]*

position, maternal habitus and foetal orientation. Figure 7 demonstrates the range of foetal positions which alter the orientation of the foetal heart.

Depending upon this orientation, the 4-chamber view is presented in a range of positions depending on foetal lie (figure 8).

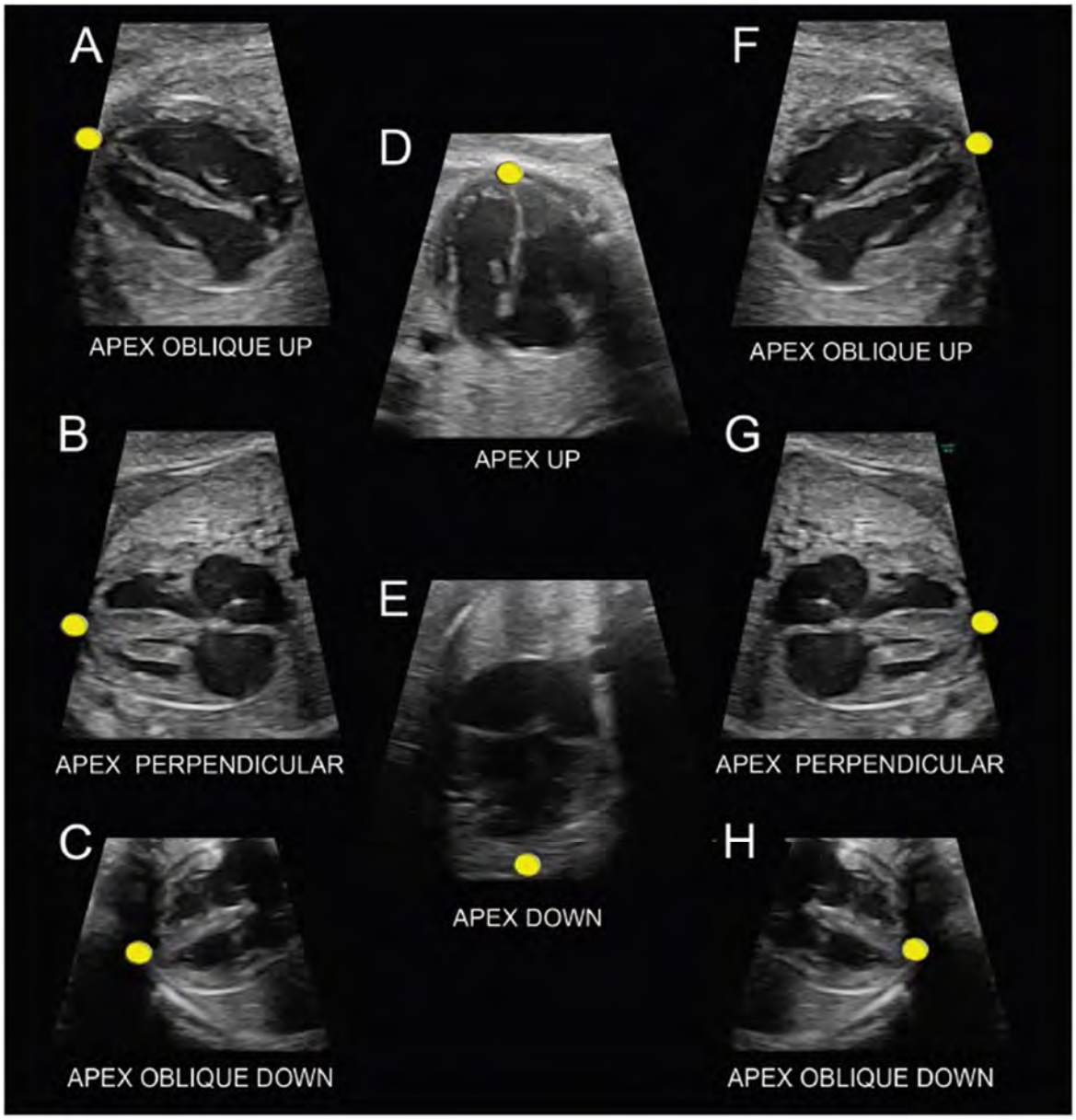



*Figure 8 Orientations of the 4-chamber view depending on foetal position*

*[images adapted from (DeVore GR P. B., 2016)]*

The foetal spine will completely obscure a 4-chamber view (as in example E, in Figure 8), when the fetus is in an occiput anterior orientation. This is largely due to the shadow

created by the dense bones of the spine. Increased maternal body mass index (BMI) requires increased ultrasound penetration which in turn compromises satisfactory resolution of the intricate cardiac structures. These factors, among others which cause suboptimal imaging, can be detrimental to performing a comprehensive examination of the cardiac anatomy. Poor imaging and foetal/maternal movement also affect the ability to perform various measures of contractility and functionality in the foetal heart.

It is also important to consider that the 20-week anomaly scan is an examination which only provides screening to exclude cardiac abnormalities which are evident at this gestation in the form of a major lesion; any minor defect which has yet to have an impact on the relative flow and functional capacity of the heart can often be missed. This is explained as a limitation to any obstetric ultrasound examination where quality and operator experience make interpretation subjective as a diagnostic tool. As such, it is often specific key markers in the anatomy which may alert the operator to an underlying abnormality rather than an obvious defect. The presentation of an imbalance between the size of the right and left ventricles in mid-pregnancy can be an important indicator of an underlying structural or functional (flow-related) abnormality (Quartermain, et al., 2009) which may otherwise be overlooked. Any subjectively visual difference in size, without an obvious cause, is very difficult to predict whether it will normalise with growth or continue to evolve into a more severe lesion.

## 1.3 FOETAL ECHOCARDIOGRAPHY

Foetal echocardiography is an advanced and specialised diagnostic investigation of the foetal cardiac anatomy which is now a fundamental part of antenatal care (Day, Charakida, & Simpson, 2019). It is a technique which combines the specialist knowledge of paediatric cardiology and congenital heart disease with foetal-perinatal medicine and obstetric ultrasound (Freud & Hornberger, 2021). Foetal echo is a technique performed across professions, but Foetal Cardiology is a service provision within a tertiary centre maintaining an authoritative lead on congenital heart disease diagnosis. Across the UK there is diversity in the geographical locations of foetal cardiology. In most tertiary centres, foetal cardiology is performed alongside the foetal medicine specialists who provide clinical care and management of women with high risk of a foetal abnormality. In other regions, the service is delivered alongside paediatric cardiology. In either domain, the team consists of a foetal/paediatric consultant cardiologist, specialist foetal cardiac sonographer/s, dedicated cardiac nurse specialist/s, psychology support, and administrative support. As well as providing a diagnostic service, there are also national standards to provide screening to women at high risk of having a baby with a congenital heart defect (NHS, 2016). These include, but are not limited to, women with a first-line family history of CHD, women who take certain medications which are known to affect cardiac development processes and women affected by an autoimmune condition.

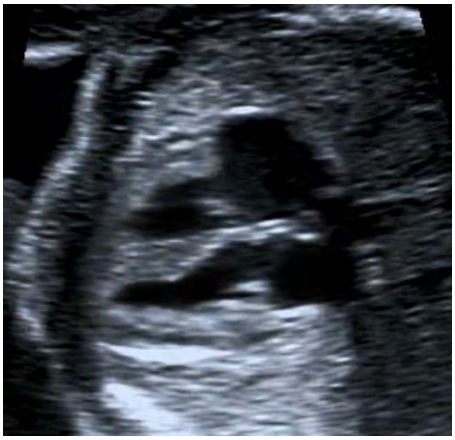
A referral for urgent evaluation should be seen within 3 calendar days (NHS, 2016) indicated by the New Congenital Heart Disease guidelines. An imbalance of the ventricles, as seen in the 4-chamber view at mid-gestation, would fall under the criteria for a significantly abnormal foetal cardiac presentation which would require a highly

specialist foetal cardiac echocardiogram and clinical consultation. Table 2 outlines a summary of the main components assessed in a foetal echocardiogram. The full UHBW Trust Standard Operating Procedure (SoP) for performing a foetal echo can be viewed in Appendix 2.

**Table 2 Summary of the foetal echo protocol**

**Each of these pointers relate to key assessment criteria for evaluating normal foetal cardiac presentation. The 4-chamber view (as demonstrated) is one of the most fundamental components of the protocol**

- Laterality demonstrated via maternal/foetal position and the Cordes technique[1]
- Situs – solitus, inversus, ambiguous (isomerism)
- Cardiac position – levo, meso or dextrocardia
- Connections – AV VA concordance
- Aortic and ductal arch side – left or right
- Systemic and pulmonary venous connections
- Axial – four chamber view



- 2D Cardiac size
- Colour Doppler – inflow TV and MV
- Pulse Doppler - inflow Doppler of TV and MV and LV inflow/outflow Doppler
- LV outflow view with colour Doppler to assess outflow
- Pulsed wave Doppler to determine aortic velocity
- RV outflow view with colour Doppler to assess outflow
- Pulsed wave Doppler to determine pulmonary artery velocity
- Foetal heart rate (BPM)
- Haemodynamic Doppler assessment of the heart rhythm
- Systemic venous Doppler – Inferior Vena Cava / Ductus Venosus
- Pulmonary venous pulse wave Doppler of the left and right sided pulmonary veins

[1] **(Cordes, O'Leary, Seward, & Hagler, 1994)** *Standardised technique for distinguishing left and right in foetal echocardiography*

### 1.3.1 Echocardiographic measures of Cardiac Function

The 3-dimensional complexity of the myocardial layers mean that functional assessment, using 2-dimensional echocardiography, requires multiple methods of assessment. The direction of myocardial lengthening and shortening can be measured to evaluate the effectiveness of contractility, but due to the differing directions of this movement (figure 9), functional assessment is made using a range of different methods which are synonymous with both paediatric and adult echocardiography.

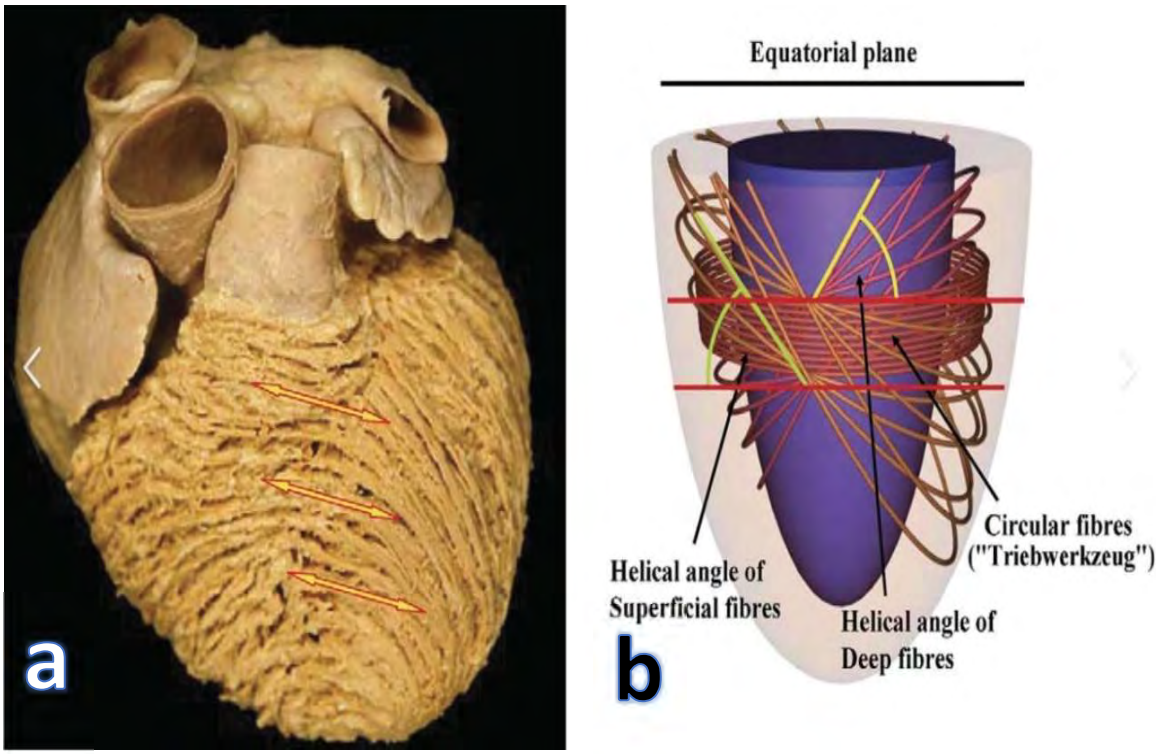



*Figure 9 Left ventricular function mechanics.*

*a) Cardiac anterior wall arrangement of the myofibres are readily seen (red/yellow arrows), enclosing both the right and left ventricles.*

*b) different angles of the myocardial fibres relative to the equatorial plane of the left ventricle, which vary at different depths within the ventricular wall. Note that the circular fibres of the middle layer are parallel to the ventricular equatorial plane.*

*Adapted from (Anderson, Yen Ho, Redmann, Sanchez-Quintana, & Lunkenheimer, 2005)*

The helical arrangement of myofibers is evident in the early stages of cardiac development and is responsible for highly efficient multi-directional contraction. The opposing rotation of the LV apex and the base of the LV creates a wringing motion during systole known as twist. Torsion is the opposing lengthening motion of this contractility or the “normalisation” of twist. Left ventricular (LV) twist and torsion are important aspect of cardiac mechanics and represents circumferential–longitudinal shear strain which greatly augments LV systolic ejection. This shear strain results from a combination of the 3 compacted myocardial layers and the opposing motion of the double helical orientation of the left ventricular architecture (Li, et al., 2017) (Lo, Lai, & Wu, 2013) (Sengupta, et al., 2006). To evaluate myocardial motion, three perpendicular orthogonal planes of myocardial deformation are identified as (A) longitudinal shortening of the endocardium, (B) radial thickening of the mid wall, and (C) circumferential shortening of the epicardium (figure 10).

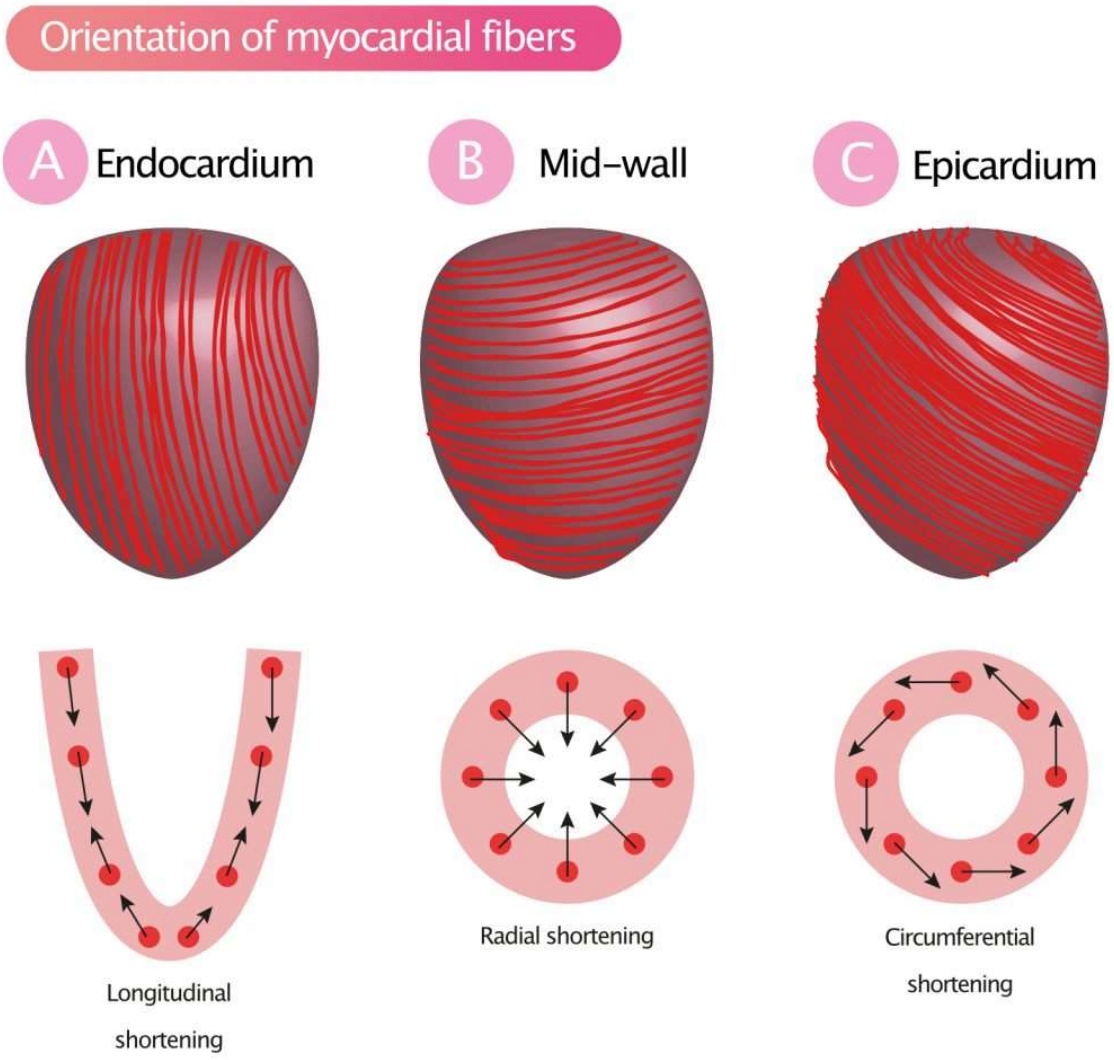



*Figure 10 3 Orthogonal planes of myocardial motion.*

*A Endocardium orientation of myocardial fibres relating to longitudinal shortening,*

*B Mid-wall orientation pertaining to radial shortening,*

*C Epicardium orientation relating to circumferential shortening*

*[adapted from (Triposkiadis, et al., 2018)]*

As well as their contractile properties, torsion and twist are fundamental to left ventricular diastolic function by generating suction for effective filling (Bansal & Kasliwal, 2013) (Lo, Lai, & Wu, 2013) (Patey, Carvalho, & Thilaganathan, 2020).

### 1.3.2 Fractional Shortening

Changes in radial motion are the most traditional method of interpreting the effectiveness of systolic contractility and are relatively easy to quantify. There are several methods used to quantify the percentage change of the left ventricle between systole and diastole. These are usually described as Fractional Shortening of the LV, the volume/area change or Ejection Fraction (Simpson, 2004). The shortening fraction is derived from measurements taken of the left ventricle m-mode to calculate the dimensional cavity change over time (figure 11). The left ventricular internal dimensions (LVID) are measured in both systole and diastole and applied to the equation below to obtain a percentage change.

$$(LVIDd - LVIDs)/LVIDd \times 100.$$

An example of this method, in a foetal echocardiogram can be seen in figure 11 with the left ventricle in a longitudinal plane at 20 weeks gestation. This method can also be measured from an m-mode recording of the left ventricle in a short axis plane as in figure 12. Fractional shortening and Ejection Fraction do not significantly change across gestational age (Eckerseley, 2017) and a Fractional shortening ≥28% is considered normal (Moon, Miyamoto, Younoszai, & Landeck, 2014).

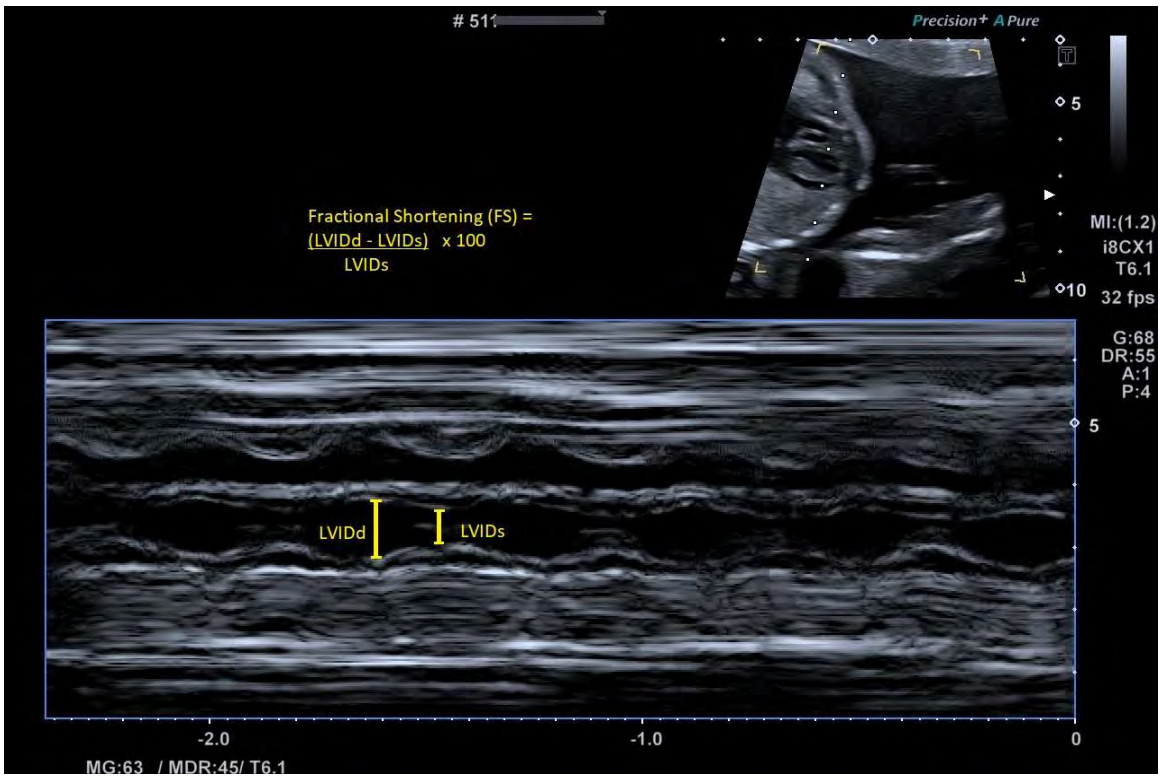



*Figure 11 M-Mode recording of left ventricular radial contractility*

*In the small imaging box, there is a dotted line through the longitudinal plane of the left ventricular cavity of the foetal heart. This line should be at right angles to the ventricular septum and is recorded over time to demonstrate motion as seen. The measurements made in yellow correspond to the internal dimensions of the left ventricle in diastole (LVIDd) and again in systole (LVIDs). The percentage change can be calculated as a fractional shortening measure of the left ventricular function.*

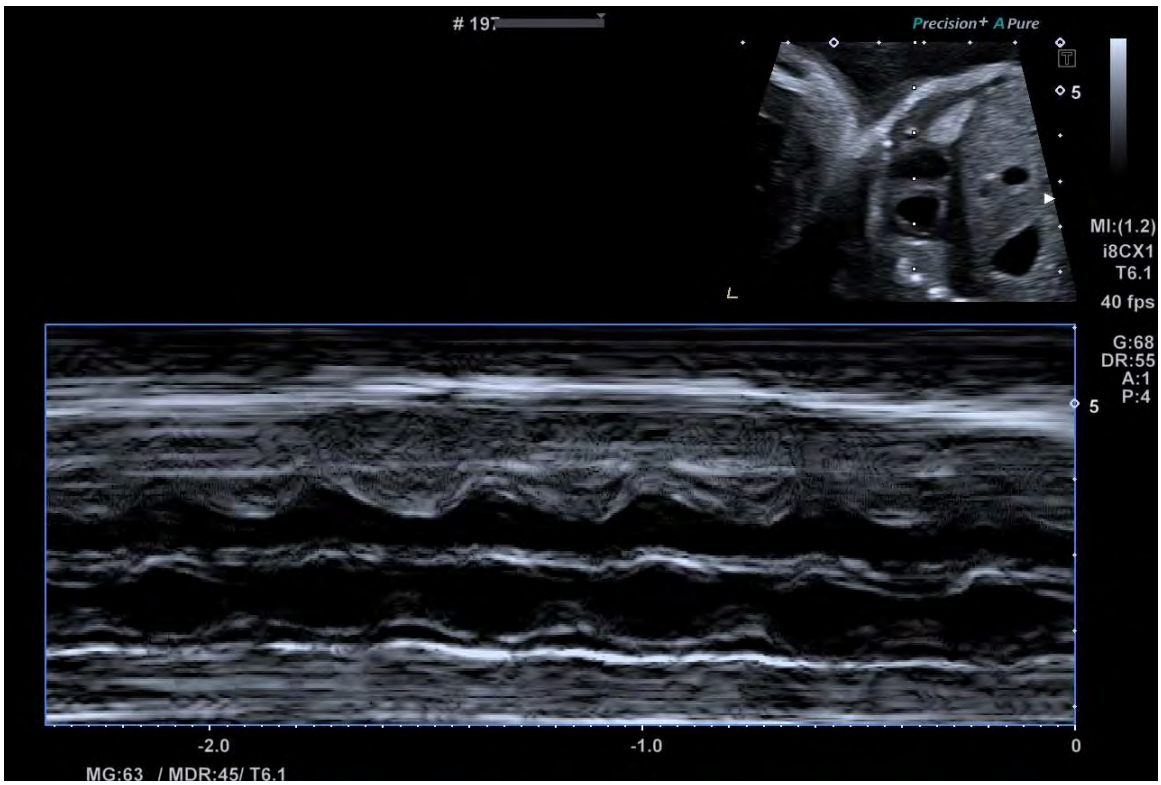



*Figure 12 Left ventricular cavity motion over time in a short axis orientation of the foetal heart*

As with figure 11, the motion of the left ventricular walls can be seen relaxing and contracting. This m-mode demonstrates 6 whole cardiac cycles and includes the motion of the right ventricular free wall.

### 1.3.3 Ejection Fraction

Calculation of the ejection fraction (%) uses a geographical assumption that the LV is of cylindrical shape. This method divides the ventricle into a series of discs at equal thickness to calculate the volume of the left or right ventricles. By manually tracing the endocardial border of the ventricle in 2 different planes, the cavity area in diastole and again in systole provides quantification of the percentage volume change using mathematical calculations. Normal ejection fractions for adults are >55%, but due to the difficulty in obtaining 2 orthogonal planes, ejection fraction is not easy to obtain in foetal echocardiography. Ejection Fraction values obtained with 4D imaging have been reported in foetuses with normal cardiac presentation with mean right and left

ventricular ejection fractions of 54 ± 11.2% and 57.5 ± 14.6%, respectively (Esh-Broder, Ushakov, Imbar, & Yagel, 2004).

### 1.3.4 Fractional Area Change

Fractional area change is an alternative method for calculating the percentage difference between diastole and systole by measurement of the 2-Dimensional cavity area (Figure 13). This calculation is commonly used to assess the change in geometric shape of the right ventricle but can be easily applied to the left ventricle. Both Ejection Fraction and Fractional Area Change quantification are accurate and reproducible tools for the assessment of systolic contractility in paediatric cardiology by way of calculating percentage change. However, both quantification tools are heavily influenced by increased/decreased loading conditions and high heart rates. This makes accurate quantification unreliable when there is a physiological change in blood flow and/or cardiac output.

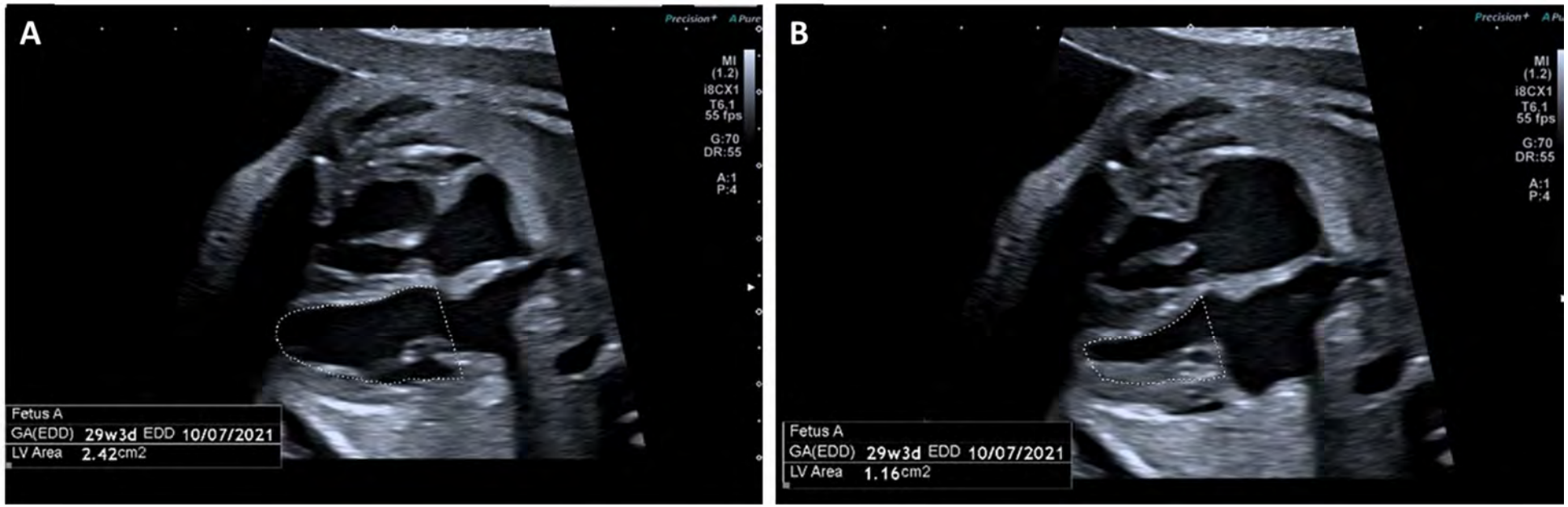



*Figure 13 Manual trace of the LV endocardial border in both diastole and systole*

*A) Example of operator performed measurement of left ventricular area in diastole. B) Measure of left ventricular area in systole. The area is converted mathematically into volume using the idea that the left ventricle is cylindrical and therefore volume is calculated by a number of discs to divide the cavity. Left Ventricular Ejection Fraction is the volume ejected in systole (stroke volume) in relation to the volume of the blood in the ventricle at the end of diastole (end-diastolic volume). Stroke volume (SV) is calculated as the difference between end-diastolic volume (EDV) and end-systolic volume (ESV). LVEF is calculated from: LVEF: [SV/EDV] x 100 (Kosaraju, Goyal, & Grigorova, 2021)*

### 1.3.5 Longitudinal Function

Longitudinal function is described as the change in the shortening length of the ventricle from base to apex (DeVore, Klas, Satou, & Sklansky, 2018) and is often the first dimension of systolic function to deteriorate (Day, Charakida, & Simpson, 2019). Quantification is made using the distance measured between the diastolic and systolic annular plane of the AV valves in an m-mode presentation. This distance represents either the mitral (left ventricular) annular plane systolic excursion (MAPSE) or tricuspid (right ventricular) annular plane excursion (TAPSE) and is used to perform serial measures to observe a decline in longitudinal function (figure 14). In the foetal heart this measurement fluctuates depending on gestational age and estimated foetal weight but at 20 weeks normal values are reported of 3.01mm +/- 1.3mm for MAPSE and TAPSE as 4.23mm +/- 1.2mm and (Lee-Tannock, Hay , Gooi, & Kumar, 2020).

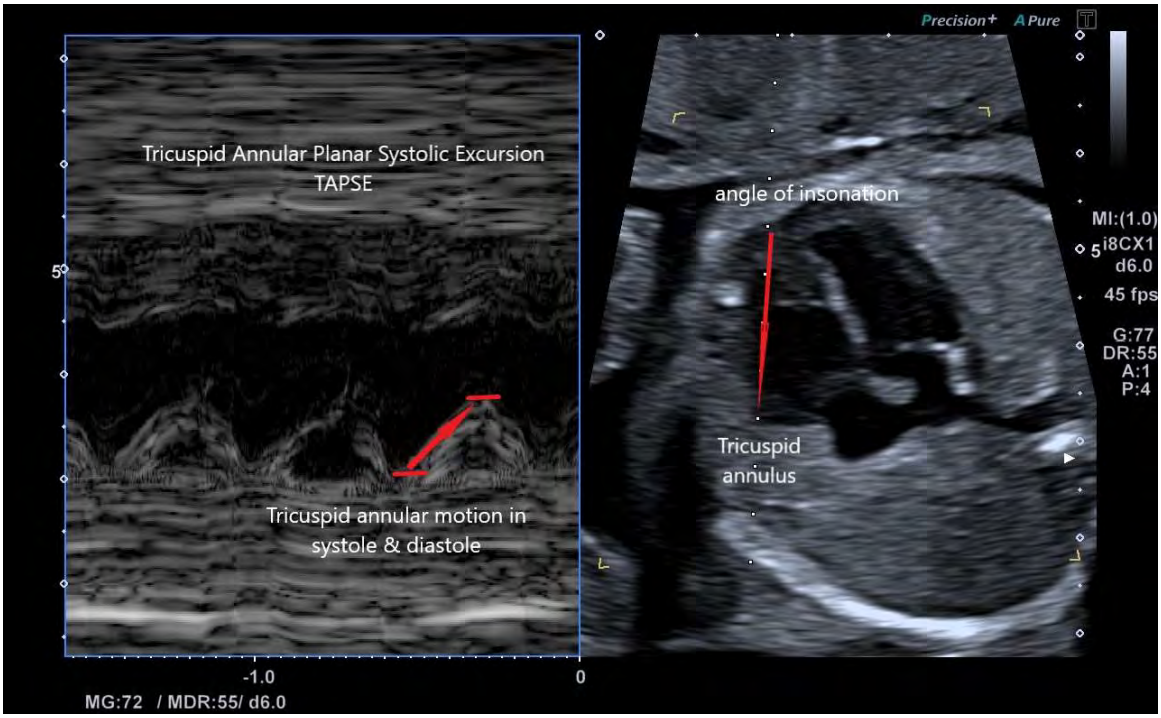



*Figure 14 Tricuspid Annular Plane of Systolic Excursion (TAPSE)*

*Here, a m-mode picture over time is depicted on the left from the dotted line (angle of insonation) on the image on the right. The dotted line runs alongside the right ventricular*

*free wall to measure the change in distance of the tricuspid annulus in systole and diastole. This measure corresponds to the longitudinal function of the ventricle.*

Whilst all these traditional methods of function quantification are feasible in foetal echocardiography, they are challenging and time consuming. They have also been shown to be insensitive to clinical outcomes (Singh, et al., 2010).

## 1.4 SPECKLE TRACKING AND STRAIN

### 1.4.1 Speckle Tracking Echocardiography

Speckle tracking echocardiography (STE) is a relatively new echocardiographic tool which has been demonstrated to be both feasible and reproducible in foetal cardiac assessment (Crispi, et al., 2012), (Di Salvo, et al., 2008) (Krause, et al., 2017). STE is an application of pattern matching technology to ultrasound cine data (Enzenberger, et al., 2017) which is more sensitive than traditional echocardiographic measures of LV function (Asthana, Mantha, Yang, Benharash, & Vorobiof, 2017) (Xiangbo, et al., 2016). It analyses 2-dimensional ultrasound clips tracking the individual speckles, created from the endocardial wall interface. Speckles are natural ultrasound reflectors within the myocardial tissue which are highly reproducible throughout the cardiac cycle (Peng, et al., 2009). The speckles are referenced back to their original positions, frame by frame, using anatomical timing to allow for percentage quantification of cardiac muscle deformation between systolic and diastolic motion regardless of the direction. STE uses the b-mode pixels of ultrasound rather than Doppler which means that the deflection is not expected to be angle dependant to the ultrasound beam. Tracking these speckles permits the calculation of the lengthening and shortening properties of cardiac muscle fibres independent of their direction (Figure 15).

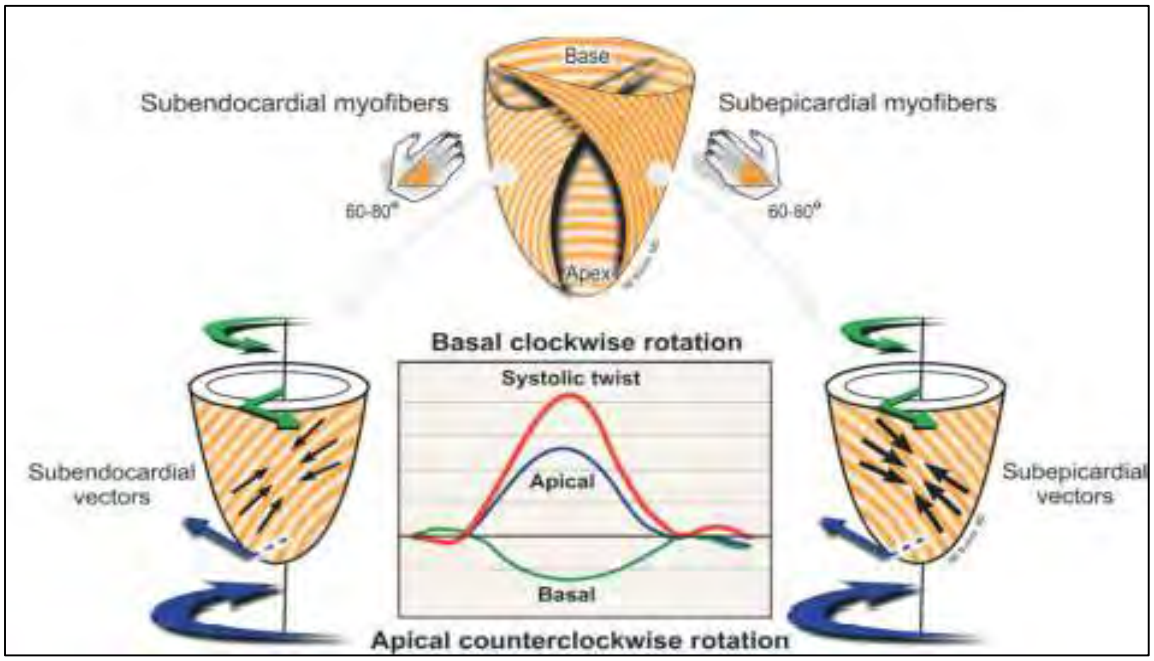



*Figure 15 Orientation of the left ventricular (LV) motion during contractility*

*These pictures demonstrate the subendocardial and subepicardial myofibres and the opposing direction and orientation of contractility. The subendocardium region shows a right-handed helical geometry which changes gradually into a left-handed helical geometry in the subepicardium (Mor-Avi, et al., 2011)*

*Fourier analysis tracking throughout the cardiac cycle provides data to calculate the magnitude of longitudinal deformation as a graphical deflection of this movement from a baseline. The 3 colours in the graph represent 3 differing cardiac vectors and their regions.*

*[picture adapted from (Lo, Lai, & Wu, 2013)]*

Figure 15 gives a pictorial representation of the directions of subendocardial and subepicardial myolayers and the multiple directions of systolic shortening/lengthening. This graphical interpretation of the changes in motion demonstrates the complexity of cardiac muscle contractility but also the influence of the twist mechanism as seen in the post processed graph of movement. The internal subendocardial layers are adversely orientated to the outer epicardial layers which causes the base of the left ventricle to have a negative directional deflection compared to the direction seen in the apex. This can be seen in the blue and green coloured lines in the graph demonstrating the opposing

amplitudes relating to positive or negative deformation. The red line denotes the contribution of systolic twist or torsion between the 2 regions. In this example, all 3 regions demonstrate a synchronous systolic peak. Peak systolic strain values and the interventicular synchronicity of the myocardium are known to be sensitive to early signs of myocardial dysfunction (Oostrum, et al., 2020) (Krause, et al., 2017).

### 1.4.2 STE in Adult and Paediatric Echocardiography

Multiple studies have also shown good correlation between cardiac strain values, clinical outcomes, and mortality in various pathological conditions (Altun, et al., 2016) (Dementgul, 2018) (Florescu, et al., 2011) (Rodrigues-Flores, Martin, & Poirier, 2015) (Xiangbo, et al., 2016). Left ventricular GLS is also strongly correlated to cardiac MRI and invasive catheter derived measures validating cardiac deformation quantification (Goudar, et al., 2016) (Singh, et al., 2010). The ability to quantify myocardial deformation has identified mechanisms of subclinical global dysfunction which have not previously been observed. It has also demonstrated that it is the subendocardial layer of the muscle which is initially affected by physiological and pathophysiological mechanical adaptations (Bansal & Kasliwal, 2013) (Semmler, et al., 2020) and interventricular dyssynchrony (Krause, et al., 2017). In addition, traditional cardiac functional tools are now known to relate more to the mid and subepicardial layers which contribute to the radial and circumferential contractility, (Bansal & Kasliwal, 2013) and their demise in function may only be seen in the later stages of left ventricular dysfunction. Studies into strain quantification between physiological and pathological processes can differentiate pathological cardiac hypertrophy (muscle thickening) from physiological hypertrophy (Xiangbo, et al., 2016) adding weight to the concept that speckle tracking analysis allows

better understanding of previously unclear mechanisms (Dementgul, 2018) (Madry & Karolczak, 2016). There is also the suggestion that myocardial strain measurements are influenced by cardiac size and growth in normal children (Dallaire, et al., 2016) (Marcus, et al., 2011) (Levy, et al., 2016) which may transpose across to the physiological growth and maturation of the foetal heart. In post-natal echocardiography, functional assessment is calculated using the average measures of the 4 different walls of the cylindrical left ventricle – anteroseptal, anterosuperior, inferior and posterolateral walls (figure 16) to obtain a global longitudinal measure of myocardial deformation.

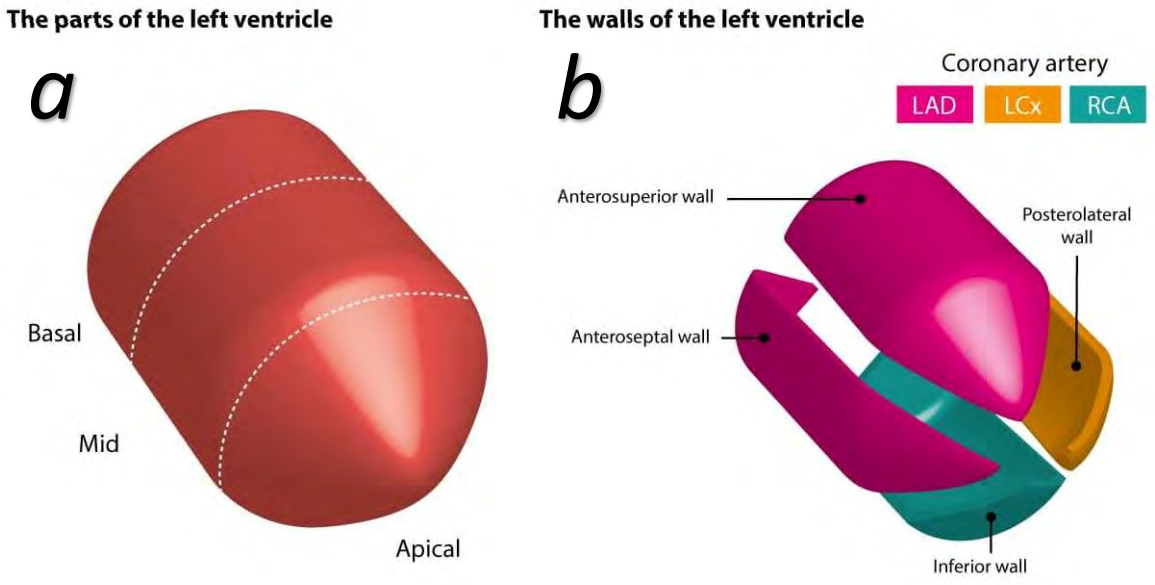



*Figure 16 Left ventricular walls and regions relating to coronary artery distribution*

*a) The left ventricle is commonly divided into three levels along its long axis – Basal, Mid and Apical/Apex*

*b) The left ventricle is further divided into territories in relation to their anatomical positions in the body – Anterosuperior, anteroseptal, posterolateral and inferior.*

*The different colours represent in b) represent the coronary perfusion of the corresponding wall – LAD = left anterior descending coronary artery, LCx = left circumflex artery, RCA = right coronary artery.*

*[Image adapted from Clinical Echocardiography, accessed from www.ecgwaves.com)]*

These walls are viewed in 2D echo by simple rotational manipulation of the ultrasound probe at the apex of the left ventricle on the chest wall of adult and paediatric patients. STE analysis is made in 3 separate planes and the global strain value is averaged from the segmentation analysis of each region (figure 17).

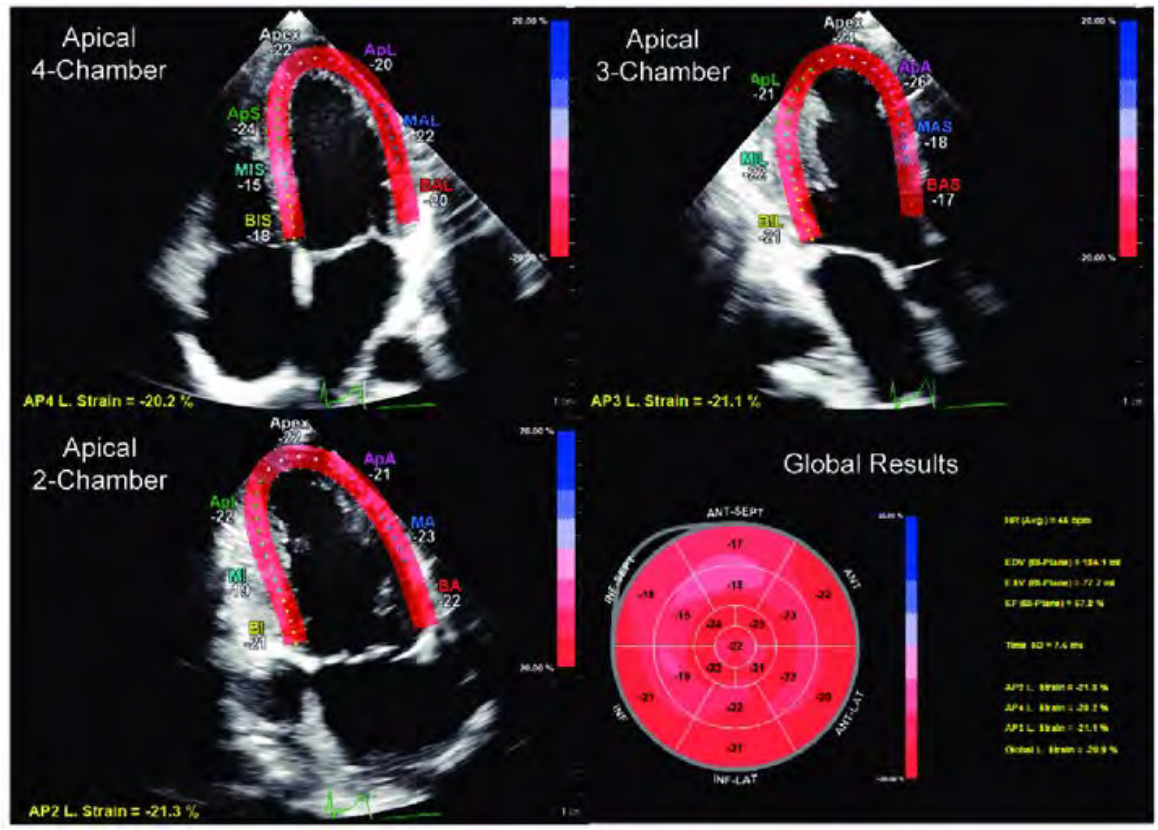



*Figure 17 Left ventricular strain*

*The images show the 3 apical views (apical 2, 3 and 4 chamber) of the left ventricle with the corresponding regional walls – anterior, anteroseptal, inferior, inferoseptal, lateral, inferolateral and anterolateral. Each of the 6 walls are assessed by 2-dimensional speckle tracking echocardiography in the basal, mid and apical portions.*

*Peak regional values are depicted in the bull's eye plot in the lower right panel. The global longitudinal strain (GLS) is-20% (normal myocardial deformation) based on the average of 17 segments.*

*[Image sourced from (Pelliccia, et al., 2017)]*

### 1.4.3 STE in Foetal Echocardiography

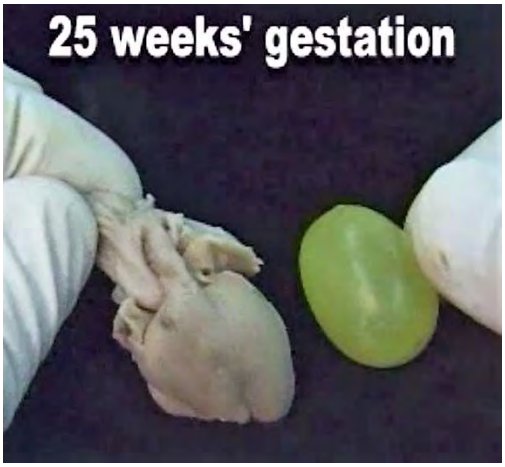



*Figure 18 Relative size of the foetal heart at 20 weeks*

At 20 weeks gestation, the foetal heart is a tenth of the size of an adult heart (figure 18) with heart rates twice the speed (160bpm). As speckle tracking relies on the ability to track individual speckles or 'kernels' of the endocardium, the size of the heart and rate of change (heart rate) play a considerable role in the feasibility of analysis (Crispi, et al., 2012) (Enzenberger, et al., 2017) (Matsuura, et al., 2019). Despite the technical challenge, strain quantification from speckle tracking is a novel approach in foetal cardiology which has the potential to measure early cardiac functional changes in deformation during cardiac maturation (Crispi, et al., 2012) (Germankis, Matsui, & Gardiner, 2012) (Maskatia, et al., 2016). One of the most important features, (unlike other echo functional modalities which rely on Doppler and the limitations of angulation) STE uses B mode imaging which is angle independent and therefore foetal orientation should not be problematic to analysis (Bansal & Kasliwal, 2013) (DeVore, Polanco, Satou, & Sklansky, 2016). Speckle tracking echo, as a means of evaluating foetal cardiac function, has provided useful information for clinical management in the presence of haemodynamic alterations in the foetal circulation. In twin-to-twin transfusion syndrome (a condition where there is an imbalance between the distribution of blood supply between 2 foetuses in a twin pregnancy) there is marked changes in foetal cardiac strain values between affected twins and controls. The haemodynamic imbalance between monochorionic twins, based on a single placenta with anastomoses, has been demonstrated to cause cardiac remodelling

(Goncalves, et al., 2011) with evidence of reduced strain values in the recipient twin (Rychik, et al., 2012). After laser treatment and improved equalisation of blood supply to each twin, strain has been reported to normalise (Harbison, et al., 2021).

This has also been demonstrated in other foetal and maternal conditions where cardiac remodelling alters myocardial deformation due to extra-cardiac haemodynamic influence (Van Miegham, Hodges, Jaeggi, & Ryan, 2014). Foetal growth restriction (FGR) is a condition predominantly due to impaired placental function at the later stages of pregnancy. A reduction in blood flow (and transportation of oxygen and nutrients) has a haemodynamic influence associated to long-term adverse cardiovascular and metabolic disease (Oostrum, et al., 2020) (Van Mieghem, Hodges, Jaeggi, & Ryan, 2014). In terms strain values, there are variable and conflicting reports on STE in FGR[1] (Oostrum, et al., 2020). Crispi, et al. and Krause K, 2017 showed no statistical difference in the strain values of growth restricted foetuses, whereas Patey, Gatzoulis, Thilagananthan, & Carvelho, demonstrated an increase (more negative value) in GLS, whilst DeVore GR P. B., 2016, demonstrated reduced RV strain values.

### 1.4.4 STE Validity

Myocardial deformation strain values obtained from STE have been validated against sonomicrometry (Enzenberger, et al., 2017) and magnetic resonance imaging in adult echocardiography (Mor-Avi, et al., 2011), but in contrast to foetal, adult echocardiography imaging is obtained from direct contact between the ultrasound probe and the thoracic wall. Whilst STE is feasible in foetal echocardiography (Crispi, et al., 2012)

[1] Myocardial deformation in foetal growth restriction has been predominantly studied in the third trimester of pregnancy

(Enzenberger, et al., 2017) (Matsuura, et al., 2019), the foetal heart is imaged via at least 4 tissue interfaces including the maternal abdominal wall, uterus, placenta and the foetal thorax which compromises accuracy and quality of the kernels or speckles throughout the cardiac cycle. Validity has, therefore, been difficult to achieve and the technique remains technically challenging for consistency and reproducibility (Crispi, et al., 2012) especially in maternal obesity. There are also technical specification implications regarding the ultrasound machine, probe selection and image optimisation which are fundamental in performing speckle tracking analysis. Curve-linear transducers used in obstetric scanning are fundamentally different to phased array probes used in both adult and paediatric cardiology. Firstly, curvi-linear probes are designed to provide wide-angle images to examine the whole fetus. They are lower frequency to permit adequate penetration through the maternal abdomen and therefore spatial resolution is suboptimal for myocardial strain accuracy despite reports of no significant difference in GLS between different probes (Rolf, et al., 2018). The impact on both the quality of foetal cardiac imaging as well as the ability to obtain adequate frame rates for accurate tracking of the kernels at high heart rates (Day, Charakida, & Simpson, 2019) has yet to be conclusively outlined. However, there are reports of significant variation between ultrasound manufacturer and Speckle Tracking software (Alessandrini, et al., 2018.) which indicate that systems and analysis packages cannot be used interchangeably (Koster, et al., 2020).

STE is considered an angle independent technique however, there has been speculation over the quality and accuracy of myocardial deformation quantification depending on the orientation of the foetal heart (Day, Charakida, & Simpson, 2019) (Derpa, et al., 2019). Speckle tracking appears to work better along the ultrasound beam rather than across beams (Voigt, et al., 2015) due to differences between spatial and lateral resolution.

Semmler, et al., 2020, found that the orientation of the foetal heart and frame rates were significant determinants of GLS due to differing resolution. There are also reports that foetal orientation affects inter-class correlation (Derpa, et al., 2019) depending on whether the foetal heart is in a vertical or horizontal plane. This suggests that STE is an angle dependent modality and reliable quantification, during foetal life, may be compromised (Semmler, et al., 2020).

In addition to this limitation, global longitudinal strain in foetal echo is restricted to only 2 dimensions of the left ventricular walls – the septal and lateral walls as seen in the standard 4 chamber view (figure 19). GLS is therefore only calculated as the difference between the minimum and the maximum peak strain using the mean value of the 6 segments. In contrast, post-natal STE and quantification of GLS is calculated from all three segments of all the 4 walls of the cylindrical left ventricle equating to 16/17 segments.

### 1.4.5 Cardiac Segmentation

As discussed, several segments of the myocardium (figure 19) can simultaneously track the lengthening/shortening properties of the muscle producing myocardial strain values (%) for each segment, and averaged for each region (Voigt, et al., 2015) (Comas & Crispi, 2012). Calculating cardiac strain deformation in this manner gives STE the potential to make measurements of cardiac function corresponding to differing areas of coronary perfusion and quantify the global changes seen when cardiac remodelling occurs (Oostrum, Guid oei, & Laar, 2019). This comprehensive analysis of the heart muscle provides information of complex multi-dimensional changes which occur in the myofiber layers of the cardiac muscle (Madry & Karolczak, 2016) and detect subtle cardiac

dysfunction and interventricular dyssynchrony in the pre-clinical stages ( (Dementgul, 2018) (Krause, et al., 2017) (Nijres, et al., 2018).

Segmentation of the ventricular walls was first introduced in adult echocardiography to provide in-depth assessment to individual segments in coronary artery disease (Norum, Ruddox, Edvardsen, & Otterstad, 2015). It is now incorporated into routine left and right ventricular function assessment linking to various physiological conditions other than just coronary artery integrity. Whilst these are easily identified in later gestation (figure 19), segmentation is challenging in the smaller foetal heart but there is a distinctive difference between strain values obtained with a bias towards higher strain values in the apex (Enzenberger, et al., 2017) (Mor-Avi, et al., 2011). However, there has yet to be consistent quantification of the differences between myocardial deformation of the basal, mid and apical regions of the foetal heart.

Figure 19 demonstrates the segmentation of the foetal heart using both the 16 segment (A) and 17 segment (B) models of the right and left ventricles. Both models evaluate the segments of the ventricular walls on the left and right, from the base to the apex and the corresponding basal, mid and apex regions. In the standard foetal cardiac 4-chamber view, the left and right septal walls can be viewed as well as the left and right lateral walls. The minor difference between the two models is the ‘apical cap’, which in the 17-segment model is a distinct and separate region of the apex for each of the corresponding walls. In this later gestation it is also possible to differentiate the 3 myocardial layers individually for strain quantification (figure 20) with reports that strain values are lower when the epicardial layers of the cardiac muscle are included in foetal cardiac strain analysis (Semmler, et al., 2020). This is an additional consideration for strain quantification in the

small foetal heart at 20 weeks, in that it may be challenging to differentiate the distinct layers of the cardiac muscle.

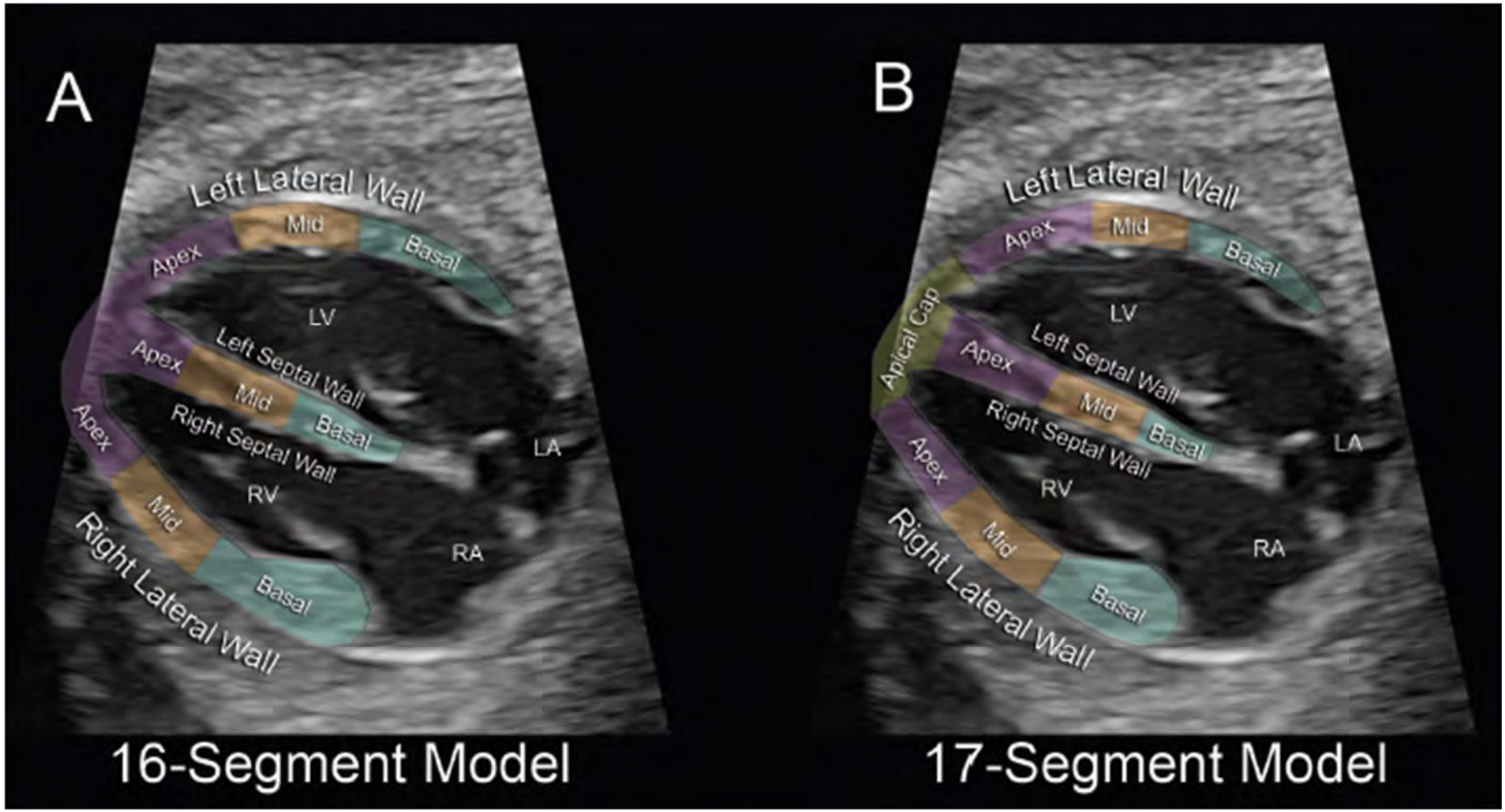



*Figure 19 Anatomical regions of the foetal myocardial walls.*

*LA indicates Left Atrium, LV; Left Ventricle, RA; Right Atrium and RV; Right ventricle [images adapted from (DeVore GR P. B., 2016)]*

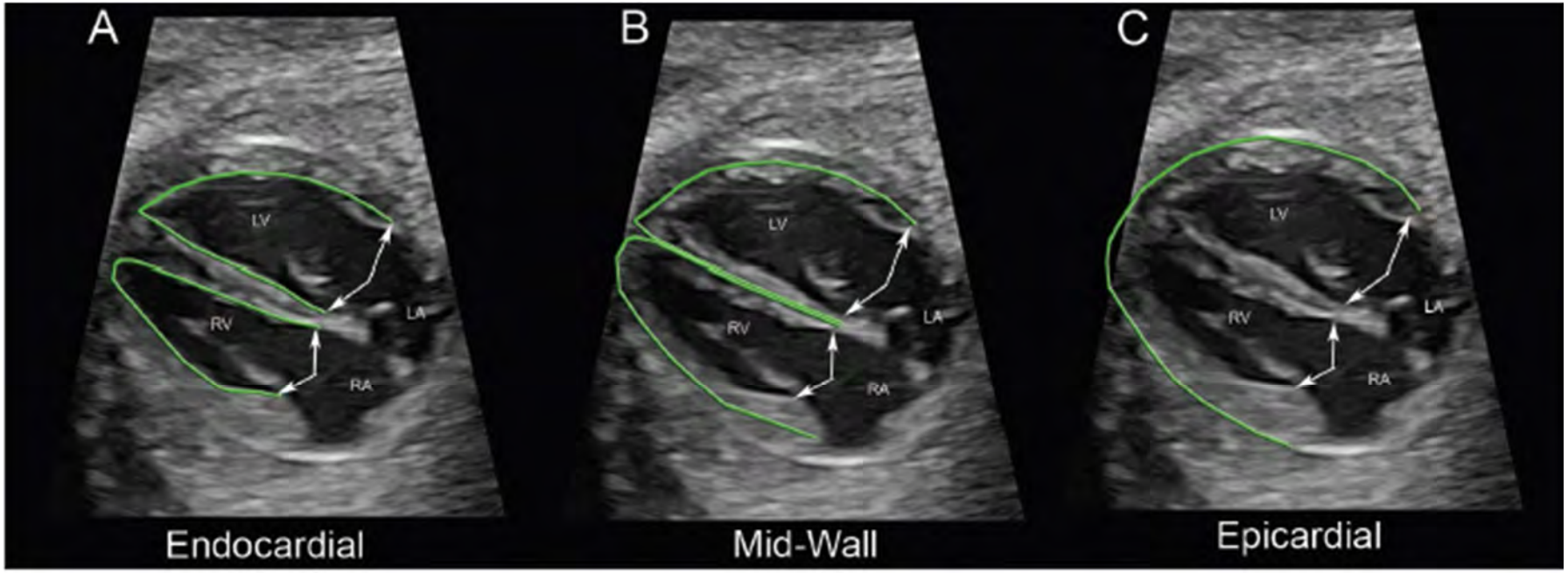



*Figure 20 2D CPA foetal cardiac border contours*

*3 layers of the foetal heart muscle in the third trimester of pregnancy*

*A = Endocardial border contour, B=Mid-myocardial border tracking, C= Epicardial border.*

*[images adapted from (DeVore GR P. B., 2016)]*

### 1.4.6 Timing

In STE, it is important to identify a reference point in time to be able to measure change in deformation. As recommended by the European association of Cardiovascular Imaging (EACVI), ventricular end-diastole is recommended as the time reference to define the zero-baseline (Bandano, et al., 2018). High quality ECG attached directly to the adult patient provides appropriate timing signals for end-diastole to ensure analysis of each segment is gated to the same frame in the cardiac cycle, despite multiple planes of acquisition. Providing the exact same baseline allows analysis of individual myocardial layers and segments of the ventricular cavity to determine overall function as a global percentage (GLS). The reference baseline point in foetal echocardiography is challenging as the baseline reference is reliant on mechanical timing rather than on more specific and reproducible electrical timing of an ECG (Crispi, et al., 2012). The 'R' wave of an ECG provides an accurate timing interval for end-diastole, whereas in foetal echo end-diastole is determined by anatomical mitral valve closure. This is therefore more susceptible to operator variability but despite the limitations, strain analysis provides valuable information regarding the functional mechanisms of the foetal heart in the presence of pathological abnormality or specific disease state (Crispi & Gratucos 2015, Miranda *et al*, 2017).

## 1.5 NORMAL STRAIN VALUES

### 1.5.1 Definition

Strain is defined as the relative shortening or deformation of an object normalised to its original shape and size (Voigt, et al., 2015) regardless of direction. It is a dimensionless entity, reported as a fraction or percentage change from the original length. Langrangian strain is calculated in the formula:

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$$\text{Langrangian Strain} = (L-L_o)/ L_o$$

*where L is the change in length of a myocardial segment as referenced to the original length ($L_o$).*

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In the heart, this percentage change is calculated using ultrasound speckles in the myocardium as they move in systole and diastole. The reference point or original length is when the endocardium is in its most relaxed state in diastole and therefore the length change is a negative percentage.

Tracking this deformation across the cardiac cycle provides strain curves (figure 21) which are uniphasic negative waves. During systole, the myocardium shortens longitudinally and strain changes from zero to negative values, reaching a peak at the end of systole. In diastole the myocardium lengthens, and strain returns to zero (Peng, et al., 2009). Longitudinal strain is computed by averaging these curves across the whole endocardial border of the cavity and calculating the percentage change from the baseline zero to the most negative deflection along any one curve or the average of curves.

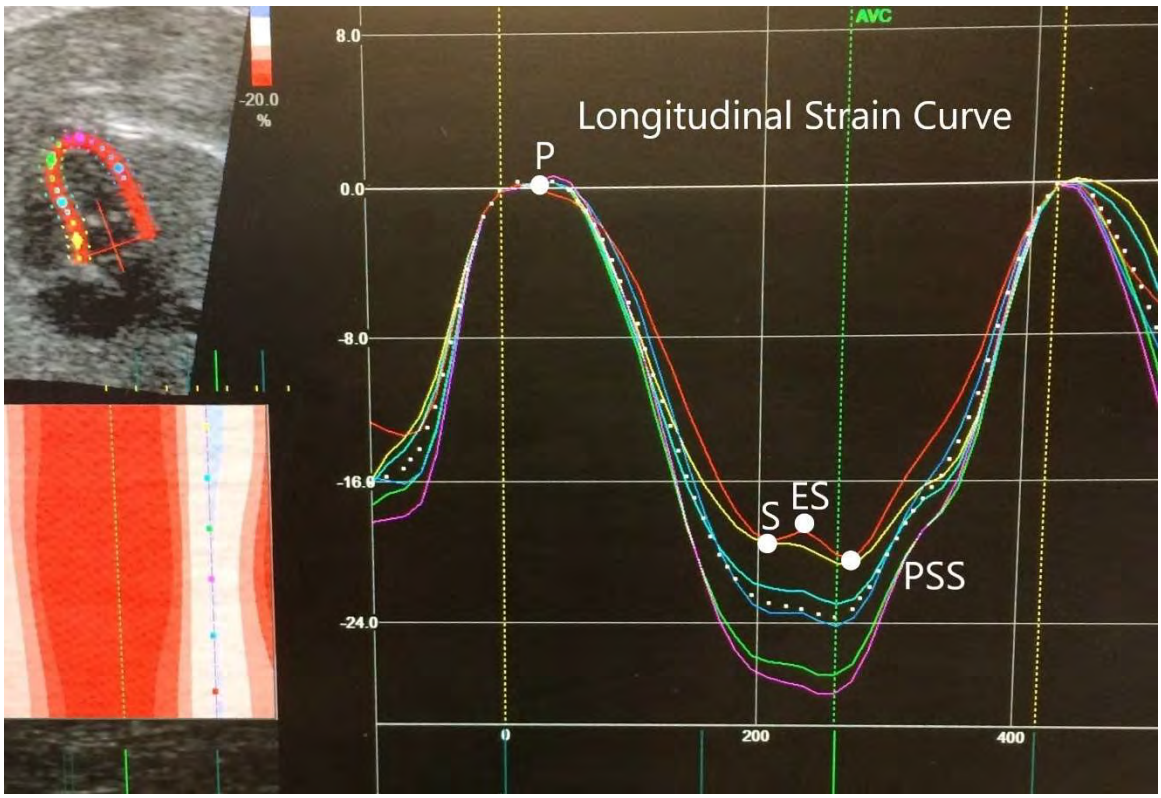


*Figure 21 Longitudinal strain curves*

*6 left ventricular segments with the clinically relevant timings P, peak positive strain; S, peak systolic strain; ES, end-systolic strain; PSS post-systolic strain*

Standardisation for 2D speckle tracking performance has been published by the European Society of Echocardiography (Bandano, et al., 2018) with normal adult cardiac strain values as published by the EACVI task force Normal Reference Ranges for Echocardiography (NORRE) study (Sugimoto, et al., 2017). This European population dataset calculated lowest expected values of LV strain as ± 1.96 standard deviations from a mean of −16.7% in men and −17.8% in women (Sugimoto, et al., 2017). Reference ranges of left ventricular strain measures by 2D STE in children reported normal mean values varied across 43 data sets from -16.7% to -23.6% (Levy, et al., 2016).

In theory, the strain value range for normal muscle deformation should translate across to the foetal myocardium but, due to the differences in circulation and loading conditions upon both the left and right ventricles, there is still no consensus for a foetal cardiac normal strain. Reports over the last 15years present widespread variation in normal foetal longitudinal strain values which, in part, can be attributed to numerous variations in manufacturer, software, methods, gestational age, and technicalities such as frame rate.

### 1.5.2 Literature Review of Foetal Cardiac Strain

Table 3 demonstrates an overview of published data on foetal cardiac speckle tracking, derived strain values and the standard deviation.

Quality assessment of the articles was performed using GRADE (Grading of Recommendations, Assessment, Development and Evaluations), which is the BMJ Best Practice tool for evaluating the quality of research papers. This assessment tool provides a reproducible and transparent framework for grading certainty in evidence. GRADEpro assessment was performed by the author and each paper was categorised into high medium, low and very low.

In this review of the literature the average left ventricular strain values are -20.09 (+/-9) and -19.9% for right ventricular strain, which aligns well to the European consensus for mean and range strain values for normal cardiac function. However, as the table denotes this data is very inconsistent and this variability is partly due to inconsistencies in study design which could explain the delays in implementing this technique into routine clinical practice (Koster, et al., 2020). There are also conflicting results regarding a difference in strain valves between the left and the right ventricles (Kapusta, et al., 2012) (Kapusta, et al., 2013) (Matsui, Germanakis, Kulinskaya, & Gardiner, 2011) and variation in the foetal

cardiac deformation changes in STE-derived longitudinal strain with increasing gestation. Kapusta, et al., 2013, Maskatia SA, 2016, and Peng, et al., 2009 report constant LV longitudinal strain between 20-40 weeks gestations whilst, Di Salvo G, 2008, Savla, et al., 2019 and Meister M, 2020 reports a positive correlation in LV longitudinal strain values with advancing gestation. There appears to be more consistency in the literature with the reducing trend of strain values obtained in the right ventricle as the foetal heart matures (Alsolai, Bligh, Greer, Gooi, & Kumar, 2018) (Di Salvo, et al., 2008) (Erickson, et al., 2019) (Kapusta, et al., 2013) (Willruth, Geipel, Fimmers, & Gembruch, 2011). The inconsistency in LV GLS, however, requires further investigation and clarification.

| Table 3 Literature review of the left ventricular (LV) and right ventricular (RV) strain values at 20 weeks (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author** | Year | n | Gestational age (weeks) | Ultra-sound system | Wall motion tracking software | LV strain | SD | RV strain | SD | Quality Assessment |
| **Di Salvo *et al*** | 2008 | 100 | 20 - 32 | GE | | -25.0 | 4.0 | -24.0 | 4.0 | Moderate |
| **Barker *et al*** | 2009 | 33 | 17 - 38 | Siemens Acuson s2000 | VVI (Siemens) | -17.7 | 6.4 | -17.4 | 6.3 | Moderate |
| **Willruth *et al*** | 2011 | 150 | 13 - 39 | Siemens Acuson s2000 | VVI (Siemens) | -27.9 | 10.5 | -35.9 | 11.2 | Moderate |
| **Germanakis *et al*** | 2012 | 144 | 14 - 39 | Siemens | | -21.9 | 3.7 | -22.0 | 3.7 | Moderate |
| **Ishii *et al*** | 2012 | 81 | 19 - 42 | Siemens Sequioa | VVI (Siemens) | -15.2 | 2.7 | -16.0 | 3.3 | Moderate |
| **Kapusta *et al*** | 2012 | 78 | 20 - 24 | GE Vivid I | STE EchoPac (GE) | -24.9 | 4.6 | -25.4 | N/A | Moderate |
| **Kapusta *et al*** | 2013 | 44 | 30 - 34 | GE Vivid I | STE EchoPac (GE) | -24.7 | 4.8 | -24.7 | 4.8 | Moderate |
| **Crispi *et al*** | 2014 | 37 | 32 | GE | | -18.2 | 4.4 | -17.3 | 4.5 | Moderate |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Maskatia *et al*** | 2016 | 60 | 20 - 38 | GE Vivid e9 | STE EchoPac (GE) | -19.6 | 3.7 | -18.8 | 3.1 | Moderate |
| **Enzenberger *et al*** | 2017 | 101 | 17 - 39 | Toshiba Artida | WMT (Canon) | -17.5 | N/A | -16.5 | N/A | Moderate |
| **Li L *et al*** | 2017 | 51 | 15 - 40 | GE Vivid e9 | STE EchoPac (GE) | -22.3 | 4.3 | - | - | Moderate |
| **Miranda *et al*** | 2017 | 12 | 19 - 33 | Toshiba | | -16.7 | N/A | -13.4 | N/A | Moderate |
| **Patey *et al*** | 2017 | 108 | 38 - 40 | Toshiba | | -11.0 | 4.0 | -11.5 | 3.8 | Moderate |
| **Alsolai *et al*** | 2018 | 276 | 36 - 40 | Siemens Acuson s2000 | VVI (Siemens) | -14.6 | 3.8 | -14.2 | 3.4 | High |
| **DeVore *et al*** | 2018 | 200 | 20 - 40 | GE Voluson e10 | STE (TomTec) | -22.9 | 3.5 | -22.7 | 4.1 | High |
| **Erickson *et al*** | 2019 | 50 | 16 - 40 | GE Vivid e9 | STE (TomTec) | - | - | 20.7 | N/A | Moderate |
| **Meister *et al*** | 2020 | 101 | 16 - 28 | Toshiba | STE (TomTec) | -17.44 | 2.29 | -17.44 | 2.29 | High |
| **Ohira *et al*** | 2020 | 109 | 20 - 38 | Toshiba | WMT (Canon) | -24 | 5.3 | -20.5 | 4.8 | High |

*LV - left ventricle; N/A - Not Achieved; RV - Right ventricle; SD - standard deviation; STE - Speckle Tracking Echocardiography; VVI - Velocity Vector Imaging; WMT - Wall Motion Tracking;*

As with many echocardiographic techniques, variation in software from a variety of manufacturers including GE, Siemens, Canon and TomTec, presents a challenge with aligning results (Koster, et al., 2020). It is well evidence that there is marked variation between vendors, and myocardial deformation quantification should not be used interchangeably (Alessandrini, et al., 2018.) (Koster, et al., 2020).

TomTec has been used extensively over the last 20years for STE and myocardial deformation quantification with good reliability and reproducibility in adult and paediatric populations (Alessandrini, et al., 2018.). In foetal echocardiography, it has gradually increased in popularity as an offline analysis tool due it being a vendor independent platform and therefore can be used despite using obstetric focused ultrasound systems. It is reportedly comparable and reliable against other software manufacturers with good reproducibility (Kraigher-Krainer, et al., 2012) and recent upgrades have focused more on foetal cardiac analysis.

## 1.6 ABNORMAL CARDIAC PRESENTATION

### 1.6.1 Ventricular Imbalance or Small LV

From the onset of the foetal heartbeat, blood flows through the primitive cardiac tube by contraction, applying a physical stimulus to the vessel wall which influences chamber formation, trabeculation, cardiomyocyte proliferation and valve formation (Granados-Riveron & Brook, 2012). The mechanical force of blood flow is sensed by cardiac tissues and induces changes in gene expression (Andres-Delgado & Mercader, 2016). Downstream signalling pathways allow them to react to environmental physical alterations, such as the shear stress applied to the endocardial wall. The changing blood flow of each cardiac contraction–relaxation cycle alters the strain to which cardiac cells are subjected. This force is known as mechanical loading (Andres-Delgado & Mercader, 2016), which influences cardiac developmental growth and maturation. Stable hemodynamic forces are needed to preserve normal and equally balanced ventricular dimensions to avoid heart adaptation (Pedrizzetti, et al., 2015) but these are switched off as a certain stage of maturation is reached or altered by obstruction to the flow haemodynamic and the unbalanced filling of the heart muscle (Drop, et al., 2019).

Extensive animal model research into the comparative circulatory physiology of the foetal lamb, conducted by Abraham Rudolph, suggests that circulatory cardiac output from the foetal circulation determines the development of a normally balanced heart (Rudolph, 2018). Blood flow and cardiac contractility regulate the shape of cardiomyocytes in the heart (Chi, et al., 2008) and changing the mechanical load impairs growth and disrupts trabecula organization. As such, reduced blood flow is an inhibitor of ventricular growth and as the fetus continues to increase in size, the left heart

structures become hypertrophied and decrease contractility (Gobergs, Salputra, & Lubaua, 2016). The severity of obstruction and underlying pathology reducing blood flow will determine the rate of change in ventricular size and shape. Research into this type of ventricular remodelling in a specific disease state found that foetuses with coarctation present biventricular structural changes with preserved fibre organisation and function in the third trimester (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020) (Fricke, Liuba, & Weismann, 2021) (Soveral, et al., 2017). In these studies, the left ventricle was seen to have an elongated shape with flattened septum in the presence of coarctation with reduced left ventricular length. They confirm that ventricular modelling does occur under an unloaded haemodynamic circulation which then undergoes further remodelling as a postnatal response to the haemodynamic adaptation (Soveral, et al., 2017). This is particularly important as it demonstrates that foetal cardiac dysfunction is susceptible to both intrinsic myocardial disease and dysfunction as a secondary remodelling/adaptive mechanism (Crispi, et al., 2012).

This has been supported by the recent work of Garcia-Canadilla *et al* 2018, who have demonstrated the potential for studying cardiac microstructure in the developing human foetal heart. In their study, they used both diffusion tensor magnetic resonance and X-ray phase-contrast to visualise of the complexity of myofiber layers in both the left and right ventricles in a normal foetal human heart and one with a complex unbalanced congenital heart defect (Figure 20). These high-resolution images comparing myocyte architecture suggests that overall, there is preservation of the change in helical angle and organisation of the myofiber layers in the morphological left ventricle despite a change in geometry. In comparison, the morphological right ventricle displays a greater disorganisation of the helical angle and organisation of the myofiber

layers in the specimen with congenital heart disease than the normal foetal heart. The findings of Garcia-Canadilla *et al*, support two notions that firstly, despite there being a ventricular imbalance, the morphological left ventricle maintains myocardial architecture and organisation to support function, whilst the right ventricular myocardial architecture is far more disorganised and therefore lacks the same myocyte organisation of the normal right ventricular structure to the increased flow volume demands. It is therefore important to recognise that the performance of the right ventricle is at least as cruical as that of the left and that cardiac dysfunction is usually first observed on the right side of the heart (Drop, et al., 2019) (Savla, et al., 2019).

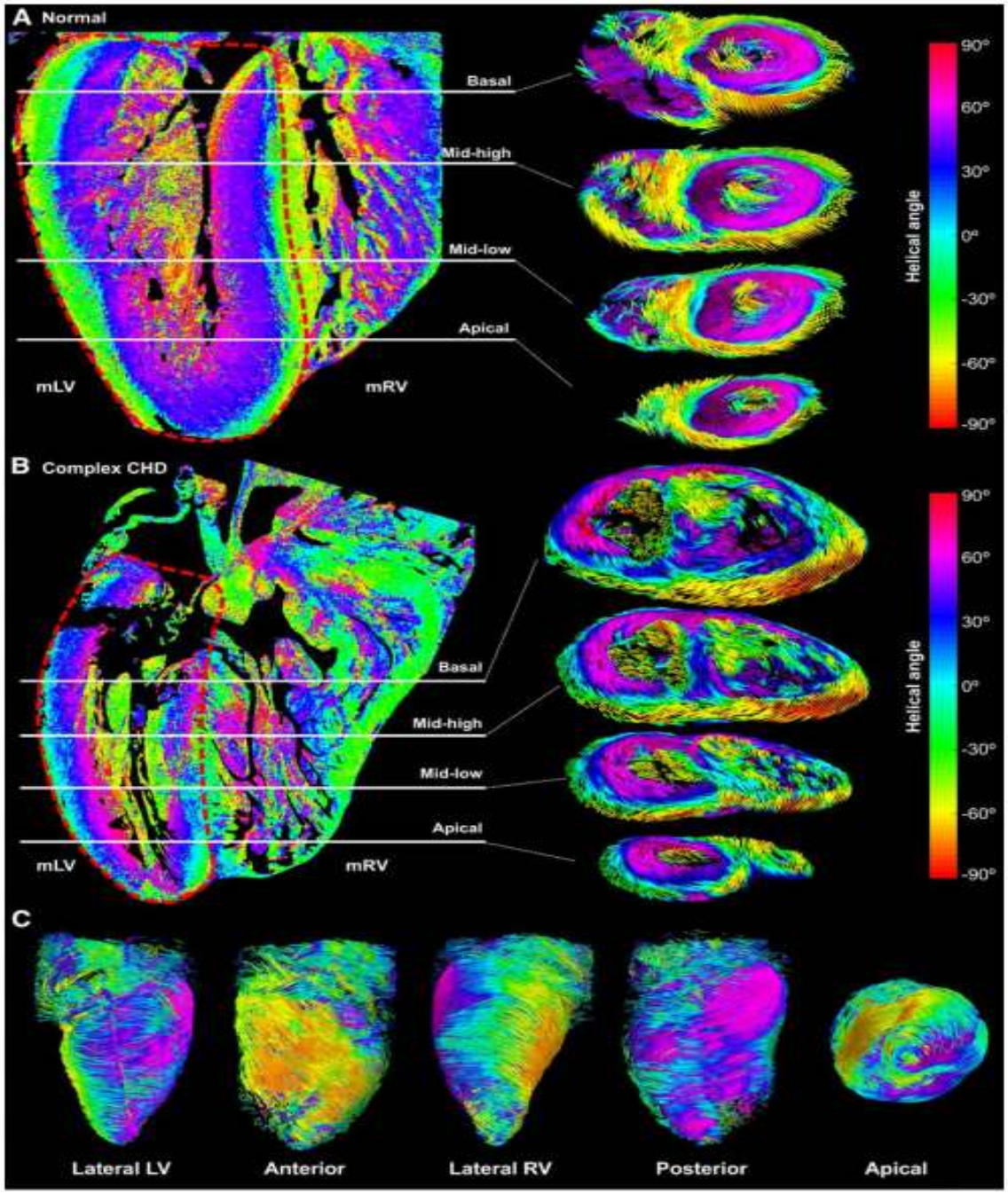


*Figure 22 Synchrotron x-ray phase contrast imaging of human foetal hearts.*

*A is a longitudinal slice of the myocyte architecture of a normal foetal heart with 4 x short axis slices to demonstrate the change in helical angle between the base and apex of the left ventricle.*

*B demonstrates the same longitudinal and short axis slices of a human foetal heart in the presence of a complex congenital heart defect with a ventricular size imbalance. The change in colour depicts the change in vector.*

*C demonstrates the external myocardial patterns of the muscle architecture rotating from the lateral, left ventricle, to the anterior, lateral right ventricle, posterior surfaces and finally the apical aspect of the foetal heart.*

*[Images taken from Garcia-Canadilla et al 2018]*

### 1.6.2 Terminology

Ventricular imbalance, ventricular size discrepancy and borderline left ventricle are all terms used in clinical practice for describing an unequal distribution of the left and right ventricular proportions in relation to the cavity base diameter and cavity length (figure 21).

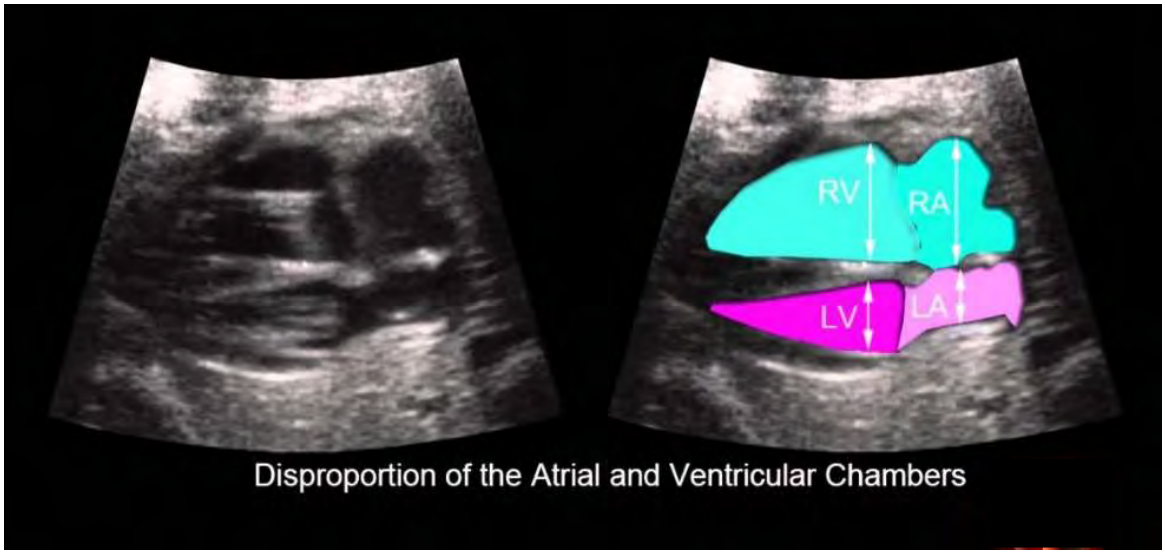


*Figure 23 Relative disproportion between the left and right sides of the foetal heart. LV=Left Ventricle, LA=Left Atrium, RV=Right Ventricle, RA= Right Atrium. [Image adapted from (DeVore, Polanco, Satou, & Sklansky, 2016)]*

In congenital cardiac lesions affecting the left sided structures of the heart, such as aortic atresia, severe aortic stenosis and severe coarctation of the aorta, there is damaged or failure of the left ventricle to grow as a consequence of a flow obstruction (Allen, 2010) (Gembruck, 2010) (Menduina, 2015) (Soveral, et al., 2017). The ability of the foetal heart to redistribute output means that a relative disproportion of the ventricles can be attributed to any anomaly along either the left or right circulatory pathway resulting in a reduced cavity area and volume of the corresponding pumping chamber. The altered circulation, either due to environmental or pathological influences, causes the cardiac

muscle fibres to remodel in response to changes in circulatory pressure and volume (Pedrizzetti, et al., 2015) (Soveral, et al., 2017). The foetal heart adapts well to congenital heart defects but typically, it is the right ventricle, as the systemic pump which remodels to compensate for adverse conditions and to preserve the perfusion of the foetal organs and placenta (Crispi, Sepulveda-Martinez, & Crovetto, 2020). Left ventricular cardiac remodelling is a phenomenon that is extensively well researched in cardiology and cardiac surgery (Corno, 2005) (Loar, et al., 2016) in response to changes in altered disease or surgical states. Often, after treating the insult in an adult heart, cardiac remodelling reverts, however significant insults to foetal cardiac development may persist even after corrections. Conversely, and unlike the adult heart, the intrinsic size and shape of the foetal heart can change as it matures so transient insults can disappear at birth (Crispi, Sepulveda-Martinez, & Crovetto, 2020).

Transient insults can include maternal conditions where haemodynamic loading conditions influence the size and function of the foetal myocardium (Erickson, et al., 2019). These include, but are not limited to renal failure, diabetes, anaemia and hypertension (Eckerseley, 2017). Foetal cardiac deformation is impaired by the maternal circulatory impact of these conditions, and several studies have shown differing GLS values in comparison to controls (Altun, et al., 2016) (Crispi, Sepulveda-Martinez, & Crovetto, 2020) (Eckerseley, 2017) (Gandhi, Zhang, & Maidman, 1995) (Pedrizzetti, et al., 2015) (Miranda, Cerqueira, Ramalho, Areias, & Henriques-Coelho, 2018) (Rolf, et al., 2018).

It is also important to note that when assessing foetal cardiac function with an altered haemodynamic circulation, the global performance depends on the interaction of the

left and right ventricles and a disproportion in size may have an impact on the function of both ventricles (Drop, et al., 2019).

The quantification of this disproportion has been widely discussed and analysed in the literature for paediatric cardiology (Corno, 2005) but due to the differing circulations, it has been challenging to transpose much of this research into foetal cardiac evaluation. Nomograms for foetal cardiac ventricular ratios suggest that a mean RV/LV ratio of 1.03 correlates well to this equal size at 20 weeks but there is evidence that this ratio increases with gestation without clinical significance (Gabbay-Benziv, Turan, Harman, & Turan, 2015).

In the foetal circulation, the right ventricle becomes the dominant and systemic pump between the second and third trimester of pregnancy with a cardiac output higher than that of the left ventricle (Simpson, 2004). The foetal circulation is also influenced by high pulmonary resistance due to inactive and deflated lung fields and a series of shunts, which actively redirect blood flow to support the nutrient and oxygen requirements of the major foetal organs. As the heart matures in pregnancy, ventricular remodelling occurs in preparation for the left ventricle to become the systemic pump. These physiological adaptations in ventricular dynamics have yet to be conclusively outlined however research suggests that there is a significant reduction in GLS with gestation (Savla, et al., 2019). Without a consensus on the GLS changes during the physiological remodelling of the foetal heart as it matures, it remains challenging to cross-compare the GLS changes in the presence of a small left ventricle. As such, predictions of possible outcomes, for a small left ventricle has stemmed from the difficulties of post-natal medical and surgical management of a borderline left ventricle (Vernandos, Colquitt, &

Morris, 2021). Corno, 2005, describes the definition of a 'borderline LV' as a "ventricle with an indeterminate position between normal and small in reference to normal ventricular dimensions and z-scores". Schneider et al (2005) published the most widely used foetal cardiac z-scores in relation to gestational age. Since then, other studies have compared these parameters with further anatomical analysis to study predictive values of outcome prognosis in relation to various pathological cardiac conditions (Altun, et al., 2016) (Dallaire, et al., 2016) (Oostrum, et al., 2020) (Patey, Gatzoulis, Thilagananthan, & Carvelho, 2017).

Whilst it is widely assumed that a ventricular size discrepancy is a left heart "problem" is also important to consider other conditions, where the underlying pathology is due to right ventricular pressure and/or overload (Brooks, Khoo, Mackie, & Hornberger, 2012) (Gabbay-Benziv, Turan, Harman, & Turan, 2015). Right ventricular dilation presents as a disproportion in ventricular size due to pulmonary or tricuspid insufficiency, ductus arteriosus restriction or severe right heart obstruction and the clinical management can be equally challenging (Corno, 2005) (Sivanandam, Nyholm, Wey, & Bass, 2015). Extra-cardiac malformations such as arteriovenous malformations or agenesis of the ductus venosus can also result in right heart dilatation which often requires similar medical management as a left heart obstructive lesion (Gabbay-Benziv, Turan, Harman, & Turan, 2015) (Menduina, 2015).

In less severe cases, the underlying mechanism for a ventricular imbalance, and the gestational age of the fetus are very difficult to evaluate clinically with little support to predict outcomes (Menduina, 2015). As such, much of the current literature focuses on

the more consistent post-natal outcomes of a small left ventricle rather than observing outcomes from a small left ventricle in utero. At birth, there is considerable changes in both circulatory and physiological adaptations to the independent neonate. Blood pressure, blood volume and lung resistances very much influence the remodelling of the cardiac muscle to adapt the left ventricle to being the systemic pump and without intervention this remains relatively stable, and outcomes are easier to predict (Crispi, et al., 2012). However, the surgical pathway for a borderline LV and choosing appropriate candidates for biventricular repair is still a very challenging (Wendt, Strunz, Rustenbach, Kroner, & Bennink, 2021). Paediatric Cardiologists often have a bias favouring biventricular repair (BVR) as an intuitive notion that “two ventricles are better than one”. However surgical reports suggest that mortality is higher in infants with borderline LV when pursuing a biventricular circulation than the overall survival rates of a univentricular pathway (UVP) (Hickey, et al., 2007). In addition, the discordant decisions made between a BVR and UVP also increase the mortality rates (Hickey, et al., 2007) and therefore morphological, functional, and pathological information is vitally important to determine the relative risks associated with the 2 pathways. There are echocardiographic clinical presentations for both antenatal and postnatal periods which are diagnostic for outcomes and a non-apex forming left ventricle with endocardial fibroelastosis has poor survival for a biventricular repair (Dias, Barros, Leite-Moreira, & Miranda, 2020). In these infants, a single ventricle surgical pathway is the most common management despite the limitations that this remains a palliative pathway (Corno, 2005) (Hickey, et al., 2007).

Up until the 1980’s mortality for single ventricle circulation was almost 100% and was significantly high for borderline LV where the left ventricle was unable to provide

adequate systemic output. Dr William Norwood was the first physician to publish a report on a successful palliative procedure for on-going survival (Norwood, Kirklin, & Sanders, 1980). This then led onto the development of the Fontan procedure which provides an alternative single ventricular circulation, albeit a palliative pathway, with high morbidity and mortality (Fontan & Baudet, 1971). More recently, surgical and hybrid procedures have evolved to provide alternative strategies for borderline LV presentations, but improved morbidity and mortality has yet to be proved.

### 1.6.3 Coarctation of the Aorta

Coarctation of the aorta is a congenital heart defect where there is a circumscribed narrowing of the descending aorta causing a left heart flow obstruction and often resulting in a smaller left ventricle (Fricke, Liuba, & Weismann, 2021). A small left ventricle or aorta is often the first indication of coarctation at 20 weeks (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020) (Fricke, Liuba, & Weismann, 2021) (Gembruck, 2010) as it is very challenging to visualise the narrowing in the descending aorta due to the dominant ductal arch (ductus arteriosus), which is a foetal circulatory shunt to by-pass the lungs. The narrowing in the aorta is easily missed by antenatal ultrasound as the ductal arch compensates and provides a superimposed good presentation of blood flow on Colour Doppler imaging (figure 24).

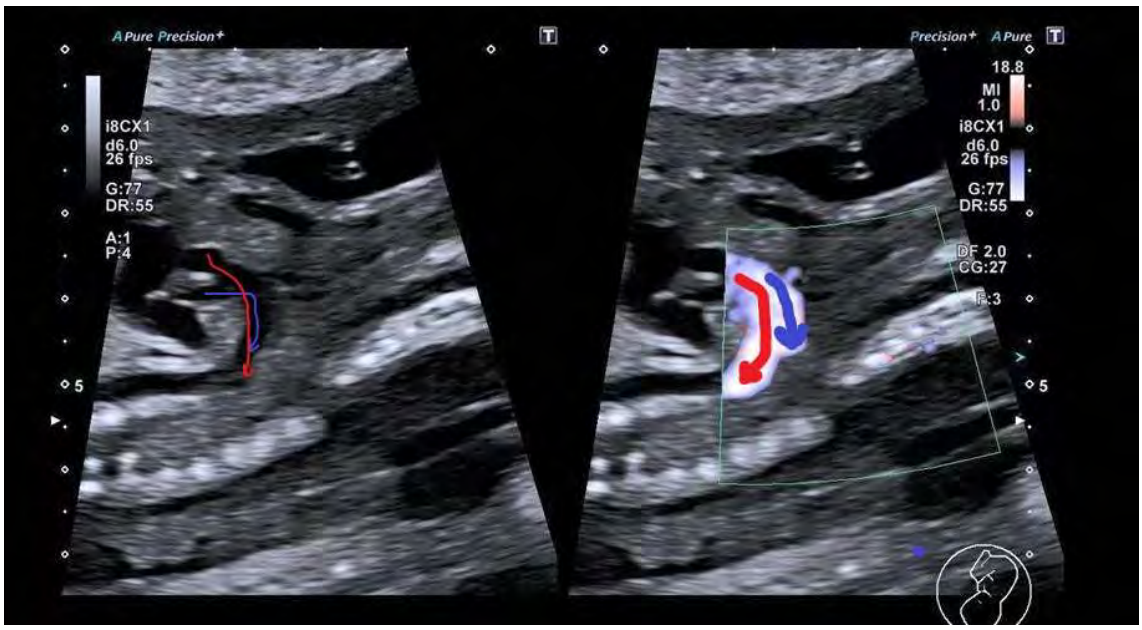


*Figure 24 Foetal Echo sagittal images of the aortic and ductal arches with and without dynamic advanced flow*

*These images show the close proximity of the arches when viewed from a sagittal plane. The red arrows demonstrate the ductal arch and the flow from pulmonary artery to the descending aorta. The blue arrows demonstrate the true aortic arch and the blood flow from the ascending aorta to descending aorta feeding the head and neck vessels.*

After birth, when the ductus arteriosus shunt closes due to the expansion of the lungs and the subsequent drop in pulmonary resistance, the narrowing in the aorta persists preventing adequate systemic circulation to the lower half of the body. If the narrowing is severe, it can cause cardiac arrest due to a loss of circulation however the timeline for this to occur may be acute or gradual depending upon how quickly ductal restriction evolves (Hornberger & Eckersley, 2021). Pre-natal recognition of coarctation is known to improve outcomes by optimising immediate post-natal management (Zeng, et al., 2017) but it is one of the most difficult congenital heart defects to diagnose prenatally (Fricke, Liuba, & Weismann, 2021) (Zeng, et al., 2017). When there is a strong pre-natal suspicion for coarctation, birth is planned in a centre with a neonatal unit where they

can provide observation whilst the duct closes and administer medication if the narrowing causes a compromise to circulation (DeVore, et al., 2020) (Zeng, et al., 2017). Surgical intervention is often performed within the first weeks of life and prenatal diagnosis is the major adjustable risk factor affecting preoperative and postoperative outcomes (Dias, Barros, Leite-Moreira, & Miranda, 2020) (Houshmandi, Eckersley, & Hornberger, 2021).

Coarctation of the aorta is a condition where often the first sign is a size imbalance between the left and right sides of the heart due to the obstruction in blood flow (Gembruck, 2010). However, it is also worth noting that the false-positive rate for a foetal cardiac diagnosis of coarctation has been reported between 50-75% (Fricke, Liuba, & Weismann, 2021) (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013) (DeVore, et al., 2020) (Tibaldi, et al., 2011) and in one study only one third of those diagnosed with coarctation presented with a ventricular size discrepancy (Houshmandi, Eckersley, & Hornberger, 2021). Foetal cardiac observations during the second and third trimester of pregnancy, and the initial post-natal echo permit a more definitive diagnosis of coarctation (Miranda, et al., 2017) however, this later diagnosis reduces options for parents on the fate of the pregnancy and the management after birth. If the size of the left ventricle diminishes significantly during the later stages of pregnancy and a bi-ventricular circulation is compromised, the clinical management is far more high risk for successful short- and long-term morbidity and mortality. Parental choice to discontinue the pregnancy or defer medical intervention is also compromised due to maternal safety relating to late gestation termination.

Coarctation in the foetal heart has been referred to as the foetal cardiologists “Achilles Heel” (Hornberger & Eckersley, 2021) due to the difficulties of accurate diagnosis. A

more diagnostic tool for assessing coarctation and the long term outcomes at mid-gestation may help to guide clinicians towards predicting where on the severity spectrum each individual case may be.

It is suggested that STE may help with a pre-natal diagnosis by identifying early subclinical dysfunction or dyssynchrony, but the published study populations are still low in numbers and there has yet been an agreement on normal myocardial deformation values. With a more evidence towards a diagnosis of a structural heart defect, it may also help decrease the number of pregnancies who required delivery at a tertiary surgical site (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013).

### 1.6.4 Congenital Heart Disease and a small LV

Congenital heart disease is a collective term for a range of conditions relating to an abnormal presentation of the structural and functional properties of the heart. Coarctation is a congenital condition which can be due to molecular level discordance of the forming aorta in embryological life (Gembruck, 2010), or it can be an acquired condition as a result of excessive tissue remaining from the ductus arteriosus. As with all cardiac abnormalities, successful treatment is highly dependent on cardiac remodelling after correction, most specifically the ability for the cardiac muscle to sustain a systemic circulation which meets the demands of the body. The outcome from any congenital heart defect is, therefore, crucially balanced on the long-term adaptation of LV structure and function as a systemic pump. Whilst, right ventricular size and function is important for an adequate pulmonary circulation, this low-pressure system is more adept to physiological fluctuations and remodelling. In contrast, the size and function of the left ventricle determines the fate of a dual circulation. Mortality and

morbidity rates are far less favourable in any congenital heart defect if the left ventricle is of inadequate size or fails to maintain cardiac output to meet oxygen and nutritional requirements. Left ventricular size and function is, therefore, a fundamental component in counselling families on the impact of congenital heart disease in their pregnancy.

In the presence of a pathological cardiac defect, there are various independent and multivariate factors which influence a long-term outcome in the presence of a ventricular size imbalance or small left ventricle. A small left ventricle with reduced volume and the presence of endocardial fibroelastosis (EFE) is shown to increase mortality in infants with aortic stenosis (Chung M, 2010) (Corno, 2005). Whilst EFE alongside increased left ventricular diastolic pressures and an ejection fraction of less than 42% demonstrated further increases risk of mortality (Gundry & Behrendt, 1987). For post-natal outcomes, the cavity length, the mitral valve orifice diameter and whether the apex was formed by the right ventricle instead of the normally related left, showed the need for an alternative approach to infants with critical aortic stenosis in the form of either a uni or single ventricular circulation or cardiac transplant (Familiari, et al., 2017) (Loar, et al., 2016). The Boston group published a very extensive analysis in 1991 of infants with critical aortic stenosis. It led to the development of an equation named the “Score of Rhodes” to help drive clinical management in preventing death after biventricular repair (Rhodes, Colan, Perry, Jonas, & Sanders, 1991). Using a scoring system, the ratio between left sided structure dimensions, LV, MV annulus and aortic annulus when indexed to infant body surface area provide a guide to long term outcomes. The more negative the score, the higher the in-hospital mortality which has influenced congenital heart surgery in North America.

Diagnostic key markers form this scoring system are now used for risk stratification in evaluating the adequacy of a systemic circulation when observed in the fetus at 20 weeks gestation (Vernandos, Colquitt, & Morris, 2021). The markers are also a key determinate on the immediate post-natal management of an infant with congenital heart disease. Where there are concerns regarding the ability of the left ventricle to maintain a systemic circulation, post-natal cardiovascular disease may alter prognosis due to haemodynamic instability and poor tissue perfusion (Cohen, et al., 2013) (Van Mieghem, Hodges, Jaeggi, & Ryan, 2014). Admission to hospital for an induced labour may be required for the delivery of the baby to provide intensive care observation and surveillance, medical and/or therapeutic interventions if required. This is particularly relevant in such conditions as an atrio-ventricular septal defect (AVSD), where a small left ventricle presents a far greater need for optimised post-natal care than and a balanced AVSD or an AVSD with a dominant left ventricle (Cohen, et al., 2013) (Corno, 2005) (Jegatheeswaran, et al., 2010). The prognosis of congenital heart disease is also negatively impacted by a small left due to increased RV pressure or volume. Reports have highlighted mortality of up to 60% in children with RV dominance in the presence of an unbalance AVSD. These statistics may alter the decisions families make at 20 weeks of pregnancy as outcomes are negatively impacted by a small, dysfunctional LV. Surgical pathways may also be reviewed depending on the size and function of the left ventricle and the duration of diastolic dysfunction due to prolonged adaptation and remodelling of the heart. Prolonged LV overload and distention may result in a non-compliant LV post-surgery, which subsequently leads to severe left atrial hypertension and late, pulmonary hypertension (Corno, 2005). Surgical “correction” may be compromised and alternative pathways towards palliation may be more appropriate.

## 1.7 RESEARCH QUESTION, STUDY AIMS AND OBJECTIVES

### 1.7.1 Research Question

Prenatal ventricular size discrepancy with disproportionately smaller left ventricle than right is an important marker of left-sided structural heart disease (Quartermain, et al., 2009) however this indirect sign is not an accurate predictor for outcomes (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013). Currently, it is very challenging to differentiate a ventricular size discrepancy at 20 weeks of pregnancy into a normal or abnormal outcome for congenital heart disease. This research looks at whether speckle tracking echo (STE) derived strain values can differentiate outcomes of a cohort of pregnancies where there is a foetal cardiac ventricular size imbalance with a smaller left ventricle than right.

### 1.7.2 Aim 1

Evaluate the feasibility and repeatability of performing STE in foetal echocardiography at mid-gestation in pregnancy.

#### 1.7.2.1 Objective

Analyse the right and left ventricles using speckle tracking echo (STE) to obtain structural dimensions, functional parameters and a percentage strain in a 4 chamber view of a foetal echocardiogram at mid-gestation. Compare the strain values obtained in the control group with those in the literature.

#### 1.7.2.2 Hypothesis

The hypothesis is that measures obtained in the left and right ventricles will closely align to the normal distribution of average values seen in the literature.

### 1.7.3 Aim 2

Aim 2 of the study is to investigate whether foetal cardiac STE, at mid-gestation, can predict a congenital heart defect where there is a cardiac ventricular size imbalance of unknown aetiology against those who are subsequently found to have normal post-natal cardiac anatomy.

#### 1.7.3.1 Objective

Where there is a disproportionally smaller left ventricle in a 4 chamber view of a foetal echocardiogram at mid-gestation, Cardiac Performance Analysis will obtain dimensions of the right and left foetal heart structures, function and the myocardial deformation by calculating percentage strain.

#### 1.7.3.2 Hypothesis

The hypothesis is that there will be a correlation between right and left ventricular strain values and the foetal cardiac measures where the ventricular size discrepancy is due to an underlying pathological defect (as diagnosed after birth) compared to those who are not affected by a defect. Those babies who had a small left ventricle at 20 weeks but went on to have no heart defect will have functional measures and strain values more closely aligned to the normal cohort.

#### 1.7.3.3 Alternative Hypothesis

The alternative hypothesis is that there will be no significant difference between right and left global longitudinal strain or functional measures despite an abnormal size discrepancy between the size and volume of the foetal heart.

### 1.7.4 Aim 3

The third aim is to investigate an age-matched cohort of foetuses with a confirmed foetal cardiac diagnosis of a congenital heart defect with an imbalanced, smaller left ventricle for comparison of strain values found in group with left ventricular imbalance (LVIM) and an abnormal outcome. The rationale for including a cohort with a congenital heart defect with ventricular imbalance is that this group provides a comparative "abnormal" cohort which is distinct from both the normal/control and the LVIM cohort where an abnormality is unknown.

#### 1.7.4.1 Objective

Analyse the myocardial deformation of a cohort of foetuses with a congenital heart defect and unbalanced ventricles (disproportionally smaller left ventricle) in a 4 chamber view of a foetal echocardiogram at mid-gestation. Again, Cardiac Performance Analysis (CPA) will obtain foetal cardiac dimensions, measures of function and a percentage strain in the left and right ventricles.

#### 1.7.4.2 Hypothesis

The hypothesis is that, due to the congenital heart defect, strain values will be abnormal and therefore there will be correlations between the strain values of the group with a congenital heart defect + small left ventricle and the strain values of the group with LVIM and post-natal confirmation of CHD.

#### 1.7.4.3 Alternative Hypothesis

There will be no significant difference of strain values or a correlation between groups.

### 1.7.5 Aim 4

Investigate pregnancy outcomes of the whole cohort in relation to the measures obtained by cardiac performance analysis to identify any correlation between foetal cardiac dimensions, functional measures and longitudinal strain and outcome.

#### 1.7.5.1 Objective

Investigate pregnancy outcomes and categorise them into

1) Normal cardiac anatomy

2) Congenital Heart Disease (CHD) with observation only

3) CHD requiring intervention

5) Death

#### 1.7.5.2 Hypothesis

The hypothesis is that the smaller the left ventricular dimensions, reduced functional measures and impaired longitudinal strain will result in poorer outcomes.

# CHAPTER 2 METHODS AND STUDY DESIGN

## 2.1 STUDY DESIGN, SETTING AND POPULATION

### 2.1.1 Study Design

The study design was a retrospective collection of ultrasound images for analysis with the speckle tracking software TomTec.

### 2.1.2 Setting

Setting for data collection, and the analysis of the images was performed on NHS premises at St Michael's Hospital, Bristol.

### 2.1.3 Population

Data was obtained from a population of pregnancies between 2017-2020, where the foetal echocardiogram DICOM images were obtained and stored as part of a foetal cardiology consultation. These were women who had been seen in foetal cardiology for a clinical indication and the images stored for clinical purposes. There are numerous clinical indications for a referral to foetal cardiology, but in this population, referrals were for abnormal anatomical presentation detected from a foetal cardiac examination, or from referral for screening women with a high risk for having a baby with a congenital heart defect. Abnormal cardiac presentation can be a referral from sonographers performing the PHE screening anomaly scan between $18^{+0}$ and $21^{+6}$ or from an obstetric/ foetal medicine specialist examination in and around the southwest of England.

### 2.1.4 Outcomes

Post-natal outcomes, which are routinely collated and stored on the foetal cardiology database, provides a means for review, audit and quality assurance in clinical practice.

The outcome information for this study was obtained from this database and that of the UHBW electronic patient records – Medway Maternity and/or Evolve. Further to this information, National Institute for Clinical Outcomes Records (NICOR) was accessed to determine whether there were any additional outcomes identified by patients having required a cardiological intervention in the first year of life.

## 2.2 INCLUSION/EXCLUSIONS

### 2.2.1. Inclusion Criteria

- Foetal echo studies with an observable imbalance between the right and the left ventricles due to.
    - the presence of a congenital heart defect
    - unknown aetiology
- Foetal echo studies with a standard normal 4 chamber image in an axial plane without sonography modification (which is performed for suboptimal foetal position) at mid-gestation between 15-26 weeks
- Maternal age >18yrs to avoid conflicting governance on the use of paediatric data
- For the normal cohort – uneventful singleton pregnancy with a gestational age between 15-26 weeks gestation

### 2.2.2. Exclusion Criteria

Any of the following conditions were excluded due to the influence it may have as an independent variable.

- Foetal cardiac diagnosis of hypoplastic left heart syndrome
- Foetal congenital heart defect affecting the AV or VA concordance (i.e., double inlet left ventricle)
- Known foetal extra-cardiac abnormalities, such as congenital diaphragmatic hernia,
- Foetal conditions which affect haemodynamic circulation such as foetal growth restriction or foetal anaemia
- Maternal conditions which alter the foetal circulatory loading, such as maternal hypertension, diabetes or placental disease
- Multiple pregnancies due to the potential for circulatory misdistribution
- Suboptimal imaging of the 4-chamber view

## 2.3 DATA COLLECTION

### 2.3.1. Data Collection

A search was conducted of the foetal cardiology excel outcomes database to identify pregnancies for the study which were seen at St Michael's between September 2017 and November 2020. This database contains information on patient and pregnancy demographics on all aspects of foetal cardiology referrals, the foetal cardiology consultations, antenatal diagnosis and outcome. This is vitally important to observe the accuracy of the antenatal diagnosis against post-natal diagnoses of structural malformations and chromosomal anomalies. This service provision ensures close monitoring and quality assurance on pre- and post-natal CHD diagnostics and was used for such purposes in this study.

### 2.3.2. Cohort 1 - Left Ventricular Imbalance

A search criterion was selected which filtered both the clinical indications and the diagnosis columns of the database using the following identifying terms:

- ? LV>RV imbalance, ventricular size imbalance **and/or** discrepancy, unbalanced RV/LV **and/or** Ventricles, small LV, ventricular disproportion

The search on these terms resulted in only 17 patients and therefore the search was further extended to terms relating to potential left sided obstructive lesions including:

- ? Coarctation, small aorta, small arch, hypoplasia of the aorta, turbulent flow in the isthmus

This produced 54 potential patients for STE and strain analysis. After reviewing all the images for suitability, 14 were excluded based on both the exclusion criteria and poor imaging of the foetal cardiac 4 chamber view.

### 2.3.3 Cohort 2 - Congenital Heart Disease with Unbalanced Ventricles

- The same foetal cardiac database was used to identify patients seen with a foetal cardiac diagnosis of a congenital heart defect with an associated ventricular imbalance. The search filters were applied again to the antenatal diagnosis column to include any conditions which included the terms listed above in addition to the following congenital heart defects. Unbalanced AVSD, small RV, PA +/- IVS, small LV

These foetal echocardiograms were confirmed as being abnormal having identified a congenital heart defect as well as having ventricular imbalance.

### 2.3.4 Cohort 3 - Structurally Normal Foetal Cardiac Anatomy

Unlike the data collection in the previous 2 cohorts, the foetal echocardiograms which were considered normal were extracted from the Phillips ISCV imaging platform. Here, studies are annotated and highlighted as good quality under the patient demographics. Therefore, the normal cohort were selected by being categorised as having good quality imaging and demonstrating a normal foetal cardiac presentation. They were later confirmed as being normal after birth on electronic patient records by way of the New-born and Infant Physical Examination (NIPE) (PHE P. , 2014). The NIPE is another screening programme by public health England which is performed by either a paediatrician or GP to screens babies within 72 hours of birth (new-born examination) and then once again between 6 and 8 weeks of age. The infant examination is an early clinical assessment used to identify any abnormalities or any clinical concerns relating to four main anatomical features:

**Eyes**: about 2 - 3 in 10,000 babies have problems with their eyes that require treatment.

**Heart**: about 1 in 200 babies may have a heart problem.

**Hips**: about 1 or 2 in 1,000 babies have hip problems that require treatment.

**Testes**: about 1 in 100 baby boys have problems with their testes that require treatment.

This screening tool uses clinical expertise and examination to ensure normality (PHE P. , 2014).

## 2.4 PATIENT AND MATERNAL DEMOGRAPHICS

Maternal demographics were collected to include hospital number identification, the indication for referral to foetal cardiology, gestational age of the fetus, the 4-chamber imaging frame rate and the foetal heart rate.

## 2.5 ULTRASOUND IMAGING

### 2.5.1 Setting and Equipment

The ultrasound setting in foetal cardiology permits 45minutes per foetal echocardiogram, using 2 dedicated obstetric ultrasound machines, Canon Aplio 800i™. Women are seen, ideally, for their first consultation from 18weeks gestation, although referrals are accepted up until 40 weeks. Occasionally, women are seen at an earlier gestation, 16weeks if it is clinically indicated or if it is specifically requested. Pregnant women are scanned in a semi-recumbent position with a conductive water-based gel to optimise imaging. The foetal echo examination follows, but is not restricted to, the service foetal echo SOP which aligns to local, national, and international guidelines (appendix 2). All ultrasound and cardiac examinations were performed by one of 2 experienced foetal cardiac sonographers – AH and the author (JEJ). As previously summarised (table 2) the foetal cardiac protocol was performed using a Canon i8cx1 matrix convex probe with a frequency range of 1.8 - 6.2MHz. Additional measurements were post-processed offline using TomTec software which can be seen in table 4.

| Table 4 Summary of the post-processed research components on TomTec |
| --- |
| • Store a 3 second (at least) DICOM loop of the four-chamber view to perform speckle tracking analysis and longitudinal strain quantification using analysis software TomTec<br>• Measure 2D cardiac dimensions of tricuspid valve annulus (basal RV), mitral valve annulus (basal LV), right ventricular and left ventricular lengths<br>• All measured in end-diastole and referenced to z-scores<br>• Determine a single cardiac cycle using m-mode to determine the heart rate and differing phases of systole and diastole<br>• Mark reference points onto the basal septum and lateral internal walls of the left and right ventricle and the apex to guide the software to the endocardial contour.<br>• Adjust the borders, where necessary, to ensure appropriate tracking of the endocardial wall throughout the entire cardiac cycle.<br>• Start speckle tracking analysis and manually adjust timings and tracking to ensure accuracy.<br>• Use 'quiver' function where border definition is challenging.<br>• Store the numerical results under the study identification. |

### 2.5.2 Cardiac Anatomy

Ultrasound imaging of the 4-chamber view of the foetal heart is obtained by directing the ultrasound beam perpendicular to the foetal chest to obtain an axial plane, ensuring that the ribs are seen in cross section. Optimisation of the ultrasound image is

performed as standard for clinical diagnostic assessment although, as this was a retrospective collection, the images were not specifically optimised for speckle tracking as the primary objective. Cardiac measurements were performed in relation to the standardised positions recommended by Schneider, 2005 (figure 25) and collated against age-related z-scores of the same source.

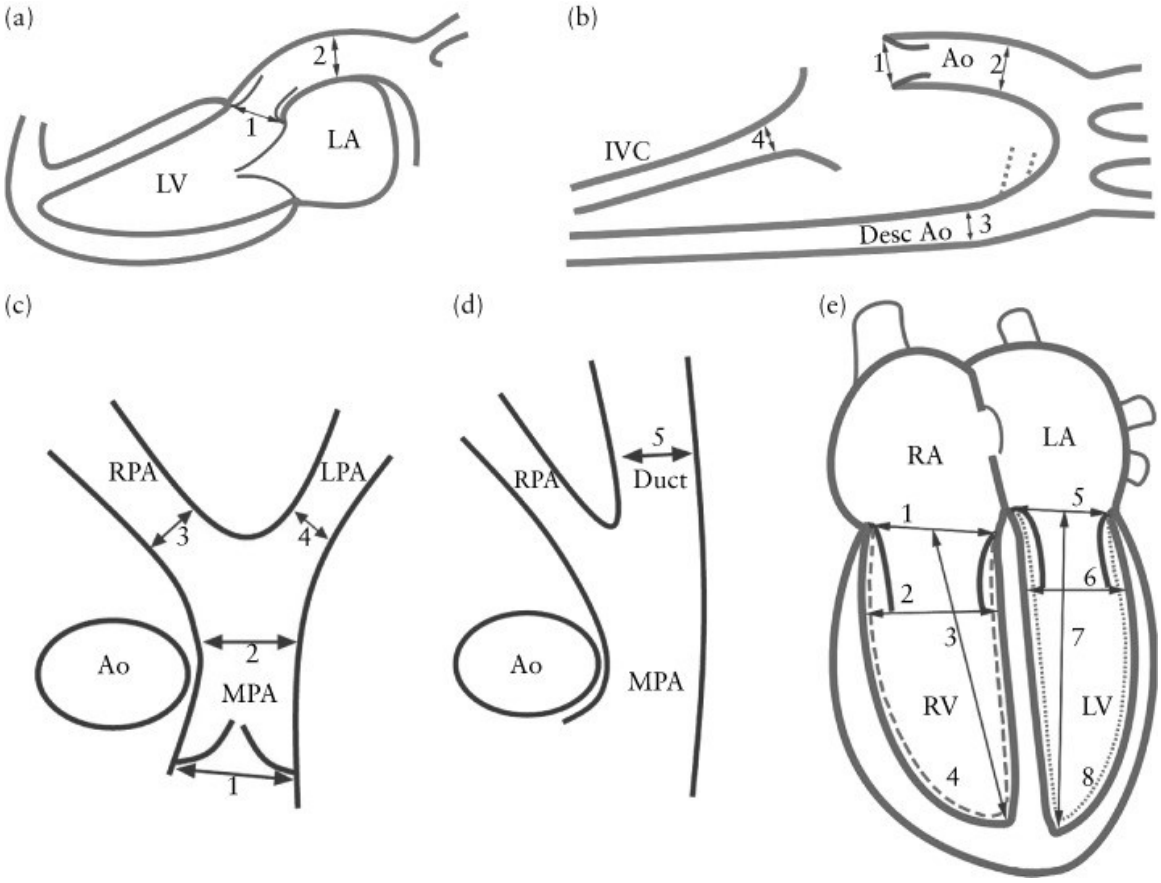


*Figure 25 Anatomical positions for foetal cardiac dimensions. (Schneider 2005)*

*Foetal echocardiographic views from which the cardiac structures and dimensions were measured. (a) Long-axis view of the left ventricle showing the aortic valve (1) and ascending aorta (2). (b) Aortic arch view showing the aortic valve (1), ascending aorta (2), descending aorta (3) and inferior vena cava (4). (c) Short-axis view, showing the pulmonary valve (1), main (2), right (3) and left (4) pulmonary arteries. (d) Oblique short-axis view, showing the pulmonary trunk and the arterial duct (5). (e) Four-chamber view, showing the tricuspid valve (1), right ventricular end-diastolic dimension (2), right ventricular inlet length (3), right ventricular area (dashed line) (4), mitral valve (5), left ventricular end-diastolic dimension (6), left ventricular inlet length (7) and left ventricular area (dotted line) (8). Ao, aorta; Desc Ao, descending aorta; IVC, inferior vena cava; LA, left atrium; LPA, left pulmonary artery; LV, left ventricle; MPA, main pulmonary artery; RA, right atrium; RPA, right pulmonary artery; RV, right ventricle.*

### 2.5.3 Image Optimisation

Optimisation of ultrasound involves adapting the settings based on the fundamental principles of ultrasound physics to ensure that imaging is of optimum quality when considering depth, motion and clarity of the anatomy being imaged. Figure 26 illustrates recommendations for image optimisation which is performed in all foetal echocardiograms to produce the best quality imaging for clinical assessment. These steps are also recommended for accurate speckle tracking echocardiography and use in performing strain analysis using 2D CPA TomTec software (Bandano, et al., 2018).

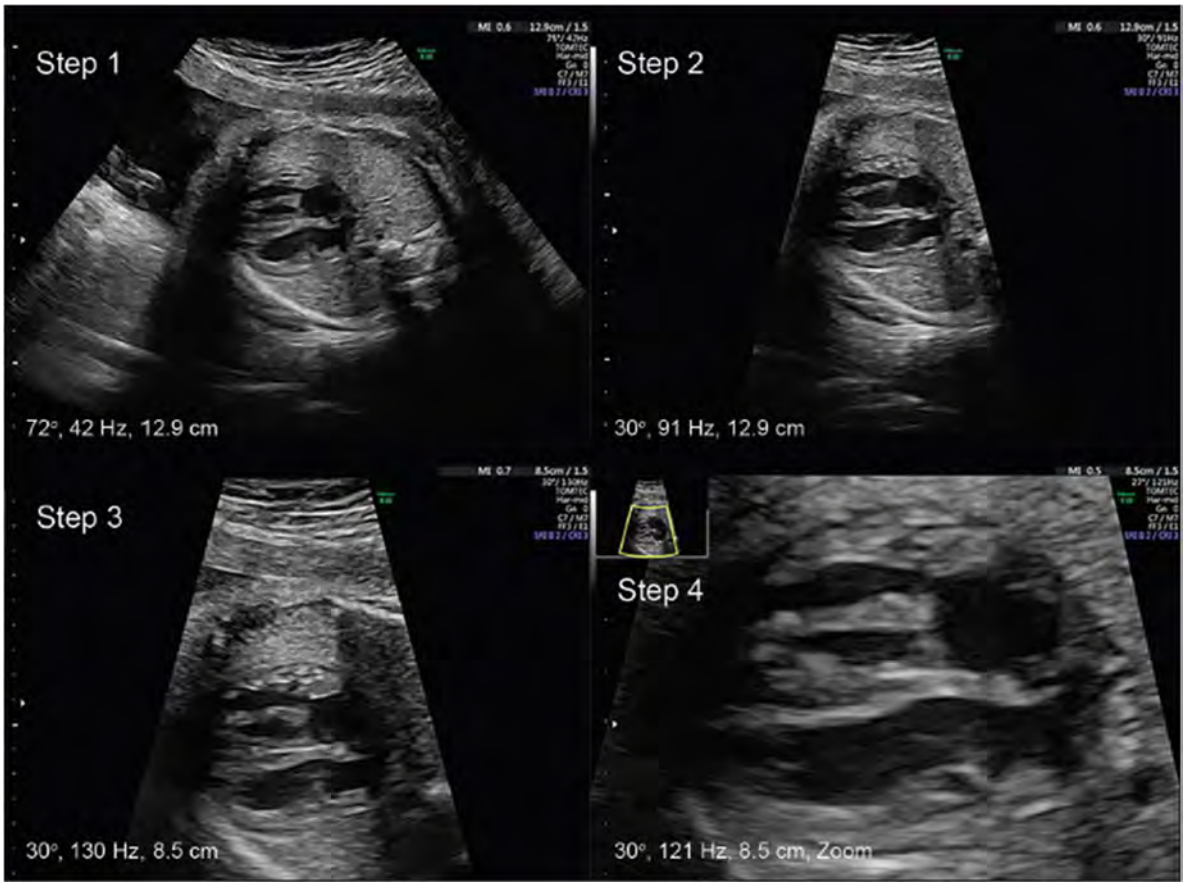


*Figure 26 Optimisation of the 4-chamber view*

*(DeVore GR P. B., 2016)*

*Step 1. Obtain a wide-angle image of the fetus in an axial plane at the level of the foetal heart.*

*Step 2. Reduce the sector width to increase the ultrasound frame rate (number of frames acquired/second across the sector).*

*Step 3. Adjust the depth of the image to further increase the frame rate and reduce extra-cardiac anatomy from view.*

*Step 4. Apply a 'spot zoom' function to the image to enlarge and visualise only the foetal cardiac anatomy.*

### 2.5.4 Digital Clips

Around 3-5 seconds of imaging is stored in a standard digital image format DICOM loop. The DICOM clip is acquired during foetal and maternal rest to prevent motion artefact. The whole foetal echocardiogram is stored and is also automatically transferred from the ultrasound machine onto the Phillips imaging viewing platform Intellispace Cardiovascular (ISCV). The images are later transferred onto the UHBW Trust PACs system for long term storage, as a standard of practice.

### 2.5.5 Study Selection

In women who had more than one foetal echocardiogram, the earliest foetal echo from the pregnancy was used for analysis. This was deliberate as the aims of this study were to assess STE and strain values at mid-gestation ($18^{+0}$ to $21^{+6}$ weeks) and not on subsequent foetal echoes where the foetal cardiac presentation may have altered.

### 2.5.6 Clip Selection

The 4-chamber view was evaluated for suitability, anonymised and allocated a study identification label. It was then transferred to a secure folder on the UHBW password protected server in preparation for offline analysis.

## 2.6 OFFLINE IMAGE ANALYSIS TOOL

TomTec imaging systems GmbH (Munich, Germany) is an internationally recognised provider of cardiac speckle tracking software – 2D cardiac performance analysis (CPA) version 1.2. The speckle tracking software, which is owned by the foetal medicine team, is located on a password protected PC at St Michael's hospital. The software was purchased for analysing STE in a local twin-to-twin transfusion study but TomTec has since had a software version upgrade which is considered superior for foetal cardiac analysis. For the purposes of the study, TomTec provided a system upgrade to the latest version for greater accuracy and enhanced measure of foetal cardiac anatomy. This software also permits quantification of the cardiac volumes based on mathematical assumptions of the LV cavity and anatomical calculations from tracking the endocardial wall. All 120 foetal echoes were imported and stored in the Tomtec 2D CPA software.

## 2.7 TOMTEC ANALYSIS

### 2.7.1 Analysis

Left and right ventricular myocardial mechanics were analysed by Cardiac Performance Analysis (CPA), which produced quantitative data on endocardial shape, size, and contractility measures from a single/or multiple cardiac cycles. Myocardial strain by STE was calculated using a semi-automatic method whereby the software identifies the interface between the endocardial borders and the blood-filled ventricular cavity.

Segmentation of the basal, mid, and apical regions were automatically derived and positive/negative peak defections in motion used to calculate the percentage

difference. As this force was relating to a baseline zero in diastole, the peak percentage difference for systole was a negative value. The more negative the value the higher the muscle deformation. Global longitudinal strain values were calculated based on the endocardium (inner layer) from the minimum and maximum mean peak from the 6 regions of the left and right ventricles. This is in accordance with the standard of the EACVI/ASE working group on strain quantification (Voigt, et al., 2015).

**2.7.2 Timing**

Cardiac timing is essential for analysis to ensure that accurate and consistent start and end points of the cardiac cycle were observed. In the absence of an ECG (electrocardiogram) in foetal echocardiography, the R-R interval of the cardiac cycle was pre-determined using a m-mode technique to subjectively plot diastole and systole at the point of mitral valve closure (end-diastole) and opening (aortic valve closure, end-systole) respectively. A cursor (yellow line) on the top right-hand image (Figure 27) was positioned across the basal-mid ventricular cavity, perpendicular to the septum. This section was then selected to view the structures in motion (Figure 27). Markers were manually placed to indicate one cardiac cycle (blue lines), providing sample selection

and heart rate for foetal cardiac analysis. Interval allocation was based on the best quality m-mode and b-mode imaging of the cardiac cycle as interpreted by the operator.

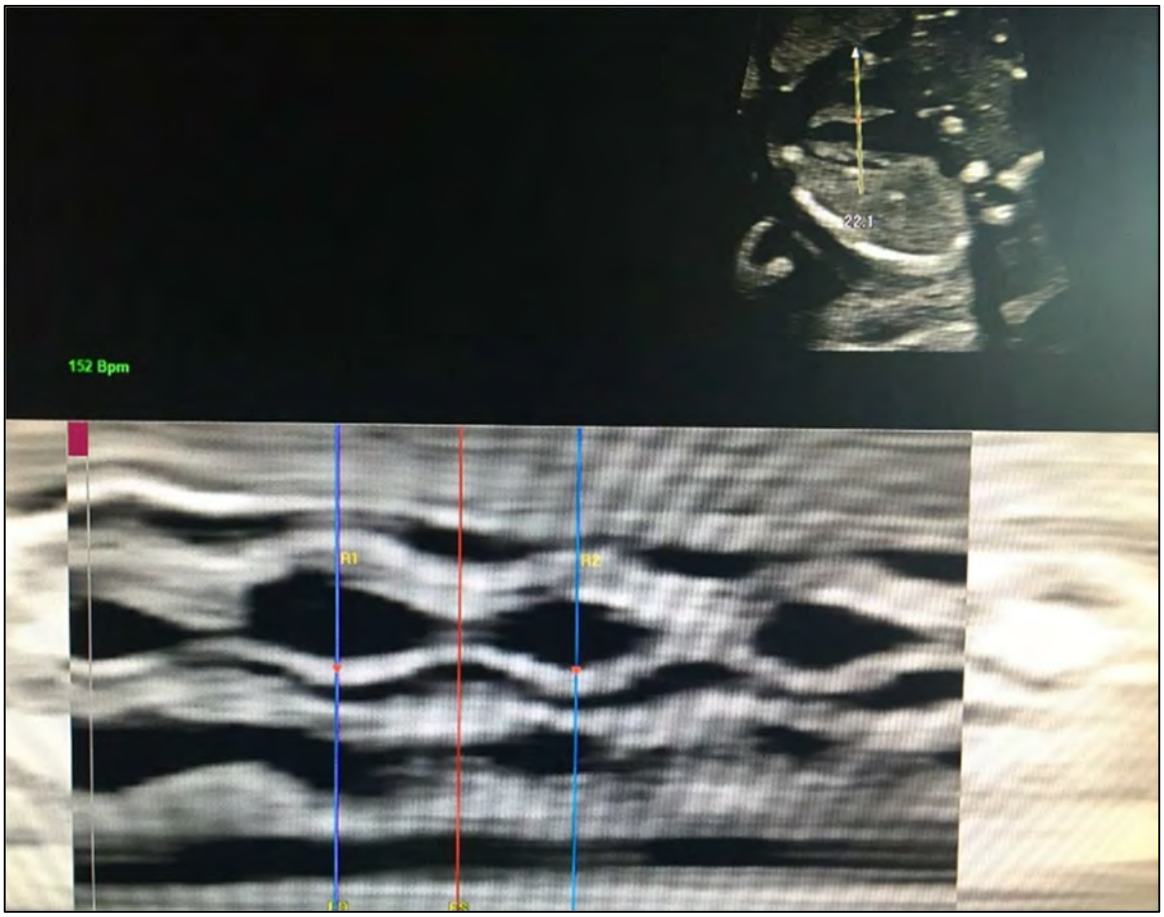


*Figure 27 2D CPA m-mode timing*

*Method for manually calculating end-systolic (ES), red line and end-diastolic (ED) intervals blue lines. R1 and R2 represents one cardiac cycle.*

Anatomical closure and opening of the mitral and tricuspid valves were determined on the 2D image to signify end-diastole and end-systole respectively. The accuracy of these markers was imperative for quantification of peak systolic and diastolic values which would be used for timing points for peak deformation. The black and white segment of the m-mode was selected for 2D CPA analysis. Depending on the quality and stability of the m-mode, CPA analysis was performed on one or more cardiac cycles. More cycles

demonstrated consistency of tracking across the selected cycles and averaged the strain values.

### 2.7.3 Endocardial Border

Once the cycle selection was made, the endocardial border was defined using 3 anatomical points as landmarks for the lateral free wall at the mitral annular junction, the septal/mitral annular junction, and the apex of the left ventricle (figure 28). 2D CPA provides a “quiver” function to allow for accurate placement, by the operator, between two or more frames. Quiver provides the operator with the option to allow the cine loop to hoover backwards and forwards between frames to better determine the endocardial border definition. Studies have demonstrated that the accuracy of anatomical placement is vastly improved using this mechanism (DeVore, Satou, & Sklansky, 2021).

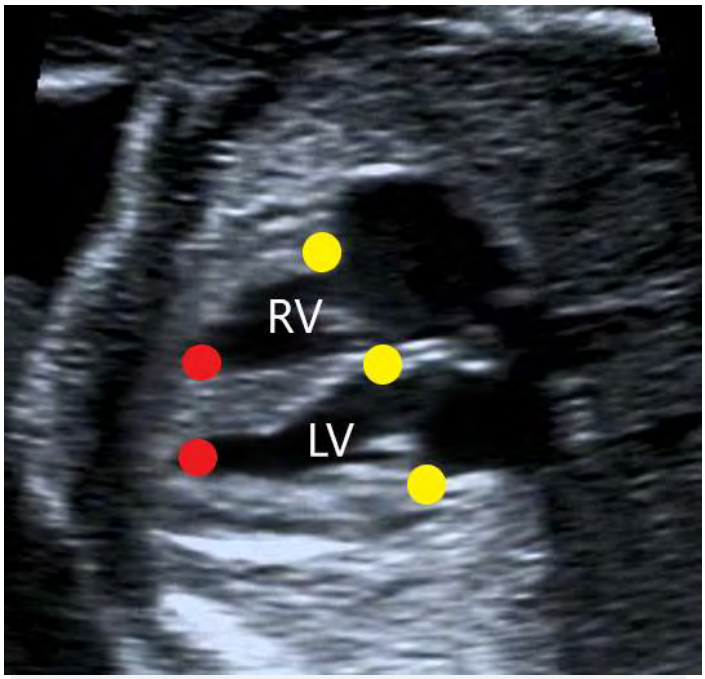


Figure 28 Anatomical markers for CPA analysis.

Yellow markers indicating left and right annular points, red markers identifying the apex of the corresponding ventricle

### 2.7.4 Automated Tracing

Once these landmarks were appropriately placed, an automated trace was produced by the software to depict the endocardial wall interface in systole. This process was then repeated at end-diastole to trace the endocardium. Where there was any deviation from the automated recognition of the wall interface, manual manipulation was made by the operator to correct any inaccuracy. This was apparent, particularly in the right ventricle where the moderator band was interpreted as the RV apex. Figure 29 illustrates the process of manipulating the tracking of the left ventricular endocardium where there is failure to automatically track the LV apex.

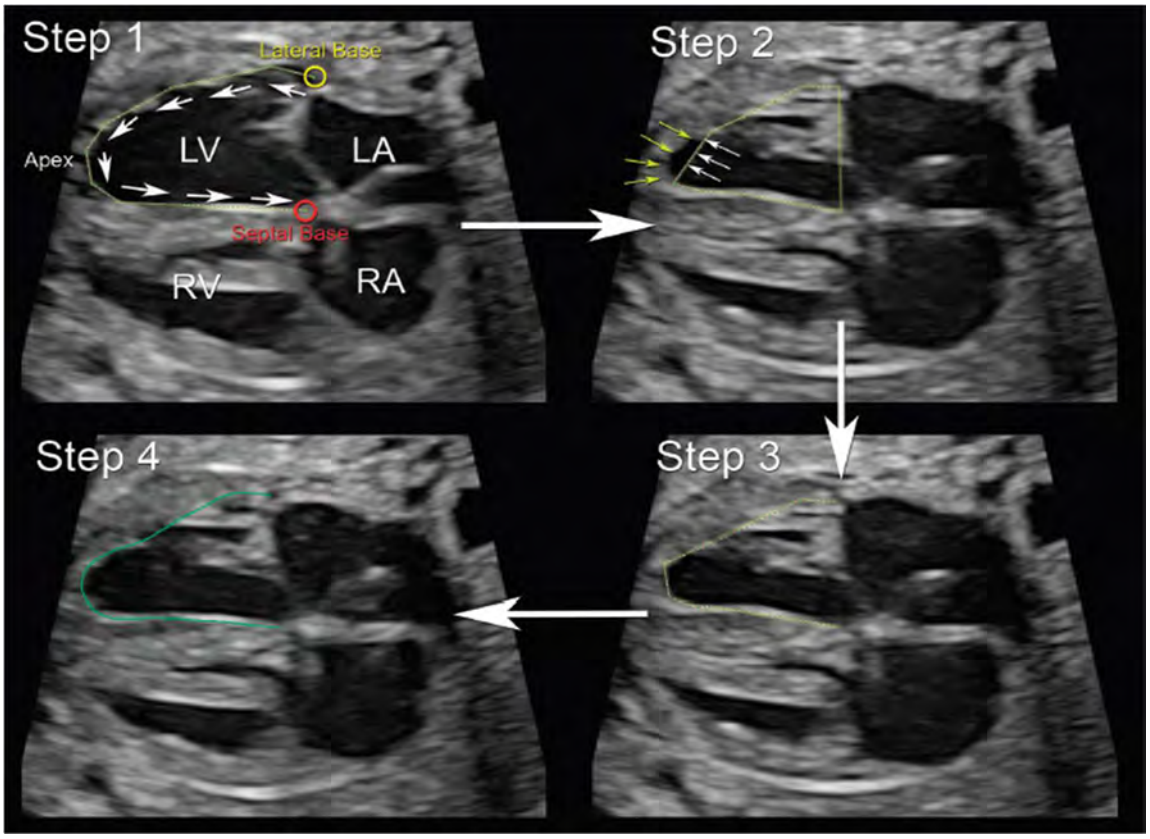


*Figure 29 Process of manually adjusting endocardial border definition*

*Step 1 illustrates the 3-point positioning of anatomical landmarks. The yellow circle identifies the position of the lateral wall/mitral annular junction, the red circle denotes the septal wall/mitral annular junction, and the third point is the LV apex.*

*Step 2 shows the automated tracking has missed a region at the LV apex where the automated line (white arrows) is not aligned to the apical endocardial wall (yellow arrows).*

*Step 3 shows manual operator adjustment to follow the LV apical contour and re-activation of the analysis. At this point, it is also important to observe correct tracking of the wall interface throughout the whole cardiac cycle. This is paramount for ensuring that tracking is limited to the endocardial wall speckles rather than the speckles detected of the valvular apparatus, or additional muscle bundles at every frame throughout systole and diastole.*

*Step 4 shows the final (green line) tracking interface of the endocardium which is used for tracking the speckles throughout the cardiac cycle in both systole and diastole.*

*[Figure taken from DeVore, Polanco, Satou, & Sklansky, 2016]*

### 2.7.5 Deformation Curves

Deformation of the muscle from zero in diastole to a peak percentage change in systole was tracked throughout the cardiac cycle for each region and averaged. Tracking was displayed in graphical form (Figure 30) where the top trace shows myocardial deformation of both the Endocardial Global Longitudinal Strain (Endo GLS, in orange) and the Endocardial Global Cardiac Strain (Endo GCS, in pink). Note the peak negative deflection of end-systole (eS) at approx. -30%, and the timing of end-diastole (eD) at

baseline zero. Deviation of deformation into the positive were attributed to pre-systolic physiological changes which would artificially obscure percentage change.

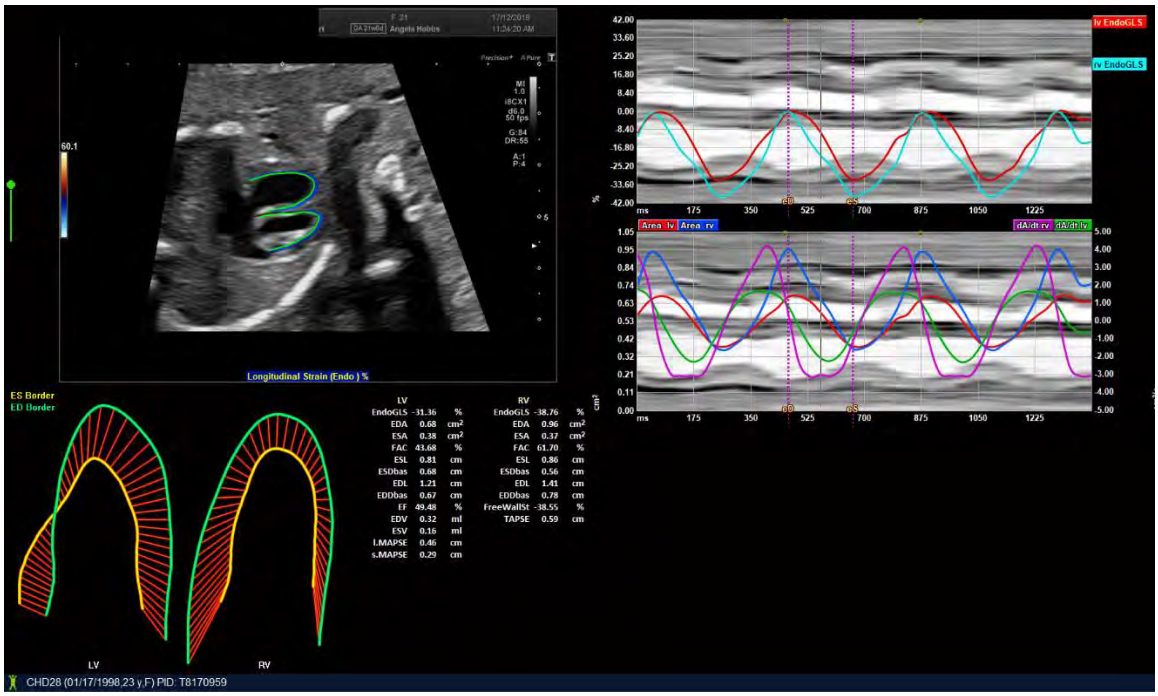


*Figure 30 Graphical representation of the percentage change in strain across 4 cardiac cycles with the automated quantification.*

### 2.7.6 Flipping Tool for Segmental Analysis.

In addition to functional strain quantification of the left ventricle the software automatically flips the images to the correct orientation for segmental analysis of the endocardial wall segments. Thus, deformation of the left and right ventricular walls can demonstrate the percentage strain for all the anatomical regions of the cardiac muscle. Figure 31 demonstrates both the flipping process required to ensure that the software can analyse the image and the output data generated from the segmental analysis.

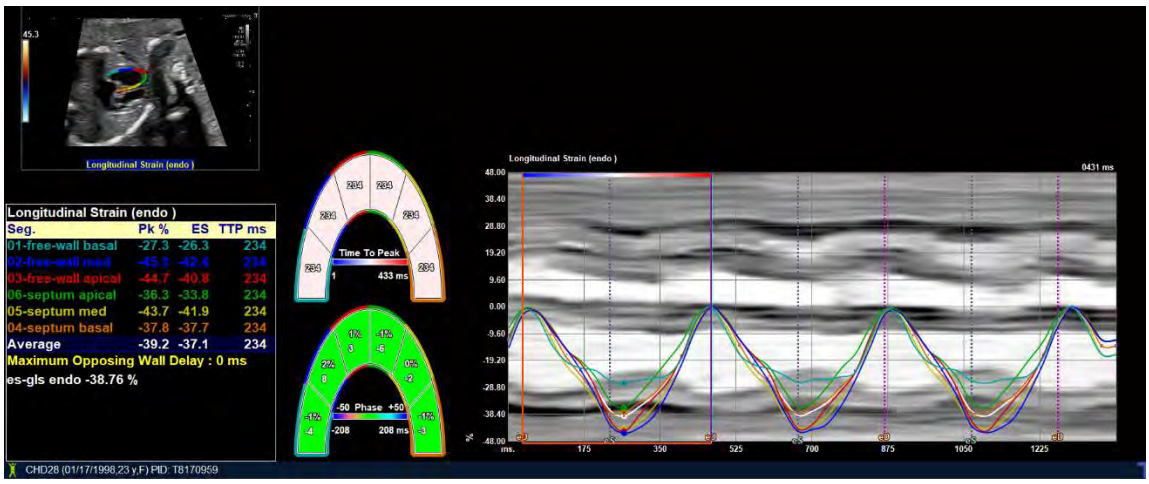


*Figure 31 Flipping process and analysis of the LV*

### 2.7.7 Size, Volume and Functional Quantification.

Once satisfactory and accurate speckle tracking analysis is performed, the software calculates several functional and volumetric parameters which are generated using mathematical and theoretical assumptions of cardiac anatomy. This provides diagnostic data of various functional parameters including ejection fraction (%), displacement of the mitral and tricuspid annulus towards the apex or longitudinal systolic function (TAPSE and MAPSE (mm)), fractional area change (%), as well as the calculated cavity and wall dimensions. These dimensions are used to describe patterns of cardiac remodelling. The data output from cardiac performance analysis on TomTec is summarised in Table 5.

**Table 5 Data output obtained from 2D CPA analysis, TomTec Arena.**

| **Index** | Units | LV | RV |
|---|---|---|---|
| **EndoGLS** | % | -17.66 | -40.95 |
| **EDA** | $cm^2$ | 0.66 | 0.15 |

| | | | |
|---|---|---|---|
| **ESA** | $cm^2$ | 0.32 | 0.25 |
| **FAC** | % | 52.32 | 66.23 |
| **ESL** | cm | 1.29 | 1.11 |
| **ESDbas** | cm | 0.48 | 0.37 |
| **ESDmid** | cm | 0.25 | 0.24 |
| **EDL** | cm | 1.56 | 0.77 |
| **EDDbas** | cm | 0.62 | 0.39 |
| **EDDmid** | cm | 0.51 | 0.21 |
| **EF** | % | 71.55 | 43.84 |
| **EDV** | mL | 0.25 | 0.33 |
| **ESV** | mL | 0.07 | |
| **l.MAPSE** | cm | 0.25 | |
| **s.MAPSE** | cm | 0.30 | |
| **TAPSE** | cm | | 0.33 |

*EndoGLS=Endocardial global longitudinal strain, EDA=End-Diastolic Area, ESA=End-Systolic Area, FAC= Fractional Area Change, ESL= End-Systolic Length, ESDbas=End-Systolic Diameter Basal, ESDmid=End-Systolic Diameter Mid, EDL=End-Diastolic Length, EDDbas=End-Diastolic Dimension Basal, EDDmid=End-Diastolic Dimension Mid, EF=Ejection Fraction, EDV=End-Diastolic Volume, ESV=End-Systolic Volume, l.MAPSE=Lateral Mitral Annular Plane Systolic Excursion, s.MAPSE=Septal Mitral Annular Plane Systolic Excursion, TAPSE=Tricuspid Annular Plane Systolic Excursion*

Using the same methodology, the right ventricular endocardial wall was identified using landmarks at the septal wall/tricuspid annular junction, the lateral wall/tricuspid annular junction, and the RV apex. Again, where possible, repeatability was assessed across multiple cardiac cycles to observe inappropriate fluctuations due to artefact.

The strain curves, from the analysis, were visually inspected by the operator to ensure appropriate timing and accurate tracking. All the numerical values were collated in an

excel spreadsheet and transferred to a secure location for further review and data analysis.

## 2.8 DATA ANALYSIS

### 2.8.1 Data

The numerical data collected from the 2D CPA analysis was saved and stored in an Excel results database. Basic analysis was performed to include information on groups of data between the 3 cohorts and then summarised to identify key findings, ranges, averages, and median values. All the cardiac dimensions were cross referenced to gestational-related z-scores (figure 32) using the web base app Foetal Echo Z-Score App, fetal.parameterz.com using Schneider, 2005 as the source. +/- 2.0 or 2 standard deviations from the mean, is universally used as a cut off for significantly larger (=) or small (-) based on an individual measurement which exceeds 90% of the upper interval of normal distribution.

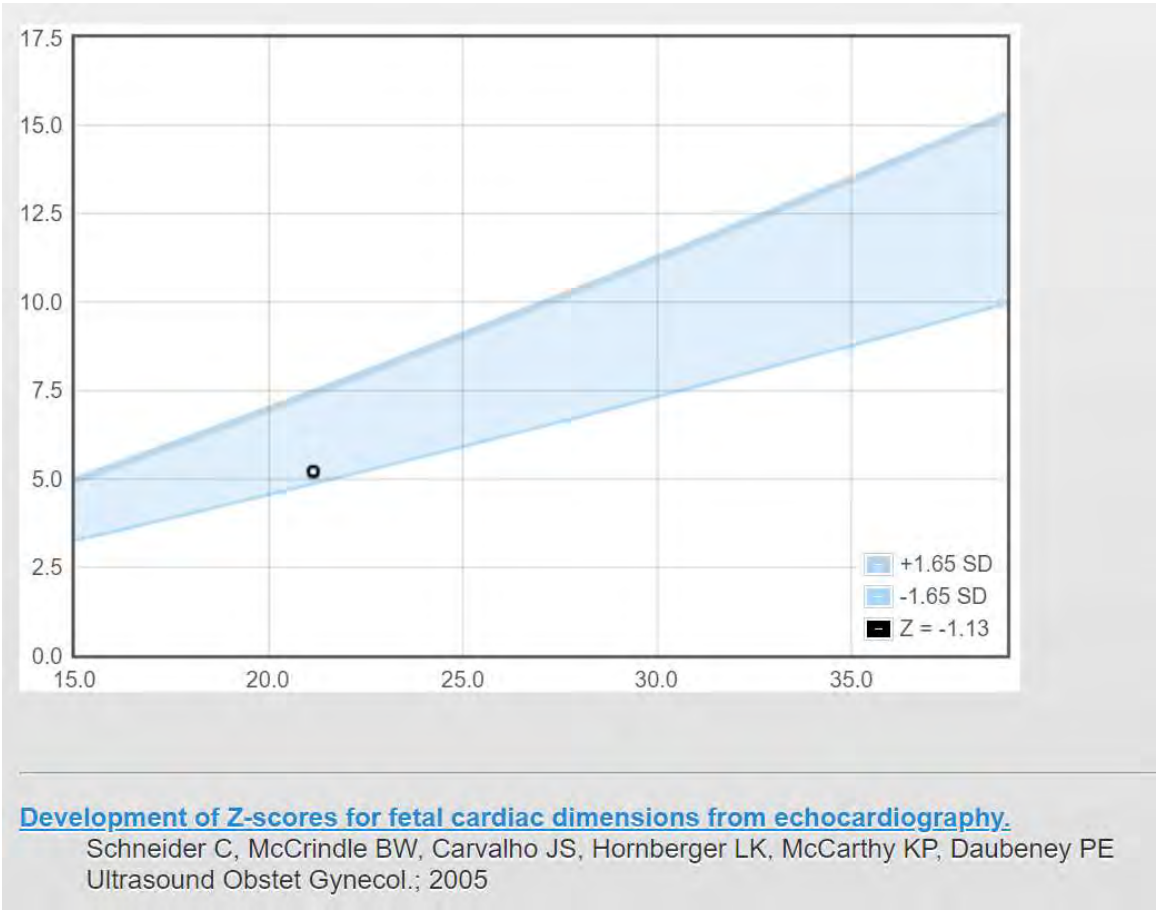


*Figure 32 Example of age-related reference ranges for foetal cardiac measurements [taken from Foetal Echo Z-Score App | fetal.parameterz.com]*

### 2.8.2 Graphpad

Statistical analysis was supported by Dr Adrian Kendrick and performed using GraphPad Prism 9 (v9.2.0 July 15th, 2021).

#### 2.8.2.1 Descriptive Statistics

All collected data was analysed to assess for normal distribution using the Anderson-Darling Test. Descriptive statistics are based to some extent on the outcome of this normality test. Where the test indicated a normal distribution of data, then mean ± standard deviation (SD) was used to describe the data. Where the test indicated a non-normal distribution, then the median and interquartile range (IQR) was reported.

#### 2.8.2.2 Inter-Rater Reliability

This is a measure of the variation between two assessors that are measuring the same subjects. In this study, two operators (JEJ and SN) independently reassessed the same recordings on two separate occasions blindly. The results of the measurements of LV Strain%, RV Strain% and RV Free wall Strain% were assessed in 12 subjects, four from each group of LVIM, CHD and Controls. Therefore, there were n=8 subjects per measurement.

The Pearson's Product Moment Correlation (*r*) gives a correlation that quantifies the direction (+ or -) and the strength of the relationship by estimation of the linear relationship which is between +1 to -1 (Vaz et al, 2013). This reflects how closely a set of paired observations (test-retest) relate to a straight line and is independent of the slope of that line.

The intraclass correlation coefficient (ICC) is similar to *r* but is calculated more precisely according to specific rules (Koo & Li, 2016)., which are based on the definitions of McGraw and Wong (1996) that defines the "Model", the "Type" and the "Definition". The flowchart within Koo & Li (2016) was used to guide the pathway analysis and concluded that this was a two-way mixed effect, absolute agreement, single rater/measurement form. For this a two-way ANOVA was used and the ICC calculated as follows.

**Table 6 Two-Way Anova for ICC**

| **ANOVA table** | | SS (Type III) | DF | MS |
|---|---|---|---|---|
| **Row Factor** | **RF** | 601.9 | 7 | 85.99 |
| **Column Factor** | **CF** | 176.2 | 1 | 176.2 |
| **Error** | **E** | 902.6 | 7 | 128.9 |

$$ICC = \frac{MS_{RF} - MS_E}{MS_R + (k - 1)MS_E + (k/n)(MS_{CF} - MS_E)}$$

Where k is the number of operators (k = 2), n = number of subjects (n = 8). For each index, the ICC was calculated from the above equation, having firstly obtained the table from the 2-way ANOVA. Values of ICC obtained are categorized according to the following –

| Table 7 ICC Score and corresponding reliability | |
|---|---|
| **ICC Score** | Reliability |
| >0.90 | Excellent |
| 0.75 to 0.90 | Good |
| 0.5 to 0.75 | Moderate |
| < 0.50 | Poor |

The repeatability coefficient (CR) quantifies absolute reliability and is the value below which the absolute differences between two measures lie with 0.95 probability. This is obtained by multiplying the within subject standard deviation (SD) obtained from the repeated measures ANOVA mean square residual and then multiplied by 2.83, i.e., $2.83S_w$. So, for LV Strain in the LVIM group –

| Table 8 Repeatability coefficient for reliability | | | |
|---|---|---|---|
| **ANOVA table** | SS | DF | MS |
| Treatment (between columns) | 471.4 | 7 | 67.34 |

| | | | |
|---|---|---|---|
| Individual (between rows) | 0.7613 | 1 | 0.7613 |
| Residual (random) | 53.93 | 7 | 7.705 |
| Total | 526.1 | 15 | |

$$S_w = \sqrt{7.705} = 2.78.$$

$$CR = 2.83S_w = 2.83 \times 2.78 = 7.86$$

In addition to the CR, a Bland-Altman plot was used to compare the results (Bland & Altman, 2003). This plots the difference between the two numbers against the mean of the two numbers and estimates the bias, the mean difference and the 95% limits of agreement (± 1.96 SD). An example plot of this is shown in Figure 33.

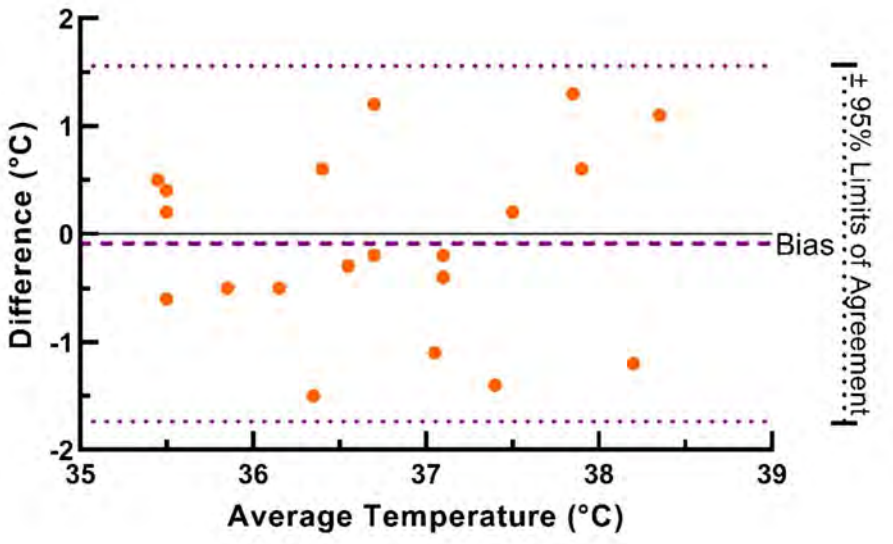


*Figure 33 Bland-Altman Example*

*Example plot of temperature measurement assessed twice on the same subject 5 minutes apart. Bias is -0.09°C, 95% limits of agreement of -1.738 to +1.558 °C.*

*Data courtesy Perpigilium Brown.*

### 2.8.2.3 Group Comparison Analysis

Comparison of data between Control, CHD, LVIM + CHD and LVIM no CHD was undertaken using ordinary one-way ANOVA with the Bonferroni multiple comparison test, comparing each group to each of the other groups. This testing produced an overall p value for the ANOVA test and for each comparison a p value. Graphically, the data are represented as shown in the Figure 34. The global analysis of comparing all four groups shows that there is a significant difference between the groups ($p = 0.0011$). For each comparison, the graphic shows the degree of significance by providing the actual p value. P values observed as $p < 0.05$ are regarded as statistically significant differences.

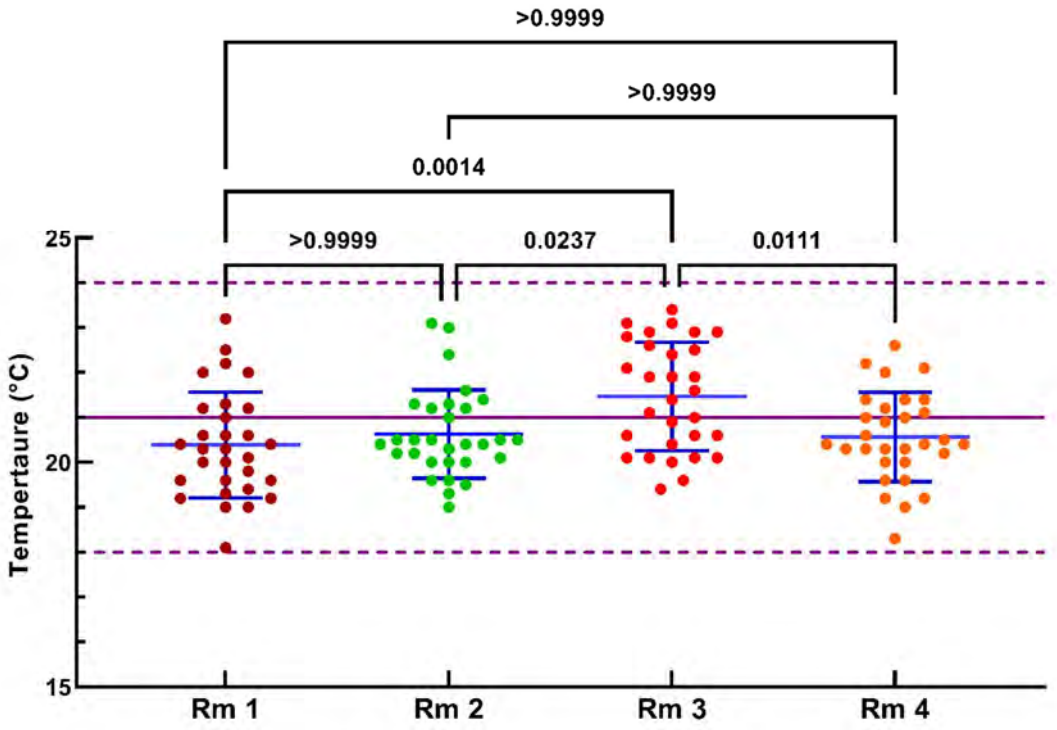


*Figure 34 Example of a one-way ANOVA with Bonferroni multiple comparison test*

*This example assesses the differences in room temperature over 30 consecutive days. The target temperature was 21˚C with a range of 18 to 23˚C. Rm 3 was overall higher, but always within the target range. Data courtesy of Perpigilium Brown.*

## 2.9 ETHICAL ISSUES

### 2.9.1 Sponsor

Sponsorship (Appendix 3) was granted by University Hospitals of Bristol and Weston NHS Foundation Trust (UHBW) who confirmed that Organisational Information Document (OID) or a Schedule of Events (SoECAT) was not required as part of HRA application (Appendix 4)

### 2.9.2 Ethics

This study was a retrospective observational study and was accepted by the Health Research Association (HRA) and Health and Care Research Wales (HCRW) without the need for a research ethics committee (REC) review (Appendix 5).

### 2.9.3 Confidentiality

All the foetal echo images that were analysed for the study were previously collected and stored securely for clinical purposes. Foetal echocardiograms were pseudo-anonymised for the study using a unique study identification and no patient identifiable material left NHS Trust premises. Pregnancy outcome data was identified from NHS maternity sources. This included the maternity Medway system at St Michael's and other comparative databases from the hospitals across the Southwest of England.

### 2.9.4 Consent

Any images used within this document or used for future publication will be from patients who gave written consent at the time of the foetal cardiology consultation. Evidence of this consent is stored on the UHBW electronic patient records – Evolve. As a teaching hospital it is common practice for the foetal cardiologists to obtain UHBW

Level 3 consent for any foetal echo studies which are particularly useful for teaching or publication.

# CHAPTER 3 RESULTS

## 3.1 RECRUITMENT AND DEMOGRAPHICS

### 3.1.1 Recruitment

In total, 112 pregnant women were identified with foetal echo findings which matched the inclusion/exclusion criteria for the 2 cohorts of a ventricular size imbalance. The ultrasound images were reviewed and 80 out of 112 were deemed to have adequate quality imaging for STE - 40 with Left Ventricular Imbalance (LVIM) and no confirmed diagnosis of a congenital heart defect and 40 foetal echoes with congenital heart disease and a ventricular size imbalance. 40 foetal echoes with normal cardiac anatomy were also identified as a control group. All foetal echoes were allocated a study number. Each foetal echo was cross-referenced to the pregnancy and maternal information stored and z-scores were calculated for all the ventricular measurements (Schneider, 2005).

### 3.1.2 Demographics – All Groups

Overall, 120 foetal echoes were recruited for STE analysis. The gestation range of the foetal echoes was between $15^{+3}$ and $25^{+5}$ with a median gestation of $20^{+4}$, mean 20.78 ± 1.51 weeks. Frame rates for the whole cohort were between 22 – 84Hz with a mean frame rate of 52.9. Foetal heart rates were between 108bpm – 182bpm with a mean of 148. There was no statistical difference between the gestation, frame rate or heart rate between the groups. The results are summarized in Table 9.

In those indices, when compared between the four groups, and where $p < 0.05$ is observed, then the results are presented in red. These results were further analysed using one-way ANOVA plots to identify the parameters where the significant differences lie within the four groups 1) Control, 2) CHD, 3) LVIM +CHD, 4) LVIM + normal (figure 35

& 36). The outcomes for all 120 pregnancies included in the study can be seen in figure 37. All the cardiac dimensions were cross-reference against age-related normative data (Schneider, 2005) to provide z-scores for those measurements. These can be seen in Table 10.

**Table 9 Summary of data from each cohort giving mean ± SD for each index**

**The p-value for the one-way ANOVA is shown when comparing the 4 groups. Those highlighted in red are where differences occur between some of the groups, which will be highlighted within each group section.**

| Index | Units | Control Group | CHD | LVIM + CHD | LVIM + normal | One-way ANOVA |
|---|---|---|---|---|---|---|
| **n** | | 40 | 38 | 33 | 6 | |
| **GLS Frame Rate** | fps | 52.33 ± 10.60 | 52.16 ± 12.86 | 55.24 ± 11.83 | 52.5 ± 15.32 | p = 0.6895 |
| **Gestational Age** | Weeks | 20.92 ± 1.76 | 20.62 ± 1.60 | 20.72 ± 0.96 | 21.15 ± 1.90 | p = 0.7623 |
| **Heart Rate** | bpm | 147.7 ± 10.68 | 144.7 ± 12.35 | 145.4 ± 12.21 | 154.2 ± 13.11 | p = 0.2543 |
| **LV Diastolic Length** | mm | 12.00 ± 2.23 | 11.03 ± 3.48 | 9.56 ± 1.77 | 11.63 ± 1.89 | **p = 0.0015** |
| **RV Diastolic Length** | mm | 10.79 ± 2.10 | 10.62 ± 2.46 | 10.58 ± 1.75 | 10.68 ± 2.30 | p = 0.9763 |
| **RV: LV ratio** | | 0.91 ± 0.14 | 1.07 ± 0.52 | 1.14 ± 0.26 | 0.93 ± 0.19 | **p = 0.0287** |
| **MV Diameter** | mm | 6.70 ± 1.62 | 5.35 ± 1.58 | 5.01 ± 1.07 | 5.68 ± 1.35 | **p < 0.0001** |
| **TV Diameter** | mm | 6.94 ± 1.48 | 7.61 ± 2.48 | 7.19 ± 1.19 | 7.03 ± 1.41 | p = 0.4319 |
| **MV: TV ratio** | | 0.974 ± 0.18 | 0.753 ± 0.30 | 0.703 ± 0.14 | 0.823 ± 0.24 | **p < 0.0001** |
| **LVd Volume** | mL | 0.946 ± 1.48 | 0.208 ± 0.17 | 0.234 ± 0.19 | 0.208 ± 0.12 | **p = 0.0008** |
| **LVs Volume** | mL | 0.356 ± 0.63 | 0.096 ± 0.10 | 0.098 ± 0.09 | 0.087 ± 0.04 | **p = 0.0075** |
| **LVEF** | % | 60.21 ± 11.1 | 60.44 ± 17.8 | 59.28 ± 13.7 | 61.52 ± 9.01 | p = 0.9786 |
| **LV GLS** | % | -23.18 ± 3.99 | -27.59 ± 8.26 | -26.49 ± 7.62 | -27.03 ± 3.90 | **p = 0.0285** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **LV ΔFractional Area** | % | 45.08 ± 9.28 | 48.36 ± 13.7 | 45.64 ± 11.3 | 47.88 ± 7.36 | p = 0.5942 |
| **LVd Length (TomTec)** | mm | 13.39 ± 3.31 | 10.48 ± 2.61 | 11.14 ± 1.86 | 13.22 ± 1.75 | **p < 0.0001** |
| **LVs Length (TomTec)** | mm | 10.46 ± 2.53 | 7.68 ± 2.05 | 8.01 ± 1.49 | 9.73 ± 1.37 | **p < 0.0001** |
| **MAPSE Lateral** | mm | 3.27 ± 1.15 | 3.03 ± 1.44 | 3.29 ± 1.23 | 4.48 ± 1.01 | p = 0.0845 |
| **MAPSE Septal** | mm | 2.97 ± 1.29 | 2.61 ± 1.31 | 3.05 ± 1.08 | 3.58 ± 0.75 | p = 0.2128 |
| **RV GLS** | % | -23.55 ± 6.28 | -20.47 ± 8.01 | -22.51 ± 5.23 | -23.38 ± 2.94 | p = 0.2073 |
| **RV Free wall LS** | % | -26.2 ± 10.17 | -23.04 ± 10.64 | -22.86 ± 6.77 | -22.15 ± 5.97 | p = 0.5368 |
| **RV FAC** | % | 39.70 ± 14.8 | 35.57 ± 12.4 | 36.04 ± 10.50 | 30.90 ± 10.9 | p = 0.2888 |
| **RVs Length (TomTec)** | mm | 9.36 ± 2.12 | 8.05 ± 2.29 | 9.99 ± 1.51 | 11.37 ± 2.75 | **p < 0.0001** |
| **RVd Length (TomTec)** | mm | 11.87 ± 2.43 | 10.04 ± 2.97 | 13.12 ± 2.04 | 12.80 ± 1.50 | **p < 0.0001** |
| **TAPSE** | mm | 3.25 ± 0.77 | 2.74 ± 1.59 | 3.38 ± 1.37 | 2.85 ± 1.13 | p = 0.1483 |

*LV Diastolic length = Left ventricular diastolic Length (operator measured), RV Diastolic Length = Right Ventricular Diastolic Length (operator measured), RV: LV ratio = Ratio between Right Ventricular and Left Ventricular length, MV Diameter = Mitral Valve Diameter, TV Diameter = Tricuspid Valve Diameter, MV: TV ratio = ratio between the Mitral Valve annulus and Tricuspid Valve Annulus, LVd Volume = Left ventricular diastolic Volume, LVs Volume = Left Ventricular Systolic Volume, LVEF = Left Ventricular Ejection Fraction, LV GLS = Left Ventricular Global longitudinal Strain, LV Fractional Area Change = Left Ventricular Fractional Area Change, LVd Length (TomTec) = Left Ventricular Diastolic Length as automated by TomTec Cardiac Performance Analysis, LVs Length (TomTec) = Left Ventricular Systolic Length as automated by TomTec Cardiac Performance Analysis, MAPSE Lateral = Mitral Annular Plane Systolic Excursion Lateral Free wall, MAPSE Septal = Mitral Annular Plane Systolic Excursion Septal wall, RV GLS = Right Ventricular Global Longitudinal Strain, RV Free wall LS = Right Ventricular Free wall Longitudinal Strain, RV FAC = Right Ventricular Fractional Area Change, RVs Length = Right Ventricular diastolic Length as automated by TomTec Cardiac Performance Analysis, RVd Length = Right Ventricular Length as automated by TomTec Cardiac Performance Analysis, TAPSE = Tricuspid Annular Plane Systolic Excursion. P values in red denote statistical significance*

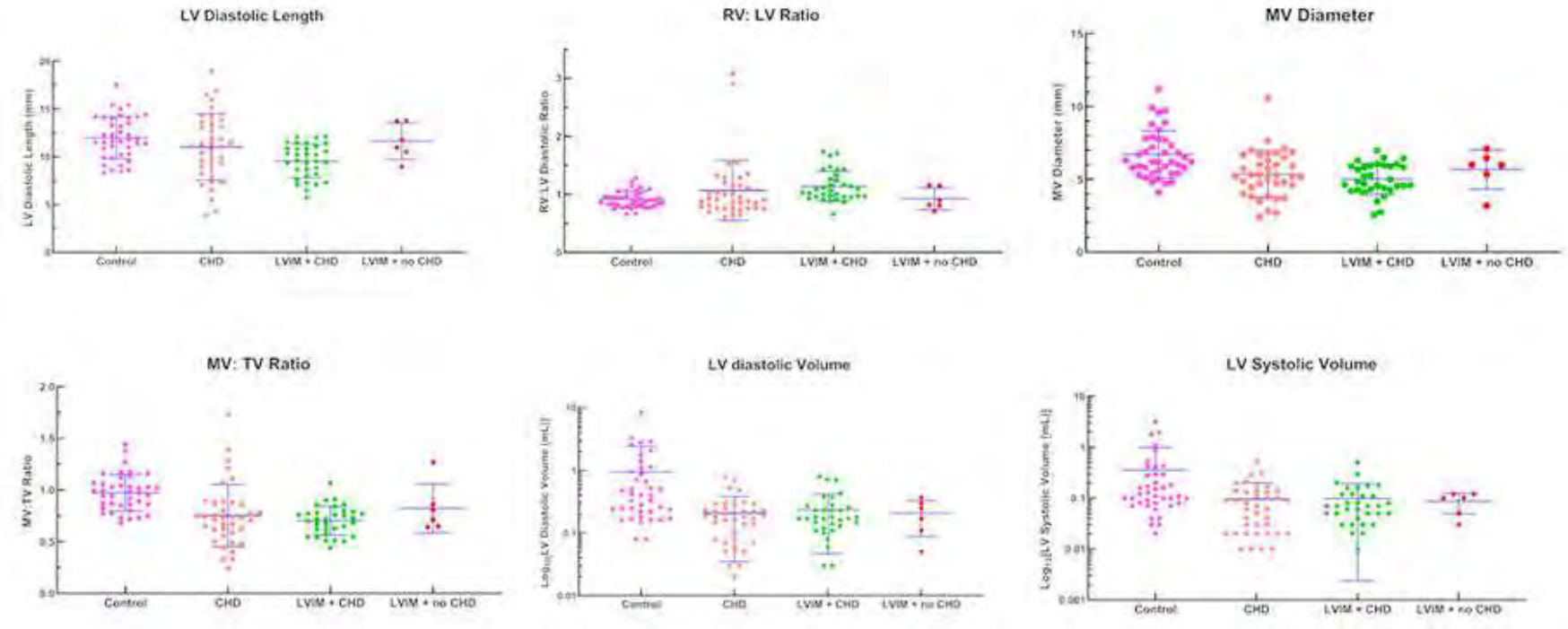


Figure 35 One-way ANOVA comparing Control, CHD, LVIM + CHD and LVIM + no CHD with foetal cardiac measurements.

Each plot shows the data points with the mean and standard deviation with red circled p values for those with statistical significance.

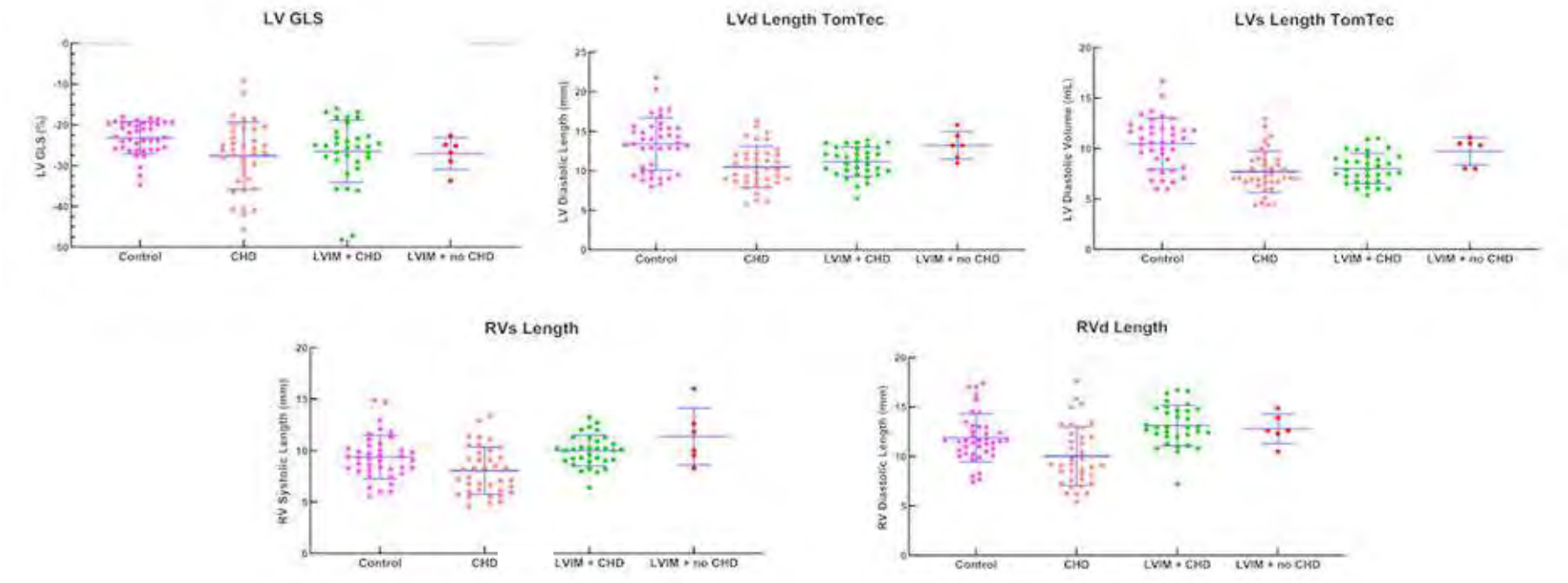


Figure 36 Comparison plots

One-way ANOVA comparing Control, CHD, LVIM + CHD and LVIM + no CHD. Each plot shows the data points with the mean and standard deviation with red circled p values for those with statistical significance.

Note LV Systolic and Diastolic volumes are $Log_{10}$ y-axis scales to allow comparison of all data points.

**Flow Chart Mapping the Outcomes of all Fetal Echo Studies Analysed by Speckle Tracking Echocardiography**

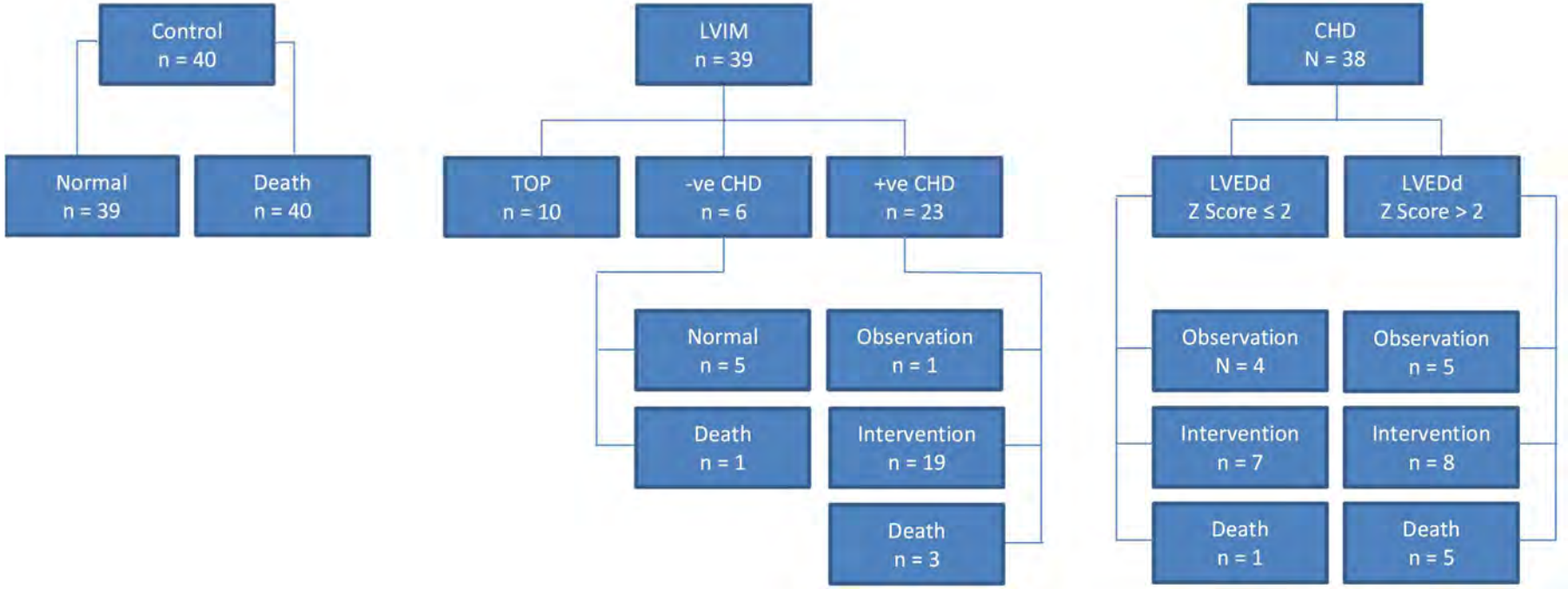


*Figure 37 Flow Chart mapping outcomes*

*All pregnancies where foetal echo studies were analysed by STE. LVIM = Left Ventricular Imbalance, CHD = =Congenital Heart Disease, LVEDd = Left Ventricular end-diastolic dimension.*

| Table 10 Summary of the z-score data Mean +/- S.D. (Schneider, 2005) | | | | | |
|---|---|---|---|---|---|
| **Index** | Control Group | CHD | LVIM + CHD | LVIM + normal | One-way ANOVA |
| **n** | 40 | 38 | 33 | 6 | |
| **MV Diameter** | 0.71 ± 1.41 | -0.99 ± 2.15 | -1.51 ± 1.74 | -0.69 ± 1.87 | **p < 0.0001** |
| **TV Diameter** | 0.97 ± 1.13 | 1.61 ± 1.74 | 1.33 ± 1.11 | 0.99 ± 0.96 | p = 0.1912 |
| **LVd Length (TomTec)** | -1.01 ± 1.74 | 2.66 ± 1.59 | -2.15 ± 1.33 | -1.00 ± 0.87 | **p < 0.0001** |
| **LVs Length (TomTec)** | 2.29 ± 1.69 | 0.40 ± 1.50 | 0.81 ± 1.27 | 1.90 ± 0.45 | **p < 0.0001** |
| **RVd Length** | -1.03 ± 1.18 | -2.05 ± 1.51 | -0.20 ± 1.08 | 0.08 ± 1.14 | **p < 0.0001** |
| **RVs Length** | 1.51 ± 1.58 | 0.57 ± 1.69 | 2.18 ± 1.12 | 2.29 ± 0.84 | **p < 0.0001** |

*MV = Mitral Valve, TV = Tricuspid Valve, LVd = Left Ventricle diastole, LVs = Left Ventricle systole, RVd = Right ventricle diastole, RVs = Right Ventricle systole, CHD = Congenital Heart Disease, LVIM = Left Ventricular Imbalance*

*P values in red denote statistical significance*

### 3.1.3 LVIM Cohort

39 out of 40 of the LVIM cohort were successfully analysed using 2D CPA giving a 97.5% success rate. The gestational age range of the foetal echoes in this group was between $18^{+3}$ and $23^{+4}$ with a median gestation of $20^{+6}$ weeks. Frame rates for this group were between 30 - 84 Hz with a mean frame rate 54.8 Hz. To compare the data between the LVIM group who were found to be either positive or negative for congenital disease, the LVIM group was split. Those at 20 weeks with a ventricular size imbalance and were found post-natally to have a congenital heart defect were re-labelled LVIM +CHD and a group who had a ventricular size imbalance at 20 weeks but who were found to have normal cardiac findings after birth LVIM +No CHD. There was no significant difference between the heart rate ($p = 0.586$), gestational age ($p > 0.999$) and frame rate ($p > 0.999$) between the two groups. Table 11 shows the results of the LVIM cohort split, giving the average and range between parameters.

Significant differences between the groups were only observed in the dimensions of the LV cavity length and the longitudinal systolic function of the LV expressed as mitral annular planar systolic excursion (MAPSE) in mm. Left ventricular length was significantly greater in the group with LVIM and a normal outcome and MAPSE of the lateral wall in this group was significantly better, but there was no significant difference in LV GLS. In the LVIM cohort with a normal outcome both the traditional functional parameters and GLS were more aligned to the normal cohort values but also comparable to the LVIM CHD group. The comparative data between z-score values demonstrated far great statistical difference between the 5 groups (Table 10).

| Table 11 Summary of the LVIM data | | | | | |
|---|---|---|---|---|---|
| **Echocardiography Parameter** | **LVIM + CHD Outcome Cohort 2** | | **LVIM + No CHD Outcome Cohort 3** | | **p-Value** |
| | **Mean** | **Range** | **Mean** | **Range** | |
| **n** | **33** | | **6** | | |
| **Frame Rate (fps)** | 55.24 | 37.0 - 84.0 | 52.50 | 30.0 – 75.0 | p = 0.6201 |
| **Foetal Heart Rate (bpm)** | 145.00 | 111.0 - 165.0 | 154.00 | 141.0 – 178.0 | p = 0.1189 |
| **Gestational age (weeks + days)** | 20.00 | 19.0 - 23.4 | 21.00 | 18.3 - 23.4 | p = 0.4019 |
| **LV diastolic Length (mm) - Operator** | 9.56 | 5.76 - 12.11 | 11.63 | 8.99 - 13.80 | **p = 0.0130** |
| **RV diastolic length (mm) - Operator** | 10.57 | 7.57 - 15.46 | 10.68 | 7.39 - 13.69 | p = 0.8999 |
| **RV: LV Length Ratio** | 1.14 | 0.66 - 1.74 | 0.93 | 0.71 - 1.16 | p = 0.0629 |
| **MV annulus (mm)** | 5.00 | 2.56 - 6.97 | 5.67 | 3.19 - 7.10 | p = 0.1848 |
| **TV annulus (mm)** | 7.18 | 5.0 - 9.61 | 7.03 | 5.01 - 8.40 | p = 0.7684 |
| **MV: TV ratio** | 0.70 | 0.44 - 1.07 | 0.82 | 0.64 - 1.27 | **p = 0.0513** |
| **LVd Volume (mL)** | 0.23 | 0.03 - 0.80 | 0.20 | 0.05 - 0.37 | p = 0.7497 |
| **LVs Volume (mL)** | 0.09 | 0.01 - 0.50 | 0.08 | 0.03 - 0.12 | p = 0.7854 |
| **LV Ejection Fraction (%)** | 59.20 | 32.57 - 81.98 | 61.50 | 49.5 - 72.7 | p = 0.7036 |
| **LV GLS (%)** | -26.49 | -16.06 - -48.17 | -27.03 | -33.7 - -22.6 | p = 0.8665 |
| **LV Fractional Area Change (%)** | 45.60 | 22.79 - 70.50 | 47.80 | 38.0 - 55.67 | p = 0.6443 |
| **LVd Length (mm) - TomTec** | 11.14 | 6.50 - 13.90 | 13.22 | 11.0 - 15.8 | **p = 0.0155** |
| **LVs Length (mm) - TomTec** | 8.01 | 5.40 -11.0 | 9.73 | 8.0 - 11.1 | **p = 0.0125** |

| | | | | | |
|---|---|---|---|---|---|
| **MAPSE Lateral (mm)** | 3.28 | 0.70 - 6.60 | 4.48 | 3.8 - 6.5 | **p = 0.0308** |
| **MAPSE Septal (mm)** | 3.04 | 1.40 - 5.80 | 3.58 | 2.8 - 4.8 | p = 0.2534 |
| **RV GLS (%)** | -22.51 | -35.30 - -15.04 | -23.38 | -27.42 - -19.99 | p = 0.6947 |
| **RV Free wall longitudinal strain (%)** | -22.85 | -10.64 - -37.50 | -22.14 | -30.72 - -14.38 | p = 0.8115 |
| **RV FAC (%)** | 36.04 | 16.05 - 56.80 | 30.90 | 14.88 - 43.29 | p = 0.2801 |
| **RVs length (mm)** | 9.98 | 6.40 - 13.20 | 11.36 | 8.3 – 16.0 | p = 0.0799 |
| **RVd length (mm)** | 13.12 | 7.20 - 16.70 | 12.80 | 10.5 - 14.9 | p = 0.7166 |
| **TAPSE (mm)** | 3.38 | 1.20 - 8.20 | 2.85 | 1.3 – 4.0 | p = 0.3788 |

*Fps = frames per second, bpm = beats per minute, LV Diastolic length = Left ventricular diastolic Length (operator measured), RV Diastolic Length = Right Ventricular Diastolic Length (operator measured), RV: LV ratio = Ratio between Right Ventricular and Left Ventricular length, MV Diameter = Mitral Valve Diameter, TV Diameter = Tricuspid Valve Diameter, MV: TV ratio = ratio between the Mitral Valve annulus and Tricuspid Valve Annulus, LVd Volume = Left ventricular diastolic Volume, LVs Volume = Left Ventricular Systolic Volume, LVEF = Left Ventricular Ejection Fraction, LV GLS = Left Ventricular Global longitudinal Strain, LV Fractional Area Change = Left Ventricular Fractional Area Change, LVd Length (TomTec) = Left Ventricular Diastolic Length as automated by TomTec Cardiac Performance Analysis, LVs Length (TomTec) = Left Ventricular Systolic Length as automated by TomTec Cardiac Performance Analysis, MAPSE Lateral = Mitral Annular Plane Systolic Excursion Lateral Free wall, MAPSE Septal = Mitral Annular Plane Systolic Excursion Septal wall, RV GLS = Right Ventricular Global Longitudinal Strain, RV Free wall LS = Right Ventricular Free wall Longitudinal Strain, RV FAC = Right Ventricular Fractional Area Change, RVs Length = Right Ventricular diastolic Length as automated by TomTec Cardiac Performance Analysis, RVd Length = Right Ventricular Length as automated by TomTec Cardiac Performance Analysis, TAPSE = Tricuspid Annular Plane Systolic Excursion.*

*P values in red denote statistical significance*

#### 3.1.3.1 LVIM outcomes

There were 6 patients within the LVIM cohort who were found to have normal cardiac anatomy on post-natal examination. One of the 6 died from non-cardiac causes, and 2 were diagnosed with chromosomal abnormalities (Turners Syndrome and Down Syndrome) after birth.

Of the remaining 33, the spectrum of subsequently diagnosed congenital heart lesions can be seen in table 12. There were 5 pregnancies who did not receive a formal diagnosis as 2 died in utero due to unknown causes and 3 failed to have a diagnosis after the termination of the pregnancy. 19 out of 20 of those diagnosed post-natally, with a heart defect, required intervention within the first year of life. Of these 19, 15 required intervention for coarctation of the aorta. The overall post-natal diagnosis of coarctation within the LVIM cohort was 50%.

Of the 10 terminations of pregnancy, 2 were found to have additional foetal anomalies in the form of congenital diaphragmatic hernia, 3 were subsequently found to have a chromosomal anomaly (2 x Downs Syndrome and 1 x Cat Eye Syndrome). Two of the deaths were unexpected in-utero foetal demise, one from non-cardiac cause with normal cardiac anatomy as previously described and the fourth death was a neo-natal death from a failed bi-ventricular repair of the RV-LV imbalance.

| Table 12 Pregnancy outcomes for the LVIM cohort | | | | | |
|---|---|---|---|---|---|
| **Post Natal Diagnosis** | No. | Obs. | Intervention | IUD or Death | TOP |
| **Normal** | 6 | - | - | 1 | - |
| **Bicuspid Aortic Valve** | 1 | 1 | - | - | - |
| **Coarctation** | 4 | | 3 | | 1 |
| **CoA + VSD** | 10 | - | 6 | 1 | 3 |
| **CoA + BAV** | 4 | - | 4 | - | - |
| **CoA + VSD + BAV** | 2 | - | 2 | - | - |
| **Hypoplastic Aortic Arch** | 2 | - | 2 | - | - |
| **VSD** | 1 | - | 1 | - | - |
| **HLHS** | 3 | - | - | - | 3 |
| **DORV** | 1 | - | 1 | - | - |
| **Unknown LVIM** | 5 | - | - | 2 | 3 |
| **TOTAL** | 39 | 1 | 19 | 4 | 10 |

*DORV = Double Outlet Right Ventricle, HLHS = Hypoplastic Left Heart Syndrome, IUD = Intrauterine death, TOP = Termination of Pregnancy, VSD = Ventricular Septal defect, CoA = Coarctation of the Aorta, LVIM = Left Ventricular Imbalance*

### 3.1.4 Congenital Heart Defect (CHD) confirmed abnormal Cohort

38 out of 40 of the CHD cohort were successfully analysed using 2D CPA giving a 95% success rate for LV and RV GLS and RV free wall longitudinal strain. The gestational age range of the foetal echoes in this group was between $15^{+3}$ and $25^{+0}$ with a median gestation of $20^{+4}$ weeks. Foetal heart rates were between 108-182 bpm with a mean of 144 bpm. Frame rates for this group were between 22-84 Hz with a mean frame rate 52.1 Hz There was no significant difference between these demographics and the other cohorts.

The CHD group were all selected due to the size discrepancy between the left and right ventricles in the presence of a congenital heart defect, but after the dimensions were z-scored there was differing significance of ventricular size imbalance. As a result, the CHD cohort was further divided into 2 groups to compare data between those with significantly small left ventricular diastolic length (LVd L) and a z-score >-2.0 and those who had a LVd L z-score <-2.0 and therefore had a less significant disproportion than previously observed. Table 13 shows a summary of all the results of the CHD cohort and the findings of the cohort when split into those with a lesser or greater LVd L z-score of -2.0 (non vs significantly small LV cavity length). There was no statistically significant difference between the gestation, heart rate or frame rate for the 2 sub-groups or the LV parameters. The RV/LV ratio was 0.61 in the CHD + LVIM group compared to 1.07. The functional parameters were reduced in the CHD + LVIM (LV EF 57% v 65%, LV FAC 46% v 52%) but not significantly, and there was no significant difference in LV GLS values between the 2 groups. There were no significant differences between the right ventricular parameters or the right ventricular functional parameters between the CHD and CHD +LVIM groups however the right ventricular and free wall longitudinal strain values were better (more negative value) in the CHD cohort with a smaller left ventricle.

| Table 13 Summary of the CHD Cohort data | | | | | |
|---|---|---|---|---|---|
| **Echocardiography Parameter** | **CHD Cohort LVd L > -2.0** | | **CHD Cohort LVd L < -2.0** | | p-Value |
| | **Mean** | **Range** | **Mean** | **Range** | |
| **n** | | **24** | | **14** | |
| **Frame Rate (fps)** | 52.56 | 22.0 - 75.0 | 51.33 | 40.0 – 84.0 | p = 0.7513 |
| **Foetal Heart Rate (bpm)** | 144.65 | 108.0 - 161.0 | 144.70 | 127.0 – 182.0 | p = 0.9832 |
| **Gestational age (weeks + days)** | 20.67 | 15.3 – 24.4 | 20.54 | 19.4 – 25.0 | p = 0.8117 |
| **LV diastolic Length (mm) - Operator** | 11.46 | 3.90 – 20.20 | 10.93 | 5.50 – 16.5 | p = 0.6803 |
| **RV diastolic length (mm) - Operator** | 10.85 | 5.20 – 17.70 | 10.23 | 7.39 - 13.69 | p = 0.4605 |
| **RV: LV Length Ratio** | 1.11 | 0.64 – 3.09 | 0.99 | 0.61 - 1.55 | p = 0.5172 |
| **MV annulus (mm)** | 5.52 | 2.80 – 10.58 | 5.05 | 2.40 - 7.00 | p = 0.3846 |
| **TV annulus (mm)** | 7.91 | 4.10 – 19.98 | 7.10 | 5.40 – 9.00 | p = 0.3441 |
| **MV: TV ratio** | 0.78 | 0.24 - 1.73 | 0.71 | 0.33 - 1.07 | p = 0.5406 |
| **LVd Volume (mL)** | 0.21 | 0.02 - 0.80 | 0.20 | 0.05 - 0.53 | p = 0.8133 |
| **LVs Volume (mL)** | 0.09 | 0.01 - 0.32 | 0.10 | 0.02 - 0.52 | p = 0.7438 |
| **LV Ejection Fraction (%)** | 57.76 | 13.47 - 82.89 | 65.02 | 39.54 - 88.07 | p = 0.2292 |
| **LV GLS (%)** | -25.94 | -40.62 - -9.23 | -30.43 | -45.66 - -17.67 | p = 0.1065 |
| **LV Fractional Area Change (%)** | 46.17 | 13.46 – 68.34 | 52.12 | 30.98 – 74.94 | p = 0.2008 |
| **LVd Length (mm) - TomTec** | 10.54 | 5.80 – 16.30 | 10.39 | 8.20 – 14.00 | p = 0.8618 |

| | | | | | |
|---|---|---|---|---|---|
| **LVs Length (mm) - TomTec** | 7.83 | 4.40 – 12.90 | 7.45 | 4.40 – 10.30 | p = 0.5897 |
| **MAPSE Lateral (mm)** | 2.85 | 0.10 – 5.70 | 3.36 | 1.10 - 6.10 | p = 0.2961 |
| **MAPSE Septal (mm)** | 2.57 | 0.02 - 5.00 | 2.69 | 0.50 – 5.90 | p = 0.7998 |
| **RV GLS (%)** | -19.03 | -30.80 - -0.57 | -22.94 | -40.96 - -10.89 | p = 0.1488 |
| **RV Free wall longitudinal strain (%)** | -21.32 | -40.30 - -2.04 | -25.98 | -42.99 - -13.21 | p = 0.1965 |
| **RV FAC (%)** | 33.45 | -0.96 – 52.52 | 39.21 | 28.52 – 52.46 | p = 0.1708 |
| **RVs length (mm)** | 8.57 | 4.60 - 13.40 | 7.17 | 5.00 – 11.10 | p = 0.0700 |
| **RVd length (mm)** | 10.79 | 5.40 – 17.60 | 8.76 | 6.20 – 13.20 | **p = 0.0405** |
| **TAPSE (mm)** | 2.86 | 0.01 – 6.80 | 2.53 | 0.17 – 6.20 | p = 0.5465 |

*Fps = frames per second, bpm = beats per minute, LV Diastolic length = Left ventricular diastolic Length (operator measured), RV Diastolic Length = Right Ventricular Diastolic Length (operator measured), RV: LV ratio = Ratio between Right Ventricular and Left Ventricular length, MV Diameter = Mitral Valve Diameter, TV Diameter = Tricuspid Valve Diameter, MV: TV ratio = ratio between the Mitral Valve annulus and Tricuspid Valve Annulus, LVd Volume = Left ventricular diastolic Volume, LVs Volume = Left Ventricular Systolic Volume, LVEF = Left Ventricular Ejection Fraction, LV GLS = Left Ventricular Global longitudinal Strain, LV Fractional Area Change = Left Ventricular Fractional Area Change, LVd Length (TomTec) = Left Ventricular Diastolic Length as automated by TomTec Cardiac Performance Analysis, LVs Length (TomTec) = Left Ventricular Systolic Length as automated by TomTec Cardiac Performance Analysis, MAPSE Lateral = Mitral Annular Plane Systolic Excursion Lateral Free wall, MAPSE Septal = Mitral Annular Plane Systolic Excursion Septal wall, RV GLS = Right Ventricular Global Longitudinal Strain, RV Free wall LS = Right Ventricular Free wall Longitudinal Strain, RV FAC = Right Ventricular Fractional Area Change, RVs Length = Right Ventricular diastolic Length as automated by TomTec Cardiac Performance Analysis, RVd Length = Right Ventricular Length as automated by TomTec Cardiac Performance Analysis, TAPSE = Tricuspid Annular Plane Systolic Excursion.*

*P values in red denote statistical significance*

### 3.1.4.1 CHD Outcomes

There was one intrauterine death while the other 6 deaths were post-natal. Six out of the 7 were diagnosed at post-mortem with extra-cardiac (1 x bowel atresia) or chromosomal abnormalities (3 x T18, 2 x T21). Of those who chose to terminate the pregnancy, all were before 24 weeks gestation and 4 out of 7 (57%) had either extra-cardiac (1 x cystic hygroma, 1 x imperforate anus/asplenia, 1 x gut rotation) or chromosomal abnormalities (1 x T21) as detected on post-mortem. The termination rate for this cohort was 25%. Details of diagnosis and the outcomes for the CHD group are summarised in table 14.

**Table 14 Confirmed post-natal congenital heart defect (CHD) diagnoses where ± indicates additional cardiac defects**

| CHD Diagnosis | No. | Obs. | Intervention | Death | TOP |
|---|---|---|---|---|---|
| **AVSD ± Complete, Partial** | 23 | 6 | 8 | 6 | 3 |
| **AVSD ± Isomerism** | 3 | - | 1 | - | 2 |
| **Coarctation ± VSD** | 4 | 1 | 3 | - | - |
| **TGA ± VSD** | 3 | - | 3 | - | - |
| **Truncus Arteriosus** | 1 | - | - | - | 1 |
| **Pulmonary Stenosis + VSD** | 2 | 1 | - | 1 | - |
| **Aortic Stenosis + VSD** | 1 | - | 1 | - | - |
| **ccTGA** | 1 | 1 | - | - | - |
| **VSD** | 1 | 1 | - | - | - |
| **Dextrocardia, Coarctation, Congenital Complete Heart Block** | 1 | - | - | - | 1 |
| **Total** | 40 | 10 | 16 | 7 | 7 |

*Obs. = Observation, TOP = Termination of Pregnancy, AVSD = Atrio-ventricular septal defect, TGA = Transposition of the Great Arteries, VSD = Ventricular septal defect, ccTGA = Congenitally Corrected Transposition of the Great Arteries*

The pregnancy outcome data was more favourable if the left ventricular cavity had a LVd Length z-score <-2.0 as can be seen in table 15.

**Table 15 CHD group sub-divided into those with a significantly small LV (z-score > -2.0) and those with a LV z-score < -2.0**

| **CHD Diagnosis** | No. | Obs | Intervention | Death |
|---|---|---|---|---|
| **LVd Length z-score > -2.0** | 24 | 6 (25%) | 8 (33%) | 5 (20%) |
| **LVd Length z-score < -2.0** | 14 | 4 (28%) | 7 (50%) | 1 (7%) |

*CHD = Congenital Heart Defect, Obs. = Observation, LV = Left Ventricle, TOP = Termination of Pregnancy, LVd = Left Ventricular Length*

*Z-scores calculated using Schneider, 2005 dataset.*

### 3.1.5 Control Cohort

100% of the control cohort were successfully analysed using 2D CPA which was anticipated as the group consisted of foetal echocardiograms of good quality imaging with apparently normal foetal cardiac anatomy. The gestational age range of the foetal echoes in this group was between $18^{+6}$ and $25^{+5}$ with a median gestation of $20^{+3}$ weeks. Foetal heart rates were between 115-170 bpm with an average of 147 bpm. Frame rates for this group were between 31-76 Hz with an average frame rate 52.3 Hz. There was no statistical difference between these parameters and the other cohorts. Table 16 summaries the parameters found in the control group.

| Table 16 Summary of the Control Data | | | |
|---|---|---|---|
| **Echocardiography Parameter** | **Normal Cohort** | | **z-Score** |
| | **Mean** | **Range** | |
| **n** | | **40** | |
| **Frame Rate (fps)** | 52.32 | 31.0 - 76.0 | |
| **Foetal Heart Rate (bpm)** | 147.67 | 115.0 - 170.0 | |
| **Gestational age (weeks + days)** | 20.35 | 18.6 – 25.5 | |
| **LV diastolic Length (mm) - Operator** | 11.99 | 8.30 – 17.50 | |
| **RV diastolic length (mm) - Operator** | 10.79 | 7.40 – 16.90 | |
| **RV: LV Length Ratio** | 0.91 | 0.66 – 1.28 | |
| **MV annulus (mm)** | 6.70 | 4.10 – 11.20 | 0.65 |
| **TV annulus (mm)** | 6.94 | 5.00 – 11.90 | 0.96 |
| **MV: TV ratio** | 0.97 | 0.68 - 1.44 | |
| **LVd Volume (mL)** | 0.95 | 0.08 – 8.40 | |
| **LVs Volume (mL)** | 0.36 | 0.02 – 3.20 | |
| **LV Ejection Fraction (%)** | 60.21 | 39.58 – 80.40 | |
| **LV GLS (%)** | -23.18 | -34.7 - -18.04 | |
| **LV Fractional Area Change (%)** | 45.08 | 29.37 – 65.20 | |
| **LVd Length (mm) - TomTec** | 13.38 | 8.00 – 21.8 | -0.94 |
| **LVs Length (mm) - TomTec** | 10.46 | 6.00 – 16.70 | 2.21 |
| **MAPSE Lateral (mm)** | 3.26 | 0.80 – 6.40 | |
| **MAPSE Septal (mm)** | 2.97 | 0.40 – 7.10 | |
| **RV GLS (%)** | -23.55 | -40.95 - -7.03 | |
| **RV Free wall longitudinal strain (%)** | -26.15 | -54.50 - -11.68 | |
| **RV FAC (%)** | 39.69 | 12.25 – 85.80 | |
| **RVs length (mm)** | 9.04 | 5.60 – 14.90 | 1.27 |
| **RVd length (mm)** | 11.87 | 7.40 – 17.40 | -0.94 |
| **TAPSE (mm)** | 3.25 | 2.10 – 5.20 | |

*Fps = frames per second, bpm = beats per minute, LV Diastolic length = Left ventricular diastolic Length (operator measured), RV Diastolic Length = Right Ventricular Diastolic Length (operator measured), RV: LV ratio = Ratio between Right Ventricular and Left Ventricular length, MV Diameter = Mitral Valve Diameter, TV Diameter = Tricuspid Valve Diameter, MV: TV ratio = ratio between the Mitral Valve annulus and Tricuspid Valve Annulus, LVd Volume = Left ventricular diastolic Volume, LVs Volume = Left Ventricular Systolic Volume, LVEF = Left Ventricular Ejection Fraction, LV GLS = Left Ventricular Global longitudinal Strain, LV Fractional Area Change = Left Ventricular Fractional Area Change, LVd Length (TomTec) = Left Ventricular Diastolic Length as automated by TomTec Cardiac Performance Analysis, LVs Length (TomTec) = Left Ventricular Systolic Length as automated by TomTec Cardiac Performance Analysis, MAPSE Lateral = Mitral Annular Plane Systolic Excursion Lateral Free wall, MAPSE Septal = Mitral Annular Plane Systolic Excursion Septal wall, RV GLS = Right Ventricular Global Longitudinal Strain, RV Free wall LS = Right Ventricular Free wall Longitudinal Strain, RV FAC = Right Ventricular Fractional Area Change, RVs Length = Right Ventricular diastolic Length as automated by TomTec Cardiac Performance Analysis, RVd Length = Right Ventricular Length as automated by TomTec Cardiac Performance Analysis, TAPSE = Tricuspid Annular Plane Systolic Excursion.*

*Z-scores calculated using Schneider, 2005 dataset.*

Post-natal cardiac outcomes were confirmed normal via documentation from the new-born screening tool on the maternity databases.

### 3.2 FEASIBILITY OF SPECKLE TRACKING ECHOCARDIOGRAPHY

Overall, 97.5% of the 120 foetal echocardiograms were analysed using CPA on TomTec 2D, and 100% analysis of the normal cohort. This demonstrates good feasibility of undertaking speckle tracking echocardiography at mid-gestation of pregnancy. Of the 3 foetal echoes which were not suitable, foetal/maternal movement was the main reason for an inability to tracking the endocardium accurately throughout the cardiac cycle.

### 3.3 ASSESSMENT OF PEAK LONGITUDINAL STRAIN

Offline analysis was performed using 2D Cardiac Performance Analysis on TomTec. This system uses a segmental averaging method to determine Longitudinal Strain as described in the American Society of Echo & the European Association of Cardiovascular Imaging recommendations (Voigt, et al., 2015). Average peak longitudinal strain was obtained in 3 anatomical regions of the foetal heart – LV, RV and the RV free wall. The mean LV GLS was obtained from the mean percentage strain from 6 individual regions of the left ventricle – the basal, mid and apical portions of the septal and lateral walls. Right ventricular strain was obtained from the mean of the peak right ventricular longitudinal strain of 6 individual regions of the right ventricle – the basal, mid and apical portions of the septal and right ventricular lateral or free wall. CPA software also calculated the average peak longitudinal strain from the 3 anatomical portions of the free wall.

For the 3 study cohorts, LVIM, CHD and the control group, the mean ± SD of peak LV longitudinal strain was -26.57 ± 7.13, -27.59 ± 8.26 and -23.18 ± 3.98 respectively (Figure 37). These numerical values imply that the longitudinal strain was higher in the abnormal groups than the control, but these failed to demonstrate statistical significance. There was a significant difference between the LV GLS values of the control group and the CHD group, but no significant difference between control vs LVIM or CHD vs LVIM

Mean ± SD right ventricular GLS was -22.64 ± 4.93, -20.47 ± 8.01 and -23.55 ± 6.28 for the 3 cohorts LVIM, CHD and control. There was no significant difference between the cohorts.

Mean ± SD right ventricular free wall longitudinal strain was -22.75 ± 6.58, -23.04 ± 10.64 and -26.16 ± 10.17 respectively. The observed values imply that RV strain is reduced in the presence of a ventricular imbalance however, there was no statistical evidence to support the significance of this.

The 2 abnormal cohorts (LVIM and CHD) were further separated to reflect the outcome and severity of the ventricular imbalance in each group. In the LVIM group, the imbalance was split into 2 groups of normal cardiac outcome against an outcome with a post-natal diagnosis of a congenital heart defect. These groups were relabelled LVIM + CHD post-natal diagnosis and LVIM + No CHD so that the all the parameters and strain values could be compared with greater influence of whether the imbalance was due to a pathological lesion or not. The results of this split did not increase the evidence towards increased myocardial deformation where there is a ventricular imbalance alongside a diagnosis of a congenital heart defect. The average LV GLS in the group with LVIM and a post-natal diagnosis of CHD was -26.48% whereas the results from the LVIM group with normal outcome was -27.03% demonstrating a marginal decrease in myocardial deformation for those LVIM with CHD but this did not reach statistical significance. Right ventricular GLS demonstrated similar strain values in the LVIM normal outcome group (-23.46%) against the values obtained from the LVIM with CHD (-22.5%). These results were also reflected in the RV free wall values where the average RV free wall strain values were comparable (-22.85 vs -22.14) despite the outcome of the LVIM group.

The CHD group was also split into the severity of the imbalance where the left ventricular diastolic length z-scores differentiated severe imbalance of the left ventricle to not so severe using -2.0 as the dividing criteria. These groups were relabelled according to the

z-score parameters >-2.0 or <-2.0 LVIM. Strain values for the groups were comparable (-27.59 vs -27.84) despite the significantly smaller cavity in the CHD group. Right ventricular strain values demonstrated a greater difference in the presence of a smaller LV. RV GLS values were -20.47% against -18.93% where the LV EDD was >-2.0. Again, RV free wall values also demonstrated lower strain values where the LV was significantly small (-23.03% vs -21.1% respectively). However, again statistical significance of these values was not supportive of the difference in values.

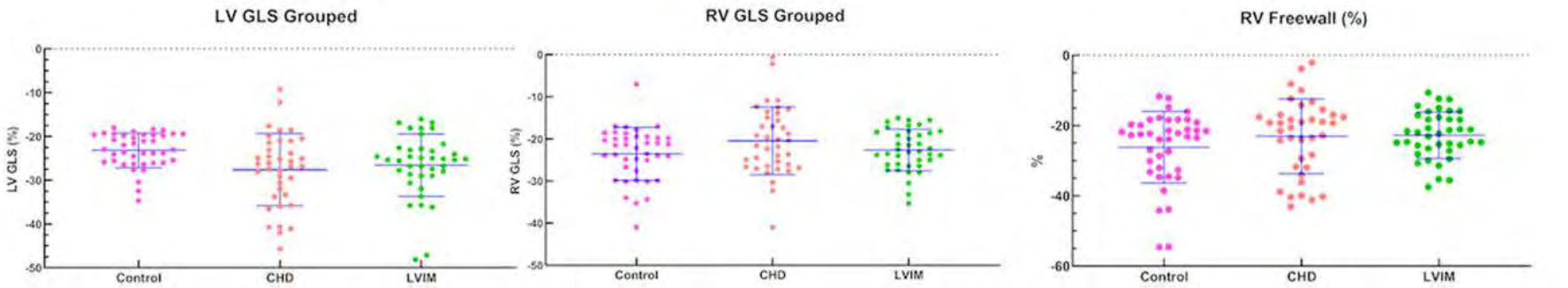


*Figure 38 GLS across initial 3 groups*

*Plots to demonstrate strain values between the 3 cohorts (Control, CHD, LVIM) and the 3 cardiac regions of interest (LV, RV and RV Free wall)*

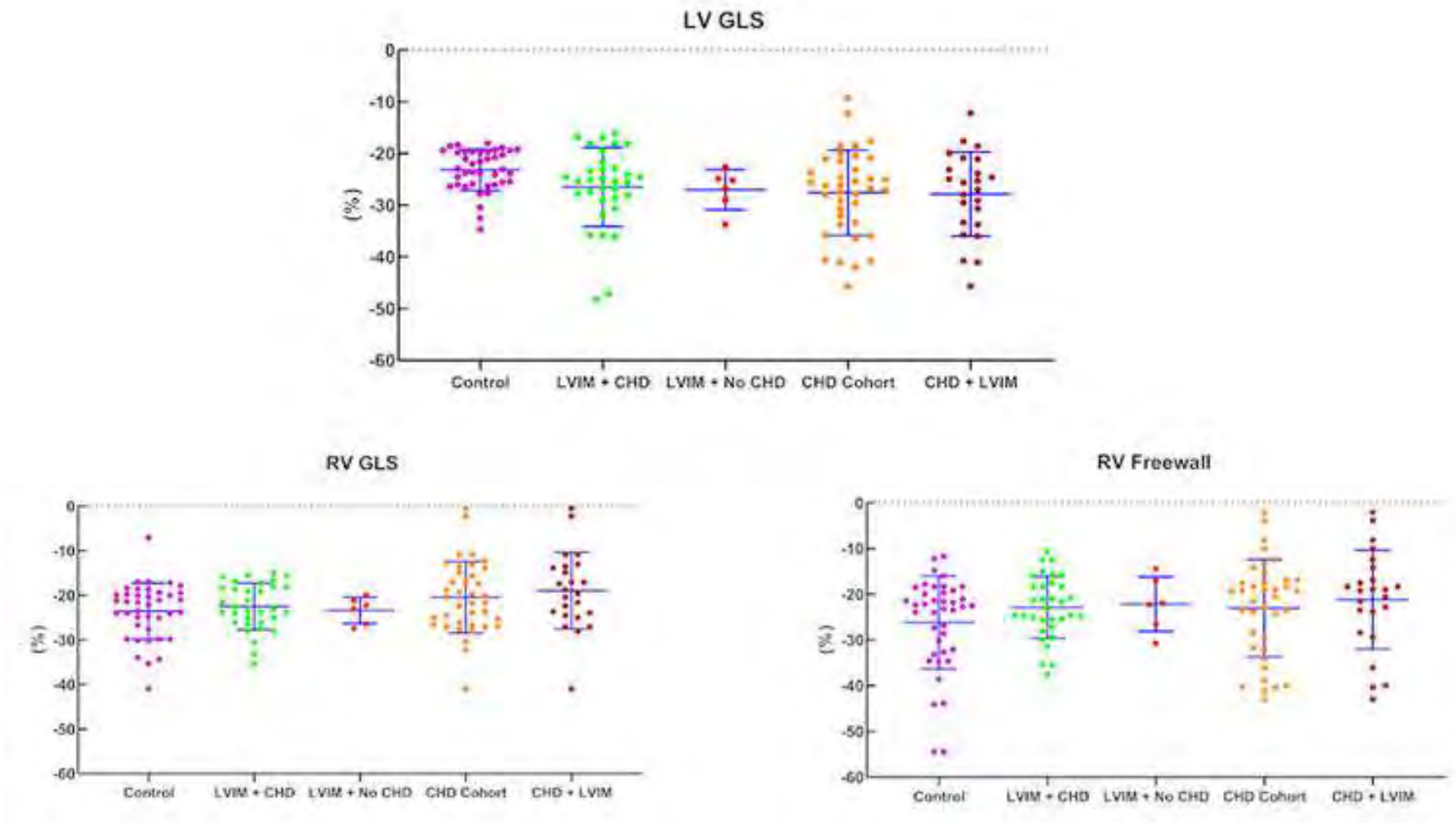


*Figure 39. Comparison of LV GLS, RV GLS and RV Free wall*

Plots comparing the difference between the strain values in the 3 cardiac regions of interest (LV, RV and RV Free wall) when the abnormal groups were further broken down into LV size significance.

### 3.3 REPEATABILITY OF MEASURES

Interobserver variability was assessed using a 10% sample of studies from each of the 3 main cohorts – LVIM, CHD and the control group. 4 out of 40 foetal echoes in each cohort were selected and blindly re-analysed on the same TomTec software v.1.2. In total, 12 out of 120 foetal echoes were successfully re-analysed by an appropriately experienced second operator. Each of the studies were analysed three times by the author (JEJ) and twice by the second operator, Dr Sarah Newell (SN), to assess intra-observer variability. The results were averaged and tabulated (Table 17).

**Table 17 Foetal Cardiac Strain Analysis Interobserver Variability**

| Foetal Study | Operator 1=JEJ; 2=SN | LV Strain % | RV Strain % | RV Free wall Strain% |
|---|---|---|---|---|
| **LVIM16** | 1 | -24.76 | -26.50 | -29.00 |
| | 2 | -20.80 | -30.20 | -36.50 |
| **LVIM19** | 1 | -22.20 | -23.63 | -25.96 |
| | 2 | -22.05 | -24.30 | -24.00 |
| **LVIM23** | 1 | -23.80 | -21.63 | -24.33 |
| | 2 | -25.70 | -23.15 | -25.15 |
| **LVIM34** | 1 | -39.20 | -19.16 | -18.20 |
| | 2 | -32.80 | -22.10 | -23.85 |
| **CHD14** | 1 | -22.00 | -17.17 | -17.17 |
| | 2 | -19.65 | -18.90 | -19.55 |
| **CHD18** | 1 | -21.93 | -21.07 | -26.63 |
| | 2 | -19.65 | -18.90 | -19.55 |
| **CHD22** | 1 | -23.27 | -22.97 | -19.37 |
| | 2 | -21.20 | -18.40 | -15.35 |
| **CHD28** | 1 | -23.27 | -22.97 | -19.37 |
| | 2 | -26.10 | -41.80 | -38.50 |
| **CON02** | 1 | -23.83 | -22.73 | -18.97 |
| | 2 | -25.35 | -20.80 | -37.55 |
| **CON18** | 1 | -23.33 | -23.23 | -30.83 |
| | 2 | -30.10 | -25.00 | -24.90 |
| **CON30** | 1 | -38.60 | -27.90 | -24.57 |
| | 2 | -30.10 | -25.00 | -24.90 |
| **CON35** | 1 | -23.47 | -27.57 | -27.70 |
| | 2 | -20.75 | -24.20 | -26.95 |

*JEJ = Joanne Elizabeth Jones, SN = Sarah Newell, LV = Left Ventricle, RV = Right Ventricle, LVIM = Left Ventricular Imbalance, CHD = Congenital Heart Defect, CON = Control*

Further analysis was performed using GraphPad to calculate the mean and standard deviation of each operator in the 3 groups. The interclass correlation coefficient (ICC) was calculated (Table 18) and Bland-Altman scatter plots produced to demonstrate repeatability measures graphically (figure 40/41).

| Table 18 GLS values obtained between operator 1 and 2 with calculated variability | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | LVIM Group | | | CHD Group | | | Control Group | | |
| **Subgroup** | LV Strain | RV Strain | RV Free wall | LV Strain | RV Strain | RV Free wall | LV Strain | RV Strain | RV Free wall |
| **Test 1 (Mean ± SD)** | 26.7 ± 5.7 | 23.1 ± 4.6 | 23.2 ± 7.2 | 23.1 ± 3.5 | 25.4 ± 9.8 | 25.9 ± 10.5 | 25.0 ± 5.0 | 24.8 ± 3.9 | 30.9 ± 14.1 |
| **Test 2 (Mean ± SD)** | 27.2 ± 6.5 | 23.0 ± 5.1 | 25.9 ± 7.4 | 25.1 ± 6.4 | 22.5 ± 7.6 | 24.7 ± 8.4 | 29.6 ± 6.8 | 25.5 ± 5.6 | 24.3 ± 4.2 |
| **r** | 0.80 | 0.36 | 0.30 | 0.08 | 0.76 | 0.52 | 0.52 | 0.48 | 0.37 |
| **ICC** | 0.81 | 0.39 | 0.31 | 0.07 | 0.72 | 0.54 | 0.40 | 0.54 | 0.19 |
| **CR** | 7.86 | 11.02 | 17.31 | 14.48 | 12.65 | 18.80 | 12.02 | 16.40 | 32.13 |
| **Bias** | 0.44 | -0.11 | 2.70 | 2.03 | -2.91 | -1.21 | 4.58 | 0.77 | 6.64 |
| **SD (Bias)** | 3.93 | 5.51 | 8.65 | 7.24 | 6.32 | 9.38 | 6.01 | 8.20 | 16.06 |
| **95% LOA LB** | -7.26 | -10.91 | -14.25 | -12.16 | -15.31 | -19.63 | -1.20 | -15.31 | -24.84 |
| **95% UOA UB** | 8.13 | 10.68 | 19.65 | 16.21 | 9.48 | 17.21 | 16.35 | 16.83 | 38.11 |

*LVIM = Left Ventricular Imbalance, CHD = Congenital Heart Disease, SD = Standard Deviation, r = ICC = Interclass correlation coefficient, LV Left Ventricle, RV = Right Ventricle*

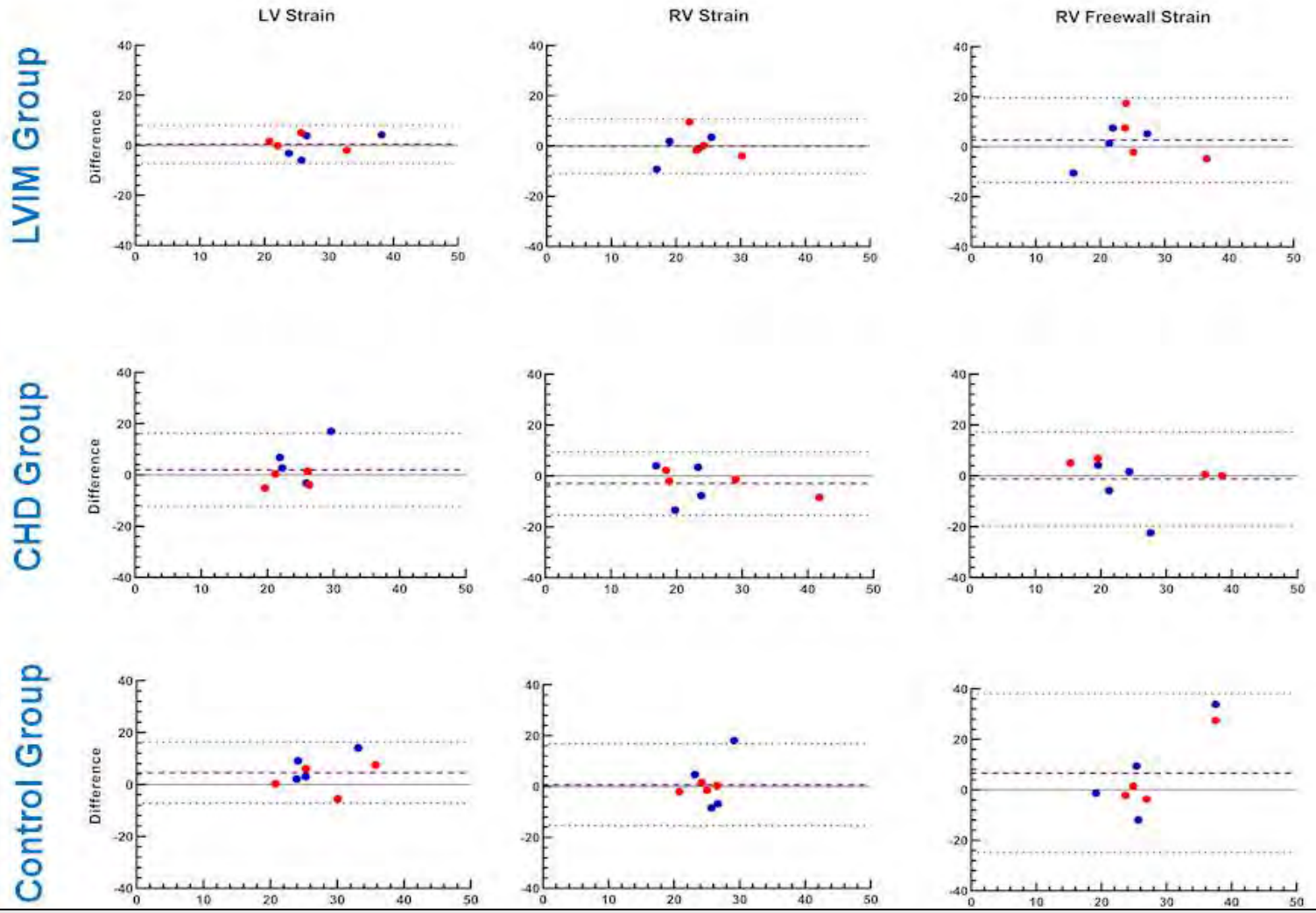


*Figure 40 Bland-Altman repeatability plots*

*LV Strain, RV Strain and RV Freewall Strain in each of the three groups for repeatability of duplicate assessments for two scorers (Blue JEJ; Red SN). Plots are difference versus the average of the two scores. The purple dashed line is the bias and the dotted purple lines are the upper and lower limits of agreement.*

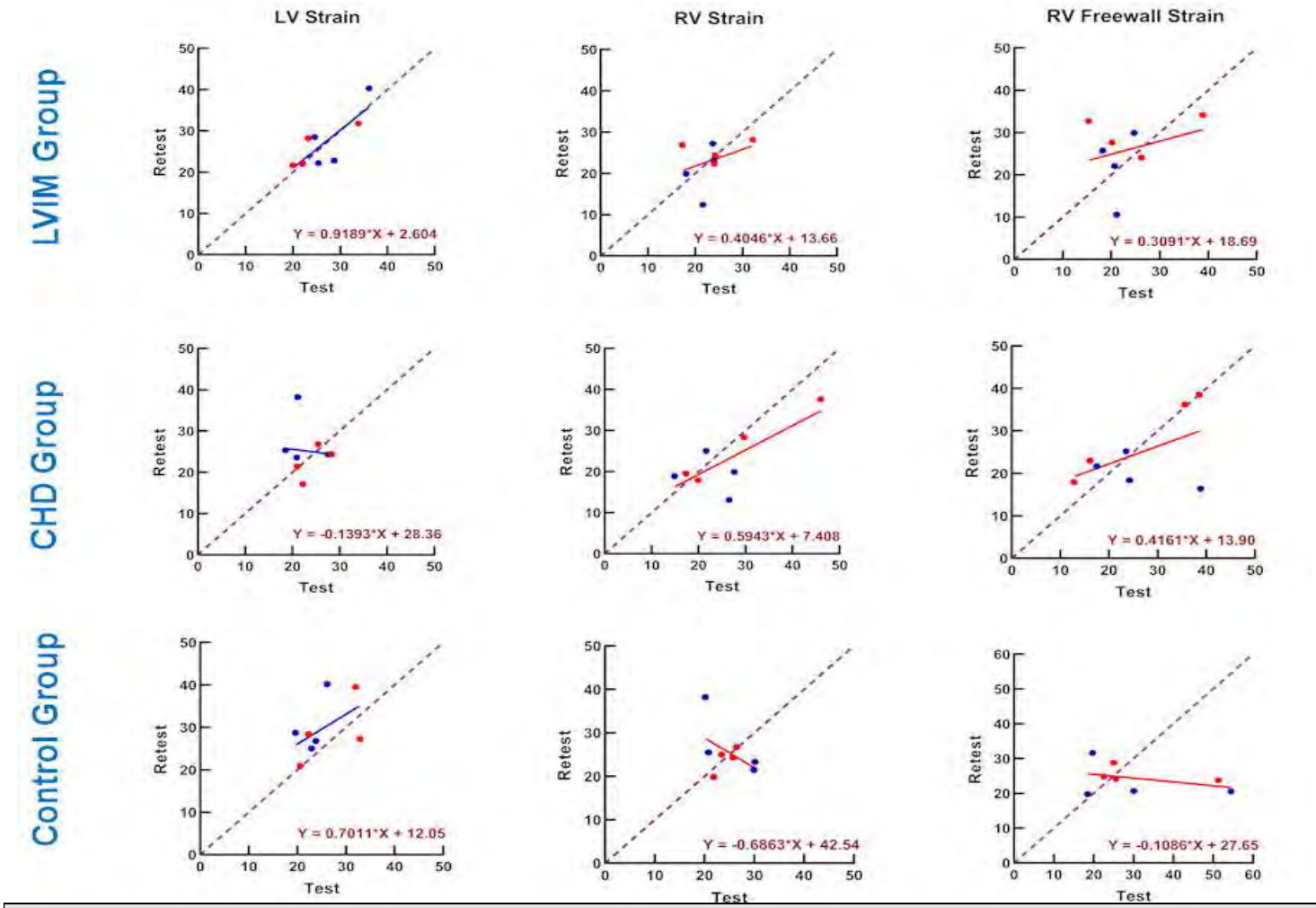


*Figure 41 Plots of GLS correlation*

*LV Strain, RV Strain and RV Freewall Strain in each of the three groups for repeatability of supplicate assessments for two scorers (Blue JEJ; Red SN). Plots are the initial scoring (Test) and the rescoring (Retest). The purple dashed line is the line of identity. For each relationship the regression equation is shown along with the regression line (red line).*

### 3.4 ASSESSMENT OF VENTRICULAR SIZE AND RATIO

Cardiac Performance Analysis (CPA) was used to quantify the endocardial RV and LV dimensions in end-diastole and end-systole. This provided structural cardiac measures for the length of the ventricles, and measures of the mitral and tricuspid annulus. Logistic regression analysis was performed on the RV/LV size ratio to identify whether it was possible to separate a normal outcome against a pathological RV/LV size imbalance due to CHD. The graphical outcome is shown in Figure 40.

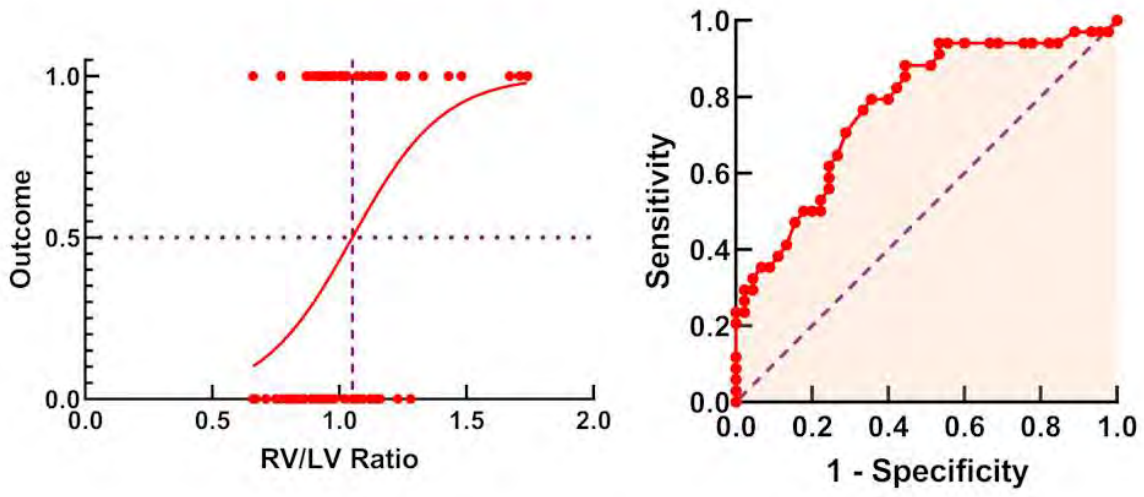


*Figure 42 Logistic regression analysis of the RV/LV ratio*

*Comparison of control plus LVIM + no CHD (score 0, n = 45) to LVIM + CHD (Score 1, n= 34). The left-hand graphic shows the logistic regression plot and highlights to 0.5 (50%) point, which has a RV/LV ratio on 1.052. The regression equation is log Odds = -5.866 + 5.579*RV/LV with a Tjur's $R^2$ of 0.2197. The right-hand plot shows the receiver operating curve (ROC) with the Area under the Curve (AUC) highlighted, along with the line of identity. The AUC was 0.7722.*

The key statistical data from this analysis are shown in Table 19

The logistic regression for RV/LV ratio indicates that the 50% point has a ratio value of 1.052. Using the relationship of this curve, a ratio of 1.30 would have a predictive probability of 80% for differentiating between those patients without an abnormality

and those with abnormality. Increasing the ratio to >1.42 would have a predictive probability of 90% or more.

| Table 19 Summary of logistic regression analysis for RV/LV ratio | |
|---|---|
| **Index** | **Value (95% CI)** |
| **Best-fit values** | |
| **β0** | -5.866 (-9.334 to -3.056) |
| **β1** | 5.579 (2.797 to 9.060) |
| **X at 50%** | 1.052 (0.9617 to 1.181) |
| **Odds ratio** | |
| **β0** | 0.002834 |
| **β1** | 264.7 |
| **Is slope significantly non-zero?** | |
| **\|Z\|** | 3.519 (p < 0.001) |
| **Deviant from zero** | Significant |
| **Likelihood ratio test** | |
| **Ratio ($G^2$)** | 19.36 (p < 0.0001) |
| **Reject null hypothesis** | Yes |
| **ROC Curve** | |
| **Area** | 0.7722 (0.6672 to 0.8773: p <0.0001) |
| **Goodness of fit** | |
| **Tjur's $R^2$** | 0.2197 |
| **N** | 79 |
| **N = 1** | 34 |
| **N = 0** | 45 |
| *CI = Confidence Interval,* | |

In terms of the ROC, this shows that the AUC is 0.7722 (CI: 0.6672 – 0.8773) which indicates a strong probability of being able to differentiate between those patients who have an abnormality and those that do not, i.e., the higher the ratio, the more likely that a disease state is present.

Logistic regression analysis was also performed on the MV/TV ratio to identify whether it was possible to identify an annular ratio which may separate a fetus with a ventricular

size imbalance between normal and abnormal outcomes. The graphical outcome is shown in Figure 43.

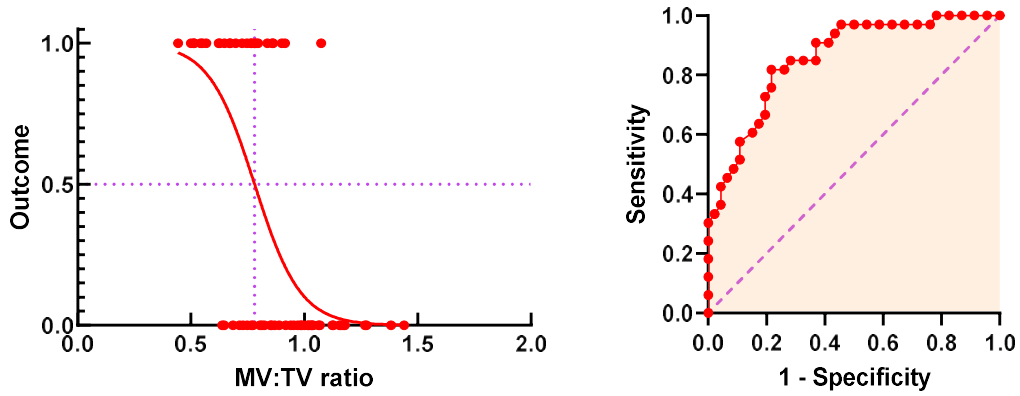


*Figure 43 Logistic regression analysis of the MV/TV ratio*

*Comparison of control plus LVIM + no CHD (score 0, n = 45) to LVIM + CHD (Score 1, n= 34). The left-hand graphic shows the logistic regression plot and highlights to 0.5 (50%) point, which has a MV/TV ratio of 0.7804. The regression equation is log Odds = 7.744 - 9.923*RV/LV with a Tjur's $R^2$ of 0.3846. The right-hand plot shows the ROC curve with the area under the curve (AUC) highlighted, along with the line of identity. The AUC was 0.8551.*

The key statistical data from this analysis are shown in Table 20

The logistic regression for MV/TV ratio indicates that the 50% point has a ratio value of 0.78. Using the relationship of this curve, a ratio of 0.64 would have a predictive probability of 80% for differentiating between those patients with an abnormality and those that do not. Decreasing the ratio to <0.57 would have a predictive probability of 90% or more.

***Table 20 Summary of logistic regression analysis for MV/TV ratio***

| Index | Value (95% CI) |
|---|---|
| **Best-fit values** | |
| **β0** | 7.744 (4.499 to 11.91) |
| **β1** | -9.923 (-15.15 to -5.909) |
| **X at 50%** | 0.7804 (0.7164 to 0.8395) |
| **Odds ratio** | |
| **β0** | 2307 |

| | |
|---|---|
| **β1** | 4.904e-005 |
| **Is slope significantly non-zero?**<br>**\|Z\|**<br>**Deviant from zero** | <br>4.266 (p < 0.001)<br>Significant |
| **Likelihood ratio test**<br>**Ratio ($G^2$)**<br>**Reject null hypothesis** | <br>35.89 (p < 0.0001)<br>Yes |
| **ROC Curve**<br>**Area** | <br>0.8551 (0.7731 to 0.9371: p <0.0001) |
| **Goodness of fit**<br>**Tjur's $R^2$** | <br>0.3846 |
| **N**<br>**N = 1**<br>**N = 0** | 79<br>34<br>45 |
| *CI = Confidence Interval,* | |

In terms of the ROC, this shows that the AUC is 0.855 (CI: 0.773 to 0.937) which indicates a strong probability of being able to differentiate between those patients who have an abnormality and those that do not, i.e., the lower the ratio, the more likely that a disease state is present.

### 3.5 ASSOCIATION BETWEEN GLS VALUES AND OUTCOMES

Further multivariable adjusted means were plotted between GLS % in foetuses with normal outcomes against those with an outcome of a congenital heart defect. 3 graphs were obtained demonstrating the mean GLS of the LV, RV and RV Free wall demonstrating the differences seen between 117 foetal echoes with CHD as a post-natal outcome and 46 foetal echoes with normal outcome (Figure 42).

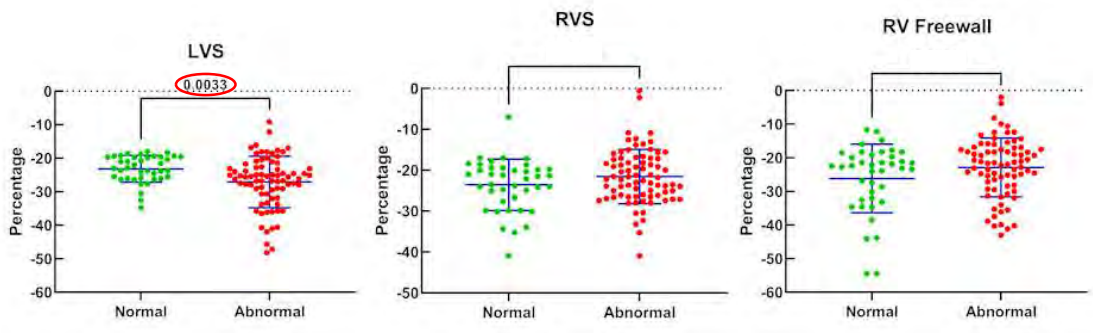


*Figure 44 Comparison of LVS, RVS and RV Free wall between subjects classified as normal (n = 40) and those classified as abnormal (n = 77) on their echo*

*There was a significant difference between the two groups for the LVS (-23.18 ± 3.99 vs -27.08 ± 7.66; p = 0.0033), but there was no significant difference for RVS (-23.55 ± 6.28 vs -21.57 ± 6.68; p = 0.1231) or RVS Free wall (-26.16 ± 10.17 vs -22.89 ± 8.76; p = 0.0730).*

GLS values for LV, RV and RV free wall were also risk stratified according to outcome – 1) Normal, 2) CHD – observation only, 3) CHD requiring intervention, 4) Death and compared using one-way ANOVA. There was no significant difference between the groups for LV, RV or RV Free wall (Figure 43)

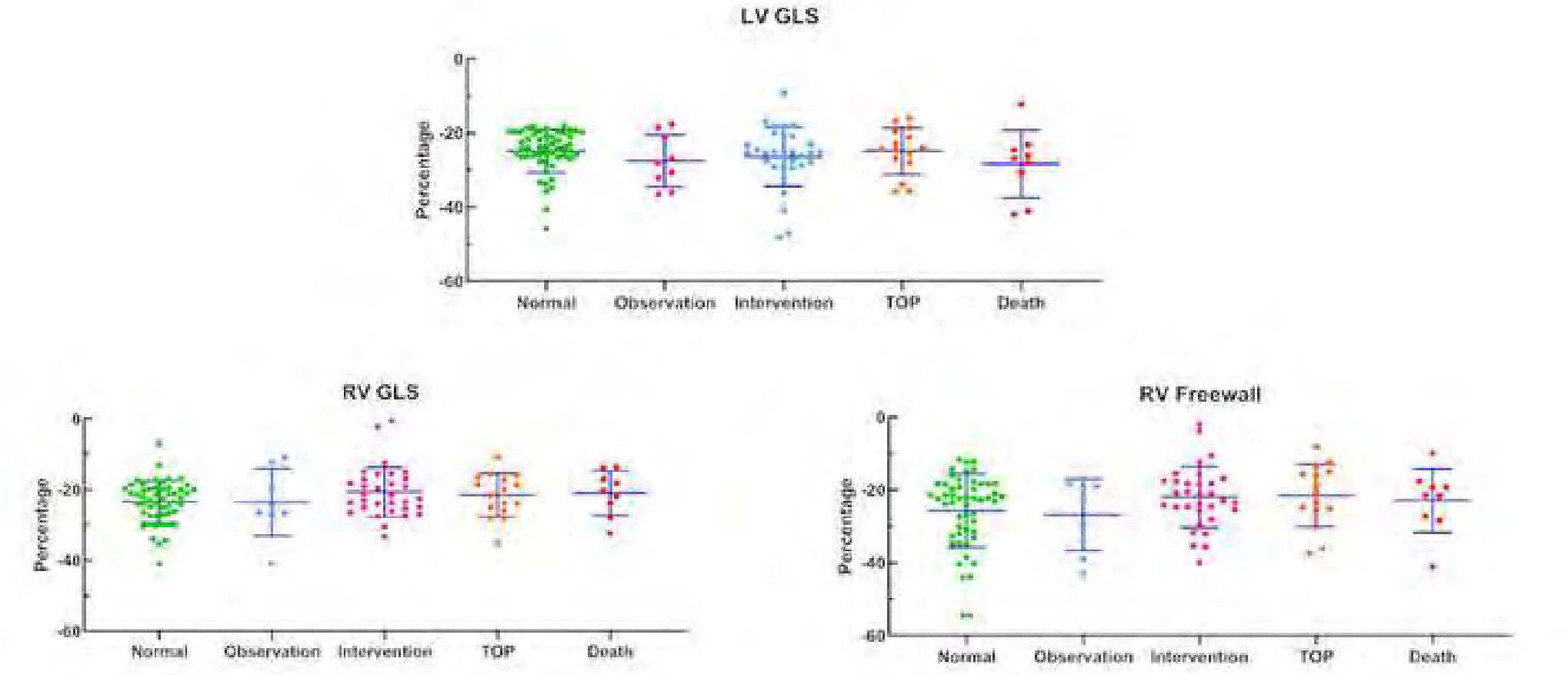


*Figure 45 Comparison of LV GLS, RV GLS and RV Free wall*

*Comparison between different subgroups – Normal, Observation, Intervention, Termination of Pregnancy (TOP) and Death.*

*One-way ANOVA showed **NO** significant differences between each of the four subgroups within each index.*

# CHAPTER 4 DISCUSSION

This study shows that ventricular strain quantification, via Foetal Cardiac Speckle Tracking, is feasible at mid-gestation of pregnancy. However, due to the high degree of variability between repeated measures, the absolute values lack accuracy. This is further confounded by the limited quality assurance of the analysis programme and technique, and lack of published normative values. Despite the limited quality control, it is concluded that there was NO significant difference between the strain values in the foetal heart where there was an observable discrepancy between the size of the right and left ventricles regardless of whether there was a congenital heart defect present or not. Current literature on foetal cardiac STE focuses on longitudinal cohorts of pregnancies between 20-40 weeks, whereas this feasibility study is a cross-sectional study focusing specifically on pregnancies at mid-gestation. This was intentional to see if STE and strain was feasible and indicative, at this stage of pregnancy, to aid counselling towards predicted outcomes during the initial consultation. The results of this study indicate that, currently, stain quantification is NOT a tool for determining whether this presentation of the foetal heart (at mid-pregnancy) is indicative for a normal or abnormal outcome.

Unlike postnatal life the foetal heart adapts and remodels to differing extents (Day, Charakida, & Simpson, 2019) due to the ability of the fetus to re-distribute flow in the “open” circulation of the foetal heart. Foetal cardiac remodelling can affect the whole heart (i.e., cardiomegaly) or either ventricle/atria, typically the right ventricle reflecting the dominance of right heart function as the ‘systemic pump’. As such, normal foetal

myocardial strain throughout pregnancy has not been conclusively evidenced (Oostrum, Guid oei, & Laar, 2019) and research is contradictory with regards to the deformation changes with growth and cardiac maturation (Di Salvo, et al., 2008) (Lee-Tannock, Hay, Gooi, & Kumar, 2019). The physiological changes in the foetal heart between 20-40 weeks include structural and functional adaptation, which alters blood flow distribution as the heart matures for an independent post-natal circulation. More recent studies with pregnancies spanning this time range have demonstrated a positive correlation to myocardial strain which suggests that left function matures gradually in late gestation (Peng, et al., 2009) (Lee-Tannock, Hay, Gooi, & Kumar, 2019) (Ohira, et al., 2020) with either stable or increasing strain. Early research reports that once myocardial contractility is established in mid-gestation, right ventricular strain values remain constant (Pu, et al., 2010), albeit lower than left ventricular strain values (Clavero Adell, Ayerza Casas, & Imenez Montanes, 2020). This was seen in the results of this study where the right ventricular values were lower in both the control group and the LVIM group. With advancing gestation, research suggests that right ventricular GLS has a negative correlation with advancing maturity (Ohira, et al., 2020). Physiological adaptation of the foetal myocardium, which is less well understood, has multiple variables due to maternal circulatory influence in comparison to a postnatal circulation. Myocardial deformation quantification is an important marker for cardiac function assessment but without a consensus on what is the normal strain pattern in the third trimester of pregnancy, it is difficult to draw comparisons against normal cardiac remodelling and remodelling which occurs because of an abnormality. In addition, normal physiological cardiac remodelling is very difficult to predict at 20 weeks, due to

factors which may arise later in the pregnancy such as impaired placental function, foetal growth restriction and acquired maternal disease.

This feasibility study is a cross-sectional representation of age-related pregnancies in mid-pregnancy analysing foetal cardiac myocardial deformation in three cohorts: 1) where foetal cardiac echocardiogram demonstrates a size discrepancy between the left and right ventricle of unknown aetiology 2) where foetal cardiac echocardiogram demonstrates an obvious abnormality (congenital heart defect) with additional ventricular size imbalance between the left and right ventricle 3) a cohort of age-related foetal cardiac echocardiograms with normal ventricular size presentation.

Left ventricular imbalance at 20 weeks is a particular presentation of the cardiac anatomy which raises concerns towards the possible progression as the heart matures. Pregnancy outcomes from a left ventricular imbalance (LVIM) range from unremarkable, as seen in 6 of the pregnancy outcomes in this study, to severely abnormal resulting in infant death, which was also documented. Despite the growing body of data for global longitudinal strain in foetal echocardiography, there has yet to be a cross-sectional study which specifically focuses on deformation at mid-pregnancy when there is an observable size discrepancy between the ventricles. This research was aimed at investigating STE in this group to try to differentiate pathological and physiological presentations to aid counselling for families at point of diagnosis.

It was found that 6 out of 40 (15%) LVIM pregnancies, where there was a discernible size disproportion between the left and right ventricles at 20 weeks gestation, subsequently went on to having babies with no cardiac anomaly. These pregnancies would have been given recommendations for further invasive testing in pregnancy (which carries a 1% risk of miscarriage), required increased hospital resources for repeated scans/appointments

and a special care baby admission, possible change in their preferred hospital of choice for delivery, as well as the option to terminate the pregnancy based on an unknown but potentially lethal cardiac anomaly. Conversely, one pregnancy within this study, where the parents would have undoubtedly been counselled along the same lines, lost their child in infancy due to a severe defect and the failure of being able to create an optimal biventricular circulation after cardiac surgery. This spectrum of severity, between normal and grossly abnormal, presents controversy amongst medics and families about how a ventricular size imbalance should be managed, hence the need for a more evidence-based diagnostic tool to identify how significant this foetal cardiac presentation is to outcomes.

To add weight to the results found in the LVIM group and to provide a direct comparison to the normal cohort, a third cohort was selected who had an obvious congenital heart defect with an associated ventricular imbalance. This was a controversial addition to the study, as this group had a variety of different cardiac pathologies and did does not allow a rigorous comparison. However, it could be argued that the mechanism by which the ventricles become imbalanced remains similar in that abnormal/reduced flow distribution around the foetal heart remains the common variable.

Numerous papers have investigated possible indicators for predicting outcomes from a ventricular size imbalance but there is still no consensus for a management pathway based on factual diagnostic evidence (Urbinelli, et al., 2015) (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013) (Loar, et al., 2016). Coarctation is considered one of the congenital cardiac conditions which is a leading cause of ventricular imbalance (Gembruck, 2010) (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013) despite a diagnosis rate which is consistently reported lower

than 50% (DeVore, et al., 2020) (Fricke, Liuba, & Weismann, 2021) (Tibaldi, et al., 2011). The diagnosis rate for coarctation in this study corresponds to these findings in that 50% of the LVIM group with an abnormal outcome, were diagnosed with coarctation in the post-natal period. The remaining pathology of this group included other significant left heart lesions, 75% of which required intervention within the first year of life. Only 20% (n=4) of those diagnosed with coarctation had the defect in isolation. The remaining post-natal diagnoses of coarctation had additional heart defects such as a bicuspid aortic valve or ventricular septal defect which can be difficult to detect in foetal echocardiography at 20weeks. This aligns to studies which also showed that coarctation was not commonly seen as an isolated cardiac condition (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020) (Beattie, Peyvandi, Ganesan, & Moon-Grady, 2017) (Familiari, et al., 2017) (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013) and that a foetal cardiac ventricular disproportion at mid-gestation is not strongly attributed to isolated coarctation (Familiari, et al., 2017).

## 4.1 KEY FINDINGS

### 4.1.1 Feasibility

Overall, STE was achievable in 97.5% of foetal echoes. This is a higher success rate than has been documented in other speckle tracking papers where there is up to a 15% failure rate in analysis (Crispi, et al., 2012) (Goudar, et al., 2016) (Enzenberger, et al., 2017) (Matsuura, et al., 2019). Feasibility was based on the ability for the software to accurately track the endocardial border after 3 attempts of optimising tracking.

### 4.1.2 Longitudinal Strain

For the 3 study cohorts, LVIM, CHD and the control group, the mean ± SD of peak LV longitudinal strain was -26.57 ± 7.13, -27.59 ± 8.26 and -23.18 ± 3.98 respectively demonstrating a significant difference between the control group and the CHD group only, but no significant difference between the LVIM group. This would imply that overall, the GLS values obtained of the left ventricle were only significantly different in the presence of a congenital heart defect but not in the presence of left ventricular imbalance.

### 4.1.3 Cardiac Dimensions

The observable size disproportion of the ventricles in the LVIM group was reflected in the dimensions obtained which were statistically different from the control group. LV length in the LVIM group with an abnormal outcome showed a significant difference from the length of the LV in the LVIM group with a normal outcome. This is clinically significant in that a smaller LV length is indicative of an abnormal outcome. In addition, it was found that the LV/RV ratio of >1.4 had a 90% probability for an abnormality and a MV/TV ratio of <0.64 had an 80% probability for an abnormality. There was also a correlation demonstrated between the size of the left ventricle and the outcomes in both the LVIM and CHD groups where the smaller LV correlated with a worse outcome. This is important information when counselling patients about the predicted outcomes of individual cases.

### 4.1.4 Functional Parameters

Left ventricular longitudinal fractional shortening, otherwise known as MAPSE was the only functional parameter which had a significantly reduced value in the LVIM group where there was an abnormal outcome. Overall, there was no significant difference

between traditional function measurements (EF% and FAC%, TAPSE) between the groups but there was evidence that reduced functional values reflected poorer outcomes. Again, this would provide support the predicting outcomes for pregnancies when counselling for congenital heart disease at 20 weeks gestation.

### 4.1.5 Operator Variability

Interobserver variation of the same cardiac cycle was variable between good and poor according to ICC reference values of 0.07-0.81. This may have a detrimental influence over the validity of results; however, similar variability is also reported in other foetal echo STE studies (Maskatia, et al., 2016) (Patey, Carvalho, & Thilaganathan, 2019). Variable and poor interchangeable correlation at 20 weeks is reported as a limitation of the technique in foetal cardiac strain analysis (Maskatia, et al., 2016) and, in making manual adjustments to the automated tracking protocol, tracking is improved but measurement variability is compromised (Koster, et al., 2020). Increasing the number of times each cardiac cycle was analysed and minimising manual adjustments would help support repeatability but in this study the loan duration of the software limited this capacity. It is also worth noting that whilst strain values of the endocardium should, in theory, remain constant between cardiac cycles, it is only same cycle repeatability which has demonstrated good repeatability (Day, Charakida, & Simpson, 2019) which questions the overall reliability of foetal cardiac strain quantification.

## 4.2 MYOCARDIAL DEFORMATION AND STRAIN ANALYSIS

This research has demonstrated several interesting findings regarding foetal cardiac myocardial deformation in mid-pregnancy.

The preliminary results of left ventricular cardiac performance analysis demonstrated that in the LVIM cohort average Left Ventricular Global Longitudinal Strain (LV GLS) values were >-18%, which is within the range of normative data for foetal cardiac strain, regardless of whether a defect was present or not. This would imply that ventricular function is preserved at this stage of pregnancy despite the presence of structural congenital heart abnormalities and/or ventricular size imbalance. Based on the lack of statistically significant differences in LV GLS, the LVIM cohort was further divided to investigate the strain values between the LVIM group with a normal outcome vs LVIM with a postnatal diagnosis of CHD, but these strain values were also comparable with no statistical significance.

It has been documented that GLS values tend to increase with a reduction in flow volume and vice versa (Eckerseley, 2017) but this was not demonstrated in the results. The ability for the foetal circulation to re-distribute cardiac output to the right side when the left is smaller may well have created a decrease in loading conditions but this may not be reflected in a reduction in strain until later gestation. Longitudinal strain analysis of this cohort may identify when this physiological adaptation in function occurs.

These physiological adaptations alter both the size and shape of the foetal heart, and recent STE research in longitudinal studies suggests that there is a significant reduction in left ventricular longitudinal strain values compared to age-related controls (DeVore, et al., 2020. DeVore, Jone, Satou, Sklansky, & Cuneo, 2020. Miranda, et al., 2017). These

studies indicate that there may be a demise in ventricular function in the presence of coarctation, but this evidence was based on strain values obtained in foetuses in the third trimester of pregnancy. Whilst this clinical information is useful at the end of the pregnancy for post-natal management, the challenge remains with providing this diagnostic evidence when the foetal presentation at mid-gestation can mislead clinicians either towards or against a possible congenital heart defect (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013) (Vernandos, Colquitt, & Morris, 2021) (Soveral, et al., 2017).

Like the strain values of the left ventricle, the right ventricular strain results did not differ significantly between a normal or abnormal outcome in the LVIM group. There was also no significant difference between the strain values of the right ventricle between the LVIM, control or abnormal cohort (CHD). The lack of statistical difference between results suggests that there is no difference in myocardial deformation despite the presence of an abnormality or an isolated physiological ventricular size imbalance. However, it is important to note that there can be statistical influences relating to the outliers or fluctuations in values which are inherent to a small sample size. In this study it was unfortunate that there were not more foetal cardiac presentations of LVIM which had a normal outcome to provide greater statistical power between groups.

In the abnormal (CHD) cohort there was a statistically significant ($p=0.02$) decrease in LV strain values compared to the control group and this corresponds to recent findings, where reduced global strain values in CHD corresponded to a deterioration of function, (Drop, et al., 2019. Germankis, Matsui, & Gardiner, 2012). However, when the CHD group was split into 2 groups with differing severity of LV size, the strain values were

comparable and not statistically significant. This suggests that the reduction in strain for the abnormal cohort was not due to ventricular size imbalance alone.

This is an important finding for future research into foetal cardiac myocardial deformation that apparent differences in strain values may not be attributed to any size, shape or physiological altered flow state. It is also important to recognise the impact small sample sizes have on results and that, with further validation on a greater sample size, there may be differences which do relate to clinical outcomes or severity. In addition, a greater sample size may help exclude the normal fluctuations in foetal STE results based on variations in method and technique which can accumulate to produce significant variation (Di Salvo, et al., 2008) (Voigt, et al., 2015).

There was no significant difference between the ventricular functional parameters (ejection fraction, fractional area change) of the left ventricle between the LVIM group with or without an abnormality. However, there was a difference in the longitudinal fractional shortening, or MAPSE, of the left ventricle which is suggested to be a similar parameter measure of the same mechanisms of longitudinal strain (DeVore, Klas, Satou, & Sklansky, 2018). MAPSE was significantly ($p=0.03$) reduced in the LVIM which was positive for CHD than in the LVIM group with a normal outcome. This may be an important parameter to distinguish normal and abnormal outcomes in foetal cardiac studies with a ventricular size imbalance. Reduced annular systolic function was also found in studies with a post-natal diagnosis of coarctation (DeVore, Klas, Satou, & Sklansky, 2018) (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020). This adds weight to research supporting a demise in left ventricular function in the presence of a defect, but at 20 weeks, the evidence is limited for a diagnosis of a congenital heart defect without additional diagnostic evidence.

There was no statistical difference between the RV functional parameters (FAC & TAPSE) in the LVIM normal cohort regardless of an outcome of congenital heart disease,. This suggests that whilst the remodelling of the RV apparently demonstrates normal myocardial deformation (where there is a ventricular disproportion) the traditional methods of function assessment may indicate compensatory mechanisms on ventricular function in the presence of a CHD. This relates to important quantification of RV cardiac function relating to a predisposition to postnatal RV dysfunction as described by Savla, et al., 2019. Certainly, in terms of the architectural structure of the right ventricle, myocardial deformation may hold more prognostic value in later gestation than left ventricular values due to underlying anatomic architecture. Significant alterations are evident in the disorganisation of cardiac muscle in the right ventricle where there is ventricular imbalance which may link to a reduction in RV strain, whereas the left ventricle maintains compact and organised structure and preserved LV GLS (Garcia-Canadilla, et al., 2018) (Zeng, et al., 2017). The preserved structure of the left ventricular myocardium also supports the theory that without a change in the number of speckles, strain values are not significantly affected, whereas in the right ventricle the disorganisation of the layers may reduce the number of speckles and thus impair myocardial deformation (Brooks, Khoo, Mackie, & Hornberger, 2012) (Crispi, Sepulveda-Martinez, & Crovetto, 2020).

The LV length was the only parameter in the LVIM group which demonstrated a significant difference (p=0.01) between those with congenital heart disease and those with a normal outcome. A reduction in left ventricular length is well evidenced as significantly different to controls in studies of coarctation (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020) (Fricke, Liuba, & Weismann, 2021). However other studies of

coarctation found that only the aortic arch measurements were significantly reduced (Johnson, Soslow, Mouledoux, Parra, & Kavanaugh-McHugh, 2013). The specific dimensions of the aorta, relating to coarctation were not measured in this study.

Significantly reduced LV length may be an early clinical indicator for congenital heart disease and where LV length has been demonstrated to correlate with LV strain values (Oostrum, et al., 2020), the demise in LV deformation may only become apparent in late gestation.

Whilst there was a statistically significant difference (LVd p=0.02, LVs p=0.01) between left ventricular volumes in the LVIM group and the control group, there was no significant difference between the left ventricular measurements in the LVIM + CHD and the LVIM + No CHD. Therefore, there was no significant difference in LV volume to differentiate pathological outcomes of the LVIM group. This aligns to measures of RV/LV area disproportion in later gestation where there is no significant difference between foetuses with coarctation or a false positive diagnosis of coarctation (DeVore, et al., 2020). Clinically, therefore, these findings lack reliability when observed in isolation.

In this study, there was no significant difference found between the mitral valve (MV) diameter for a normal or abnormal outcome, however MV z-scores were significantly lower (p=<0.0001) in the LVIM group with positive outcome for CHD. MV z-scores, calculated by comparing the mitral valve dimensions to age-related reference values, are more sensitive and specific to individual measures (Gabbay-Benziv, Turan, Harman, & Turan, 2015) (Krishnan, et al., 2016). These more accurately referenced values, are shown to be significantly lowered in foetuses with coarctation than normal cohorts of age-related foetuses (DeVore, Jone, Satou, Sklansky, & Cuneo, 2020) (Schneider, 2005) (Truong, Pinto, & Puchalski, 2013) which corresponds to the findings here that the MV

z-score is significantly lower in LVIM positive for CHD, than in LVIM with a normal outcome. Clinically, and in combination with LV length dimensions, this may support the differentiation of normal and abnormal foetal cardiac anatomy when there is observable left to right heart asymmetry (Miranda, et al., 2017).

Further investigation into the ratios between the diameter of the AV valves (MV/TV) and the length of each ventricle (RV/LV) showed some interesting criteria for predicting outcomes. RV/LV ratio measures in this study indicates that there is a 90% probability of a cardiac abnormality if this ratio is >1.4. This compares to other studies where these ratios are clinically significant towards a diagnosis of a cardiac abnormality (Familiari, et al., 2017) (Fricke, Liuba, & Weismann, 2021), and can also indicate more critical obstruction (Quartermain, et al., 2009). Measures of the mitral and tricuspid annulus ratio indicate that a MV/TV ratio <0.64 has an 80% predictive value for differentiating normal and abnormal cardiac anomalies in ventricular size imbalance. This also corresponds to studies where MV/TV ratio measures have strong probability (>80%) for abnormal outcomes with values <0.61 (Fricke, Liuba, & Weismann, 2021).

Whilst these findings indicate promising predictive values collectively, variable findings from other studies do not support these individual criteria and no single foetal echocardiographic index is an accurate predictor (Truong, Pinto, & Puchalski, 2013).

The variability of measures in GLS for both the left and right ventricles was between good and poor for statistical correlation between operators in this study. This aligns to various studies who also describe unacceptably high intraclass correlation coefficient at 20 weeks gestation (Maskatia, et al., 2016). The Bland-Altman plots, however, show that many scores were seen within a narrow window of upper and lower limits of agreement. In addition, the plots for correlation show that the mean values for repeatability were

clustered along the line of identity in most of the measures. This suggests that the majority of measures were well repeated, and it was the few outliers which would have negatively impacted the ICC. These plots provide good graphical representation of measures between operators, discounting the outliers corresponding to ICC reports in other foetal echo research (Miranda, Cerqueira, Ramalho, Areias, & Henriques-Coelho, 2018). It is also important to recognise that ICC improves with advancing age >24 weeks (Maskatia, et al., 2016) so the outliers may have reflected earlier gestation measures.

The mean values across the groups measured for repeatability were all >-18% which is comparable to normative data for foetal cardiac function (Sugimoto, et al., 2017) suggesting appropriate values were obtained for cardiac function despite the correlation being suboptimal. Research papers provide evidence of other contributing factors for high inter-operator repeatability (Huntley, Hernandez-Andrade, Soto, DeVore, & Sibai, 2021) (Voigt, et al., 2015), including gestation and foetal/maternal movement. Other factors, which are attributed to variability, include differing ultrasound machines, differing cardiac cycles and STE software (Oostrum, et al., 2020), all of which had less influence in this study as these were constant.

Cross-comparison of results to the literature is challenging due to these variations and this, in itself, is a known limitation to the interpretation of numerical values and the clinical relevance (Farsalinos, et al., 2015). In addition, repeatability of results is heavily dependent on sample size and/or the number of times the analysis was performed on each cardiac cycle. This was limited in this study due to the availability of the software which was on loan.

Operator variability on manual measures is often a result of subjective interpretation influenced by the accuracy of cardiac cycle timing, the anatomical points of reference

and the manual manipulation of the automated border definition. These were all variations which may only have minor differences between operators but collectively, these can make significant differences between GLS values. These minor variations in the technique/method, are also influenced by the operator knowledge and experience of using the software and cardiac anatomy. Poor inter observer correlation has been previously documented relating to the more complex anatomical shape of the right ventricle and structures such as the moderator band (Huntley, Hernandez-Andrade, Soto, DeVore, & Sibai, 2021) (Sengupta, et al., 2006) and in this study the poorest correlation between measures was seen in the strain values obtained in the right ventricle. Other factors which may influence repeatability are the quality of the imaging in relation to the frame rate, depth, and optimisation of the original image.

There may also be foetal or maternal haemodynamic influences which may affect GLS values (Fan, et al., 2014) (Gandhi, Zhang, & Maidman, 1995). Whilst all efforts were made to exclude pregnancies which were known to have haemodynamic influence on foetal circulation it was not possible to exclude those where these conditions were not known at the time of the analysis, such as gestational diabetes. Several papers have been reported an impact on foetal cardiac function by deformation analysis in the third trimester of pregnancy (Miranda, Cerqueira, Ramalho, Areias, & Henriques-Coelho, 2018) from such changes in foetal/maternal circulatory physiology.

Foetal growth restriction (FGR) is predominantly due to impaired placental function at the later stages of pregnancy. This reduction in blood flow to the foetal circulation has a haemodynamic influence and is associated with long-term adverse cardiovascular and metabolic disease (Oostrum, et al., 2020). FGR would not have been identified at mid-gestation and, therefore may have been an underlying haemodynamic influence on

strain values. There are also maternal influences on foetal cardiac haemodynamic which may not have been apparent, including the development of gestational diabetes, cholestasis or maternal anaemia which reportedly have a significant adverse effect on foetal cardiac function and strain values (Altun, et al., 2016) (Fan, et al., 2014) (Gandhi, Zhang, & Maidman, 1995).

In addition, extra-cardiac structural and chromosomal abnormalities which were identified in the post-natal outcomes were not known at 20 weeks. In the 117 foetal echoes which initially met the inclusion criteria in terms of having no extra-cardiac structural and chromosomal abnormalities, 24% of the whole group were found, retrospectively, to have these anomalies, and a further 21% of the whole cohort had unknown inclusion/exclusion criteria due to death/termination of pregnancy without post-mortem diagnosis.

## 4.3 CLINICAL IMPLICATIONS

### 4.3.1 Diagnostic value

Diagnostic functional tools are paramount for providing evidence to support clinicians in counselling towards a comprehensive prognosis enabling informed patient choice (Allen, 2010) (Eckerseley, 2017). At 20 weeks gestation, the information clinicians present to the family is, not only extremely important for understanding the impact of having a baby with a potential heart condition, but it is also crucial for families in decision-making options for the pregnancy (Miranda, et al., 2017). A false-negative diagnosis of aortic coarctation can alter location of birth, negatively impact maternal bonding and lead to unnecessary intensive care admission, IV access and prostaglandin

infusions (Tibaldi, et al., 2011). Conversely, a false-positive diagnosis risks cardiovascular compromise at an indeterminate time after birth (Hornberger & Eckersley, 2021) which can be missed at hospital discharge.

Comparable ventricular longitudinal strain results between LVIM and normal cohorts suggest that myocardial function is preserved in both the left and right ventricles in the presence of a ventricular size imbalance at mid-gestation. These results correlates to the preservation of the other functional parameters measured in the LVIM cohort. With this conclusion, global longitudinal strain is not a tool for differentiating normal and abnormal outcomes of foetal cardiac ventricular size discrepancies. It may be that the foetal heart has adapted well and early myocardial dysfunction or altered haemodynamic have resulted in cardiomyocyte proliferation and not impacted function at this stage (Day, Charakida, & Simpson, 2019), or that STE is not sensitive or specific enough at this gestation. GLS should, therefore be used with caution for predicting clinical outcomes at mid-gestation currently.

However, as the technology improves, techniques are standardised and larger cohorts support diagnostic findings, STE may still have future potential for aiding diagnosis and long-term outcomes, particularly in the context of a longitudinal study.

The results did demonstrate significantly reduced strain ($p=0.02$), in the group with congenital heart disease compared to control, which corresponds to studies which suggest that these lowered strain values may be an early indicator for long-term outcomes (Germankis, Matsui, & Gardiner, 2012) (Ishii, et al., 2014). This supports research into myocardial deformation as a useful indicator in congenital heart disease and there were observable differences noted in postnatal outcomes depending on the cardiac dimensions of the left ventricle and on some of the functional parameters

(DeVore, Klas, Satou, & Sklansky, 2019). These may add diagnostic value to predicting outcomes and are aligned to literature that these specific key markers hold prognostic value for predicting successful biventricular repair (Corno, 2005).

There were no significant differences in the right ventricular measures in this research focused on foetal cardiac presentation at mid-pregnancy. Again, this may represent the true preservation of function at 20weeks gestation or be due to the limited sample size.

Larger cohorts, and/or longitudinal studies maydemonstrate a significant difference in strain values which may enable the earlier identification of those predisposed to postnatal RV dysfunction (Savla, et al., 2019). Physiologically, it fits with ventricular remodelling which occurs during the last trimester of pregnancy as the left ventricle takes over as the main contributor to cardiac output (Eckerseley, 2017). If the left ventricle fails to support this function effectively, due to a smaller cavity size, then the additional RV pressure and volume may cause a reduction in effective contractility as seen as subclinical myocardial dysfunction. Whilst this was not demonstrated in this study, these preliminary results warrant further investigation in the third trimester of pregnancy to explore whether right ventricular strain is more susceptible (than the left ventricle) to early dysfunction in foetal life due to altered loading conditions.

## 4.4 LIMITATIONS

### 4.4.1 Recruitment

The images which were analysed in this study were collected retrospectively by identifying foetuses which were documented (in the foetal cardiology database) as

having a size discrepancy between the left and right ventricles. By conforming to the inclusion/exclusion criteria, a small sample size, below the anticipated recruitment numbers was obtained. The search of eligible studies was extended beyond this initial inclusion criteria to provide adequate numbers to enhance statistical significance, but this was still a small cohort. In extending the inclusion criteria terminology there may have been foetal studies which were not isolated to a ventricular imbalance but may have had a discrepancy in the size of the great arteries in addition to a ventricular disproportion. The inclusion of these outliers may have reduced the specificity and sensitivity of the cardiac presentation and impacted the results. It was important to ensure statistical power for those with a left ventricular imbalance but unfortunately there was no ability to increase the number of foetal echoes which had a normal post-natal outcome despite an observable ventricular imbalance. This cohort of 6, with a ventricular imbalance but a normal outcome, was surprisingly low and did not reach the statistical power comparison which was hoped for. This means that this group may likely be distorted by random and systematic error.

The low yield of foetal studies with a ventricular imbalance and normal outcome, may be due to a lack of documentation in the foetal cardiology database. If individual cases of a borderline LVIM were considered low risk for an abnormal outcome, they may not have been categorised as such. In a prospective collection of foetal cardiac echocardiograms, the cohort may have been larger as it would be less dependent on specific documentation in the foetal cardiology database. Additionally, a larger cohort of one particular congenital heart defect, such as unbalanced AVSD, may have provided a better comparison as an abnormal group and reduced the bias from having multiple conditions.

### 4.4.2 Imaging

Whilst all foetal echocardiograms were optimised for clinical purposes at the time of acquisition, by performing a retrospective collection of data, we were unable to ensure that the standard 4 chamber cardiac view was optimised specifically for speckle tracking echocardiography, with optimal frame rates. Research into STE frame rates have demonstrated this to be an independent variable for accurate strain values (Enzenberger, et al., 2017). Currently, there are no definitive guidelines on optimal frame rates for Foetal Cardiac STE but evidence to indicate that as mechanical events become shorter with a faster the heart rate, the frame rate should be increased to enable tracking of physiological events (Mor-Avi, et al., 2011). Consensus papers have implied that a frame rate >100fps needs to be achieved for high resting heart rates (Voigt, et al., 2015) but other papers have demonstrated there to be no significant difference (Enzenberger, et al., 2017) in GLS values between 30 – 60 fps. In this study, the images analysed were at a frame rate determined by the settings at the time of acquisition which were between 22Hz and 84Hz, with an average of 52.9Hz across all analyses. There was no significant difference between the frame rates in each group therefore removing any bias towards better or worse frame rates between groups. Average frame rates in this study were 53Hz which may be considered suboptimal for foetal heart rates averaging 158bpm, despite the observable ability of the software to track the endocardium. Indeed, a frame rate, in one of the echo images, as low as 22Hz is acknowledged as being detrimental to the accuracy of quantification, but this was a compromise required to prevent very small sample sizes within the LVIM and abnormal cohorts. This is a limitation of both the study method and the validity of the study results but with all research into foetal STE, it has yet to be evidenced whether foetal STE is

beyond the capabilities of current technology (Day, Charakida, & Simpson, 2019) and therefore this area of research is still to be proven in accuracy. In this study, speckle tracking echo was still feasible when the frame rate was as low as 22Hz, and the majority of the echo images had frame rates of at least double this.

In addition to the limitations of low frame rate, the angle of insonation was not reported in each analysis. It has been reported that variation in the foetal heart orientation, may affect GLS depending on a vertical or horizontal septum (Semmler, et al., 2020). This variable was not accounted for in this study and warrants further investigation in subsequent foetal STE analysis.

**4.4.3 CPA Analysis, Measurements, and Timing**

Anatomical placement of the cardiac landmarks was a variable relying on operator knowledge and interpretation as indeed was the manual adjustments to optimise tracking. In such a small structure, accuracy of this placement may have been compromised by image resolution, irregular endocardial surface and interference from valves and papillary muscle and stability of the foetal heart during analysis.

It was also intended that analysis was focused on the more robust strain values of the endocardium (Semmler, et al., 2020), and whilst every effort was made to ensure differentiation of the cardiac layers there may have been cross over during cardiac cycle where the semi-automated analysis was influenced by parts of the epicardium.

Anatomical measures of the foetal heart structures are subjective due to the small size and poor resolution of obstetric ultrasound and are prone to operator variability. In this study, the cardiac dimensions were made by the main investigator only, which does not provide comparative or qualitative evidence. This is a limitation to the predictive value and significance of the foetal cardiac dimension findings in this study. To validate this

data, further measures for repeatability are required to identify and quantify the inherent fluctuations of manual measures.

The absence of an ECG in foetal echocardiography is a limitation to both performing STE and cardiac strain analysis. The timing of the cardiac cycle is dependent on operator recognition and accuracy of systolic and diastolic markers on the time-in-motion study of the ventricle or motion mode (m-mode). This placement is also dependant on the quality of the m-mode recording. Foetal movement and dyssynchrony of the left and right ventricular contractions prevented consistent multi-cycle analysis and timing due to the quality of the m-mode recording. It is recognised that in a prospective study these elements may have been avoided or excluded from analysis. Accurate alignment of end-systole and end-diastole on deformation curves is also an operator dependant determinant for accurate deformation values. These minor subjective fluctuations in performance may compromise the accuracy of timing which would be a limitation for the study.

**4.4.4 CHD Cohort**

During data collection, many imbalanced or disproportionate ventricles (LVIM) were associated with an identifiable cause, such as an outflow obstruction. Due to the frequency of this presentation, this group was formed due to their abnormal presentation (CHD) with associated ventricular imbalance as documented on the database. Although the cause for the imbalance was apparent, the rationale behind this group was to provide a truly abnormal cohort of foetal cardiac presentation as a comparator to the LVIM and control groups. This approach provided 3 groups representing -ve CHD (normal), +ve CHD (abnormal), and the LVIM group which was +/- CHD (unknown normal/abnormal). The rationale was that the aims of this study were to

identify strain values in a presentation of foetal cardiac ventricular imbalance. The mechanism of the imbalance was assumed to be related to altered circulatory physiology and therefore the strain values may be comparable whether or not the cause be identified. The advantage of including this group is that there was a significant difference in strain between the CHD group which corresponded to other papers looking at strain in CHD (Drop, et al., 2019), but this difference of strain values did not translate to those with a significant imbalance. This suggests that strain quantification is sensitive for differentiating between normal and abnormal foetal cardiac presentations in the form of CHD per se, but not in foetal cardiac presentation of a ventricular size imbalance.

The limitations of an abnormal group are that CHD covers multiple presentations of abnormal cardiac anatomy which does not provide a consistent variable for comparison. Also, when it came to analysis, the observed discrepancy in size between the left and right ventricles was not always as significant when the actual ventricular cavity dimensions were reviewed. As a result, this group required additional re-grouping to identify those CHD which had a significant between the ventricles (> z-score -2). Consequently, the size of CHD cohort was reduced, and this limited the statistical power of the data.

## 4.5 FUTURE RESEARCH

### 4.5.1 Current Climate

There has been a wealth of research over the last 10 years into foetal cardiology STE but due to multiple inconsistencies in both the methods and the results, STE has still failed to be used in daily clinical practice. Presently, there appears to be too many variables in

STE across the literature to provide evidence of accuracy and reliability for clinical interpretation particularly at specific gestations *(Oostrum, Guid oei, & Laar, 2019)*. Cross sectional and longitudinal studies have made a big impact on demonstrating feasibility and providing comparative data for reference values in the third trimester of pregnancy. There is, however, limited data on left and right ventricular longitudinal strain specifically the at mid-gestation (18-21weeks) and the data that has been reported is inconsistent.

### 4.5.2 Key Findings for development

Key findings in this study suggest that longitudinal values in the left ventricle are significantly reduced in the presence of a congenital heart lesion but not with a LV size discrepancy only. There may be changes in strain values in the presence of a ventricular size discrepancy, which hold more significance, if STE was studied in greater numbers. In this study there were limited foetal echocardiograms where there was a LV size imbalance and a normal outcome. With a larger normal cardiac outcome cohort, the apparent increase in GLS in the left ventricle in the presence of an imbalance of ventricles may reach statistical significance. Equally, the apparent reduction in RV GLS may hold more validity and statistical significance with a larger cohort. This research could be developed further with a longitudinal approach to investigate the GLS values of the LVIM group with a post-natal finding of CHD throughout the pregnancy to find a significant difference in values as the heart matures. To support the validity of this data, further STE analysis at each interval may help influence more reliable repeatability between operators particularly when an interclass correlation coefficient has been shown to be better in foetuses over 24weeks gestation (Maskatia, et al., 2016).

Several studies have explored the timing difference between peak systolic strain as a predictor of cardiac dyssynchrony (Germankis, Matsui, & Gardiner, 2012) (Koster, et al., 2020) (Rolf, et al., 2018). This is easy parameter to obtain during CPA analysis which may provide insights into the mechanical changes of cardiac function. However, further research into this dyssynchrony may identify this phenomenon to be a consequence of artificial dyssynchrony and a limitation of speckle tracking imaging due to the cardiac tissues moving out of the tracking region (Alessandrini, et al., 2018.).

### 4.5.3 Future recommendations

There are a number of learnt outcomes, relating to study design and methods, which would improve the quality of the results and reduce variation in future STE research. Firstly, the comparison of strain between LVIM +ve/-ve CHD would hold more power with a larger sample size for the LVIM -ve CHD. Currently, a sample size of 6 is insufficient to draw robust statistical comparison to the +ve CHD group of 33. Based on the LVIM data output of LV strain values (mean and SD), a sample size of 62.93 with a Type I error **α** of 5% and power **1 – β** of 80% would provide greater statistical power to the p-value calculations (Power and Sample Size Calculator, n.d.). By increasing the sample size of the study, comparative data would hold more accurate significance and reduce error. Other recommendations for future study design are listed in the table below and relate both to the findings of this study and to those of other published results.

*Table 21 Recommendations for future STE study design*

| Variable | STACVI | Recommended |
|---|---|---|
| **Study Design** | Retrospective recruitment and analysis | Prospective recruitment will increase sample size and control inclusion/exclusion criteria. Prospective |

| | | |
|---|---|---|
| | | analysis will permit optimisation of images for STE and reduce the bias of cherry-picking the best images. |
| **No. of cardiac cycles sampled** | Variable between 1 and 4 | Obtaining 4 stable cardiac cycles for each analysis ensures more accurate averaging of strain values (as observed) |
| **Sample size** | LVIM +ve CHD = 33<br>LVIM -ve CDH = 6<br>Total Sample 39 | Based on the data output of the LVIM cohort, a sample size of 62 is recommended to provide the optimal statistical power. |
| **Frame Rates** | LVIM FR 30-84Hz | Optimal setting for image FR in adult echocardiography is documented 50-60 Hz (Mirea, et al., 2019) with heart rates averaging 60bpm. The standard frame rate should be increased proportionally with the expected heart rate (Voigt, et al., 2015) therefore foetal heart rates between 120-180bpm require FRs between 60-90Hz. |
| **Gestation** | $15^{+3}$ – $25^{+5}$ | Interobserver variability is reduced in foetuses >24weeks gestation (Maskatia, et al., 2016). |
| **Inter/intra operator variability** | Operator repeatability - averaging on each sample twice<br>Comparison of 10% of results | Operator repeatability and averaging on each sample 3 times.<br>Comparison of 20% of results |

| | | |
|---|---|---|
| **Foetal cardiac orientation analysis** | No analysis made | Recommend observing variation in strain values depending on a cardiac axis in a horizontal or vertical orientation in view of the differing spatial resolutions. |

To further valid STE and strain analysis in foetal echocardiography, standardised practice and protocols need development with a consensus on normative values. Quantifying the variation between different software manufacturers and optimised prospective data collection may help to reduce the technical limitations which were demonstrated in this study. To support strain quantification in foetal cardiac STE analysis, comparative foetal cardiac MRI derived data may bring confidence for implementation to clinical practice (Singh, et al., 2010) as correlation between STE and cardiac MRI is excellent (Obokata, et al., 2016). This would support publication of normal strain values in foetal cardiology.

# CHAPTER 5 CONCLUSION

The results from this study show that there is no statistically significant difference between strain values found in foetuses with an LV/RV size imbalance regardless of whether it is related to CHD or not. There was a significant difference in GLS values in the CHD group against the control group, but this did not translate across to the ventricular disproportion of the condition. These findings play an important role in recognising the limitations to strain analysis at mid-gestation in supporting clinical outcomes.

Quantification of foetal myocardial function is still challenging (Enzenberger, et al., 2017) and more evidence is required before it can be introduced into clinical practice. However, confirmation that there is a significant reduction in LV strain in congenital heart disease is a promising tool for evaluating cardiac function and warrants further investigation.

Measurements of foetal cardiac function are one of the most important components of foetal echocardiography. They can aid diagnosis, assess disease severity, predict prognosis, and direct management strategies (Patey, Carvalho, & Thilaganathan, 2019). STE in foetal echocardiography is feasible and, despite the lack of evidence for this gestational age range, it is a promising tool for detecting subtle cardiac adaptations to cardiac function. A longitudinal cross section of pregnancies with LV/RV imbalance may help differentiating a whether a ventricular size imbalance is significant to a structural congenital heart defect and predict outcomes.

The findings of this study support the further development of clinical applications in speckle tracking and strain analysis. It will increase our understanding of cardiac function in foetal life but also help to demonstrate feasibility, reproducibility and specificity of a technique which may hold important predictive value for diagnosis and prognosis. Speckle tracking and strain are not currently used in clinical practice and the results seen in this study supports a lack of evidence towards the validity and reproducibility of the technique at 20 weeks gestation. There needs to be further evidence and a reduction in contradictory and variable data, for clinical validity (Oostrum, Guid oei, & Laar, 2019).

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## APPENDICES

| Table 22 Appendices | | |
|---|---|---|
| **APPENDIX 1** | HSST Modules completed in addition to the C2 research Component | |
| **APPENDIX 2** | Foetal Echocardiogram Standard Operating Procedure | |
| **APPENDIX 3** | University hospitals of Bristol and Weston NHS Foundation trust, Research and Development Sponsorship Agreement | |
| **APPENDIX 4** | Evidence that Organisational Information Document (OID) or a Schedule of Events (SoECAT) is not required as part of your HRA application | |
| **APPENDIX 5** | Health Research Association (HRA) Study Approval | |
| **APPENDIX 6** | MMU Project Proposal | |
| **APPENDIX 7** | Manchester Metropolitan University Approval of project proposal | |

## APPENDIX 1 - HSST Modules completed in addition to the C2 research component

| Module | Description | Credit Value | Grade |
|---|---|---|---|
| A1 | Professionalism and professional development in the healthcare environment | 30 | P |
| A2 | Theoretical foundations of leadership | 20 | P |
| A3 | Personal and professional development to enhance performance | 30 | P |
| A4 | Leadership and quality improvement in the clinical and science environment | 20 | P |
| A5 | Research and innovation in health and social care | 20 | P |
| B1 | Advanced history taking and communication skills | 15 | P |
| B2 | Clinical presentation and management of cardiac disorders - 1 | 20 | P |
| B3 | Therapeutics | 10 | P |
| B4 | Diagnostics and monitoring in cardiology | 20 | P |
| B5 | Contemporary issues in healthcare science | 20 | P |
| B6 | Clinical presentation and management in Cardiology - 2 | 15 | P |
| B7 | Teaching, learning and assessment in healthcare science | 30 | P |
| B8 | Specialist Option: Service Evaluation | 30 | P |
| B9 | Specialist Option: Service Evaluation Viva | 15 | P |
| C1 | Doctoral Research and Innovation in Clinical Science | 270 | P |

## APPENDIX 2 - Foetal Echocardiogram Standard Operating Procedure for University Hospitals of Bristol and Weston

Standard Operating Procedure

# FETAL ECHOCARDIOGRAPHY

| | |
|---|---|
| **SETTING** | Fetal Cardiology Department - St Michael's Hospital, Women's & Children's. |
| **FOR STAFF** | Members of staff undertaking fetal echocardiograms for the purpose of examining fetal cardiac anatomy:- Specialist Sonographer, Specialist Radiographer Specialist Nurse Practitioners, Specialist Midwife Practitioners and doctors in training. |
| **ISSUE** | Standardising the approach to fetal echocardiography |

## Standard Operating Procedure (SOP)

Fetal echocardiography is performed in both screening and diagnostic capacities. Depending on the nature of the referral, the examination can be individualised to achieve optimal diagnostic accuracy.

This SOP details the standard examination and reporting procedures for fetal echocardiography undertaken within University Hospitals Bristol NHS Foundation Trust. It is designed to provide the operator with standardised guidance for the performance and recording of a comprehensive fetal cardiac examination, utilising some of the current recommendations from both national and international fetal echocardiography standards and guidelines[1-5]

Fetal echocardiography aims to establish either normality or the presence of congenital heart disease[1]. The examination assesses normal anatomical relationships and functional flow characteristics. Various ultrasound modalities are used with a frequency between 1-20 MHz. These include; 2 dimensional and m mode imaging, colour, pulse-wave and continuous flow Doppler. The fetal cardiac anatomy is visualised using transverse, sagittal and coronal planes ensuring a complete sequential analysis of the cardiac components and their relationship.

**General Considerations**

- Where possible screening fetal echocardiograms should be performed between 18-20$^{+6}$ week's gestation.
- Urgent Referrals with suspected CHD in fetus must be seen by a fetal cardiology specialist within 3 calendar days of referral
- Referrals with suspected CHD in fetus must be seen by specialist cardiac nurse on the day of diagnosis
- Prior to commencing the ultrasound examination, verbal consent must be obtained after a full explanation of the proceedings.
- A comprehensive maternal obstetric history must be documented
- Optimizing patient position with careful attention to comfort, dignity and safety
- Verbal communication of the results to the patient and documentation within the maternity hand held notes if available
- Transfer of all images and data to ICVS which, once finalised is saved to the trust server UPAC's for long-term storage
- Document any restriction to the views

## APPENDIX 3 - University hospitals of Bristol and Weston NHS Foundation trust, Research and Development Sponsorship Agreement

NHS
University Hospitals Bristol and Weston
NHS Foundation Trust

Research and Innovation
University Hospitals Bristol and Weston NHS Foundation Trust
Education & Research Centre Level 3
Upper Maudlin Street
Bristol BS2 8AE

Tel: 0117 342 9873

research@uhbristol.nhs.uk
http://www.uhbristol.nhs.uk/research-innovation

**Joanne Jones**
**Fetal Cardiology, Level E**
**St Michael's Hospital**
**Bristol**
**BS2 8EG**

**05/11/2020**

Dear Joanne,

**RE: CS/2020/6935 - Can speckle tracking in fetal echocardiography help to predict the outcome of cases presenting with a ventricular size imbalance in mid-pregnancy. (STACVI)**

University Hospitals Bristol and Weston NHS Foundation Trust (UHBW) has agreed to act as sponsor for the above study under the UK Policy Framework for Health and Social Care Research.

An HRA approval letter for the study must be received by the Trust within one year of the date of this letter. Otherwise, Trust agreement to act as sponsor will lapse, and a fresh application will be required.

All studies sponsored by UHBW are monitored for recruitment to time and target. The Trust may withdraw sponsorship if there are significant causes for concern.

A study specific local information pack as described on the HRA website: http://www.hra.nhs.uk/resources/hra-approval-guidance-for-sponsorschief-investigators-working-collaboratively-with-nhs-organisations-in-england/ must be submitted to ResearchApprovals@UHBristol.nhs.uk in order to complete Capacity and Capability review before the study can commence at this site. We look forward to receiving your HRA approval letter.

**Please note that no recruitment may take place until Confirmation of Capacity and Capability is received and we as sponsor have given green light.**

Yours sincerely,

pp. Jessica Bisset

Diana Benton
Head of Research and Innovation/Deputy Director of Research

## APPENDIX 4 - Evidence that Organisational Information Document (OID) or a Schedule of Events (SoECAT) is not required as part of HRA application

**Jones, Joanne**

**From:** Mulligan, Sandra
**Sent:** 04 November 2020 19:03
**To:** Jones, Joanne
**Subject:** Sponsor confirmation that OID and SoECAT not required for STACVI

Dear Joanne ,

**Re: 'Can speckle tracking in fetal echocardiography help to predict the outcome of cases presenting with a ventricular size imbalance in mid-pregnancy. (STACVI)',**

This is to confirm, that as Sponsor for the above study, we do not require the submission of an Organisational Information Document (OID) or a Schedule of Events (SoECAT) as part of your HRA application.

The study is currently a single-site study at University Hospitals Bristol and Weston NHS Foundation Trust and we have confirmed our capacity to undertake all research activities as described in the protocol.

However, should the study design change and additional sites are to be added, then these documents will be required for the amendment submission to the REC/HRA.

Please submit this e-mail as part of your HRA application.

Best wishes
Sandra

**Sandra Mulligan**
**Research Management Facilitator (0117 34 29894)**
Research & Innovation | UHBW | Level 3, Education and Research Centre | Upper Maudlin Street | Bristol | BS2 8AE
|sandra.mulligan@UHBW.nhs.uk

***Please note:*** *All future research amendments should be sent to our new inbox at the following address: researchamendments@uhbristol.nhs.uk. You no longer need to send them to researchapprovals@uhbristol.nhs.uk. Thank you.*

## APPENDIX 5 - Health Research Association (HRA) Study Approval

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Mrs Joanne Jones
Specialist Cardiac Physiologist in Congenital Heart Disease Imaging
University Hospitals Bristol and Weston NHS Foundation Trust
Fetal Cardiology, Level E
St Michael's Hospital
Bristol
BS2 8EG

Email: approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

08 December 2020

Dear Mrs Jones

**HRA and Health and Care Research Wales (HCRW) Approval Letter**

| | |
|---|---|
| **Study title:** | **Can speckle tracking in fetal echocardiography help to predict the outcome of cases presenting with a ventricular size imbalance in mid-pregnancy.** |
| **IRAS project ID:** | **263538** |
| **Protocol number:** | **CS/2020/6935** |
| **REC reference:** | **20/HRA/5753** |
| **Sponsor** | **University hospitals of Bristol and Weston NHS Foundation Trust** |

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

**How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?**
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

## APPENDIX 6 - Project Proposal

### STUDY PROPOSAL

This protocol has regard for the HRA guidance and order of content

### TITLE OF THE STUDY

Can speckle tracking in foetal echocardiography help to predict the outcome of cases presenting with a ventricular size imbalance in mid-pregnancy.

### SHORT STUDY TITLE / ACRONYM

Speckle Tracking as a tool for Assessing foetal Cardiac function in Ventricular Imbalance (STACVI)

### PROTOCOL VERSION NUMBER

| Protocol Version No. | Date issued | Author(s) of changes | Details of Changes made |
|---|---|---|---|
| **1.0** | 19/10/2020 | Jo Jones | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

### RESEARCH REFERENCE NUMBERS

| | |
|---|---|
| **IRAS Number:** | 263538 |
| **SPONSORS Number:** | CS/2020/6935 |
| | University Hospitals Bristol and Weston NHS Foundation Trust. (UHBW) |
| **FUNDERS Number:** | |
| | HSST Bursary – Health Education England |

**SIGNATURE PAGE**

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

**For and on behalf of the Study Sponsor:**

Signature: Date:

..... ...../...../.....

Name (please print):

.....

Position:

.....

**Chief Investigator:**

Signature: Date:

..... 19/10/2020

Name: (please print):

.....Joanne Jones.....

LIST of CONTENTS


| **GENERAL INFORMATION** | **Page No.** |
|---|---|
| HRA protocol compliance declaration | 1 |
| Title page | 1 |
| Signature page | 2 |
| List of contents | 3 |
| Key study contacts | 4 |
| Study summary | 5 |
| Funding | 5 |
| Role of sponsor and funder | 6 |
| Roles & responsibilities of study steering groups and individuals | 6 |
| Study flow chart | 7 |
| Timescales | 7 |
| **SECTION 2 STUDY PROTOCOL** | |
| 1. Background | 8 |
| 2. Rationale | 11 |
| 3. Theoretical framework | 11 |
| 4. Research question/aim(s) & objectives | 11 |
| 5. Study design/methods | 12 |
| 6. Protocols | 14 |
| Abbreviations | 15 |
| 7. Study setting | 16 |
| 8. Safety Reporting | 16 |
| 9. Authorisations and Research Governance Statement | 16 |
| 10. Monitoring and Audit | 17 |
| 11. Patient & public involvement | 17 |
| 12. Data Management, Handling and Protection | 17 |
| 13. Financing, Indemnity, and Insurance | 18 |
| 14. References | 19 |

KEY STUDY CONTACTS

| | |
|---|---|
| **Chief Investigator** | Mrs. Joanne Jones, BSc (Hons) Clinical Physiology 07747112503, joshack8@hotmail.com<br>joanne.jones@uhbw.nhs.uk |
| **Study Supervisors** | Dr Adrian H Kendrick BA, PhD, PhD (Applied Stats), RPGST<br>Consultant Clinical Scientist, Department of Respiratory Medicine, University Hospitals, Bristol. pyahk@bristol.ac.uk Phone: 01173421633<br>Dr Martin Stout, Manchester Metropolitan University<br>Academic Supervisor, 0161 247 1183; m.stout@mmu.ac.uk<br>Dr S Narayan, MD, Paediatric consultant cardiologist, UHBW NHS trust. srinivas.narayan@uhbw.nhs.uk, 07971695721 |
| **Sponsor** | University Hospitals Bristol and Weston NHS Foundation Trust, Research and Innovation, Level 3, UH Bristol Education and Research Centre, Upper Maudlin Street, Bristol BS2 8AE. Tel: 0117 342 0233. |
| **Joint sponsor(s)/co-sponsor(s)** | N/A |
| **Funder(s)** | HSST Bursary – Health Education England |
| **Key Protocol Contributors** | Dr Patricia Caldas, Foetal and paediatric consultant cardiologist, UHBW NHS trust. patricia.caldas@UHBW.nhs.uk |
| **Committees** | The HSST Oversight Group (HOG) is a committee which oversees the delivery of DClinSci doctoral programmes at MAHSE partner universities. |

**STUDY SUMMARY**

| | |
|---|---|
| **Study Title** | Speckle Tracking as a tool for assessing foetal cardiac function in Ventricular Imbalances (STACVI) |
| **Internal ref. no.** | CS/2020/6935 |
| **Study Design** | Retrospective observational study |
| **Study Participants** | Foetal echocardiograms of pregnant women referred to the Specialist F[etal Cardiology Service at St Michael's Hospital, Bristol |
| **Planned Size of Sample** | 120, (3 cohorts of between 20-40)<br>20-40 x foetal echocardiograms with a f[etal cardiac ventricular size imbalance of unknown aetiology<br>20-40 x foetal echocardiograms with a foetal cardiac ventricular size imbalance in the setting of a congenital heart defect<br>20-40 x control population of foetal echocardiograms referred for screening at St Michael's Hospital, Bristol. |
| **Follow up duration** | Follow up as clinically indicated in pregnancy |
| **Planned Study Period** | 01/09/2020 – 31/08/2021 |
| **Research Question/Aim(s)** | The aim of this feasibility study is to determine whether myocardial deformation, assessed by speckle tracking in foetal echocardiography, can predict the outcome of cases presenting with a ventricular size imbalance at second trimester screening. |

**FUNDING AND SUPPORT IN KIND**

| **FUNDER(S)** | **FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN** |
|---|---|
| Funding via Health Education England HSST Bursary | Non-financial support from UHBW Clinicians & research supervisors |

## ROLE OF STUDY SPONSOR AND FUNDER

University Hospitals Bristol and Weston NHS Foundation Trust (UHBW) is the sole sponsor for the study as the employer of the chief investigator and employer of the clinical academics. The organisation receives an annual bursary over five years from Health Education England in support of the investigator's participation in the Higher Scientific Specialist Training programme. The Higher Specialist Scientist Training Programme (HSST) is a workplace-based training programme supported by an underpinning doctoral-level academic programme. The academic component of HSST is known as the Doctor of Clinical Science (DClinSci) which is provided by the university partnerships at Manchester Academy of Healthcare Science (MAHSE).

It is the role and responsibility of the sponsor UHBW to over-see study design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results with academic insight from Manchester Academy of Healthcare Science. It is also the role and responsibility of the supervising clinicians to ensure controls over the final decision regarding any of these aspects of the study.

## ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

### HOG Steering Group

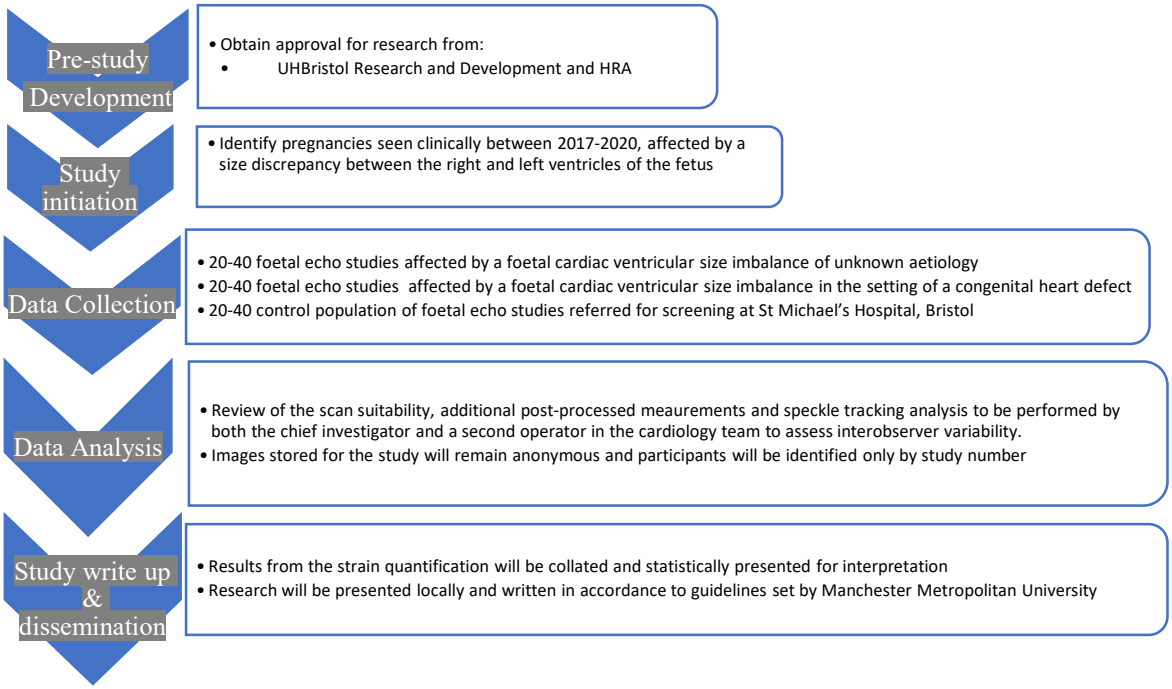
The HSST Oversight Group (HOG) is a committee which oversees the delivery of DClinSci doctoral programmes at MAHSE partner universities. Acceptance of the study was agreed after review of the project proposal and presentation to the panel.

## PROTOCOL CONTRIBUTORS

Contributors to the protocol include the study clinical supervisor's Dr A Kendrick and Dr S Narayan, and Dr Patricia Caldas. Dr Kendrick is involved in clinical research within UHBW NHS Trust and the Bristol universities. He will oversee the scientific and statistical component of research study design and conduct. Dr Narayan is a paediatric Cardiologist with an interest in cardiac imaging and functional assessment tools. He will oversee the speckle tracking and strain protocols whilst, Dr Caldas will oversee the foetal cardiology component.

**KEY WORDS:** Speckle tracking, myocardial deformation, strain, and strain rate, foetal echo, ventricular size imbalance

STUDY FLOW CHART




**TIMESCALE**

Below is a projected timescale for research procedures within the constraints of the HSST programme:

| Procedures | Time Frames | | | |
|---|---|---|---|---|
| | Sept-Dec 20 | Dec-Jun 21 | Jun-Sept 21 | Sept-Dec 21 |
| Sponsorship confirmed | x | | | |
| Screening for retrospective data | x | x | | |
| Data Collection | x | x | x | |
| Data Analysis | | x | x | |
| Research write up | | | x | x |

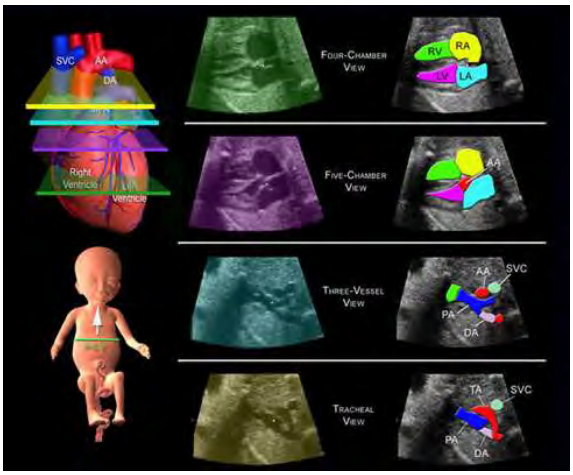
**STUDY PROTOCOL**

Speckle Tracking as a tool for Assessing foetal Cardiac function in Ventricular Imbalance (STACVI)

BACKGROUND

Foetal Anomaly Screening Programme

In England, all eligible women are offered screening to assess the chance of their baby being born with a significant chromosomal or structural abnormality. Under the expert governance of the Foetal Anomaly Screening Programme (FASP), this second scan is designed to detect foetal anomalies between $18^{+0}$ to $20^{+6}$ weeks to allow for specialist diagnosis and counselling, and (if required) further diagnostic tests. In foetal cardiology, the diagnosis of a significant congenital heart defect requires expert clinical management to provide non-directive counselling about the condition, prognosis, and long-term outcome. The wide spectrum of congenital cardiac conditions encompasses those that may benefit from treatment before or after birth, those that may require planning of the delivery site and management guidelines to optimise treatment after the baby is born or those that require various levels of palliative care. At this point, families are offered all options including, continuation with the pregnancy with a view to intervention/medical management after birth, continuation with the pregnancy with a view to provide comfort or palliative care or the option to terminate the pregnancy. This highly emotive and often, controversial aspect




*FASP foetal cardiac views*

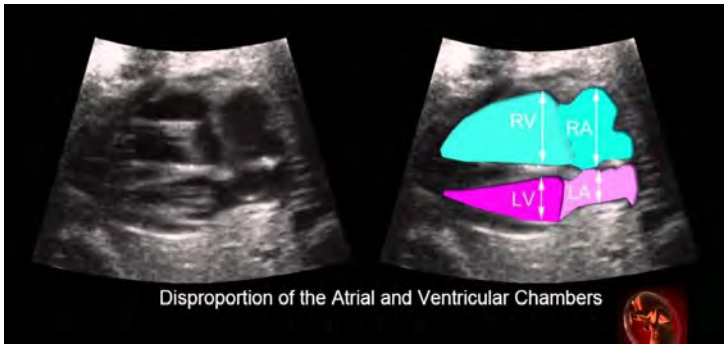
of antenatal screening places an enormous responsibility on the competency and diagnostic reliability of ultrasound images.

Normal Foetal Cardiac Protocol and presentation

At 8 weeks gestation the foetal heart is fully formed structurally and functionally providing foetal circulation. The remainder of the pregnancy is growth and maturation. Foetal Cardiac assessment by ultrasound is optimal at $18^{+0}$-$20^{+6}$ weeks due to the relative proportions of the foetal cardiac size to fetus ratio. It is also an optimal time for diagnosis and decision-making for medical and legal implications on discontinuing a pregnancy. The Abortion Act 1967 supports the decision of families to terminate a pregnancy affected by a serious cardiac abnormality which has a significant impact on quality of life. Timings, therefore, play an important component in process of an antenatal diagnosis of a cardiac anomaly.

Ventricular size imbalance at 20 weeks gestation

The presence of a ventricular size imbalance in the second trimester of pregnancy is often the first indication for a congenital heart defect (Menduina 2015 and Troung, Pinto & Puchalski, 2013) although due to the variable rates of maturation, the underlying defect may not be apparent (Johnson et al, 2013). This places a considerable burden on the clinicians to counsel patients without a known diagnosis (Menduina 2015). A ventricular imbalance (as seen in fig 2) can be inconsequential to a long-term outcome or it can be the beginning of a progressive lesion such as coarctation or

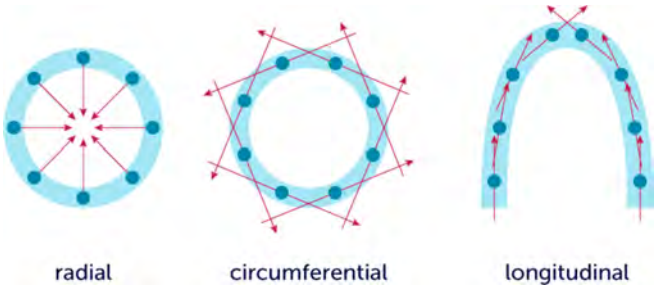



*Foetal echo LV/RV imbalance*

aortic stenosis which may lead to interventional corrective surgery or result in a hypoplastic left heart lesion requiring a 3 staged surgical palliative pathway (Menduina 2015 and Loar *et al,* 2016). In many congenital cardiac defects such as an unbalanced atrio-ventricular septal defect, the additional presence of a ventricular imbalance creates a guarded approach to counselling for a successful outcome. Ventricular imbalance can, therefore, have a marked impact on the risk stratification on long-term prognosis when counselling families (Quartermain *et al,* 2009). Immediate medical management at birth may also be dependent on the identification of lesions such as total anomalous pulmonary venous drainage to permit survival. Finding diagnostic evidence towards the diagnosis of a pathological lesion at 20weeks may improve the challenge placed upon clinicians to provide non-directive, but evidence-based counselling for families presenting with a size discrepancy between the ventricles.

Functional Cardiac Assessment

The left ventricle has a sophisticated orientation of muscle fibres, which permits the heart, not only to contract in radial direction, but also shorten its length and provide contractility in a circumferential or "twisting" mechanism (Fig 3). Several different functional echo techniques can be used to evaluate these 3 mechanisms individually but these traditional techniques (principally used in adult and paediatric echocardiography) have proved very difficult to replicate in foetal echo. Most of the techniques require the 4-chamber view to be displayed in a specific alignment – either vertical for longitudinal




*Mechanisms of left ventricular function*

excursion i.e., mitral annular planar systolic excursion (MAPSE) or horizontal as in the measurement of shortening fraction (%). A foetal 4-chamber view is obtained at an angle, dependent upon foetal orientation rather than the standard transthoracic apical view. Obstetric scanning also limits the ability for some of the functional assessments due to the suboptimal images in comparison to direct patient acquisition and both the foetal size and heart rate are challenging for ultrasound physics to accurately interpret.

Speckle Tracking strain analysis

More advanced functional assessment is performed using tissue Doppler analysis and 2D speckle tracking strain analysis (Divanovic *et al,* 2011). The baseline study and functional parameters are based on the guidance published by the American Heart Association (AHA) (Donofrio *et al*, 2014). Functional assessment of the foetal heart utilizes 2D, colour and pulsed wave Doppler to assess cardiac size and flow and 2D speckle tracking (2DSTE) is a relatively new ultrasound technique with only limited reported data in the fetus.

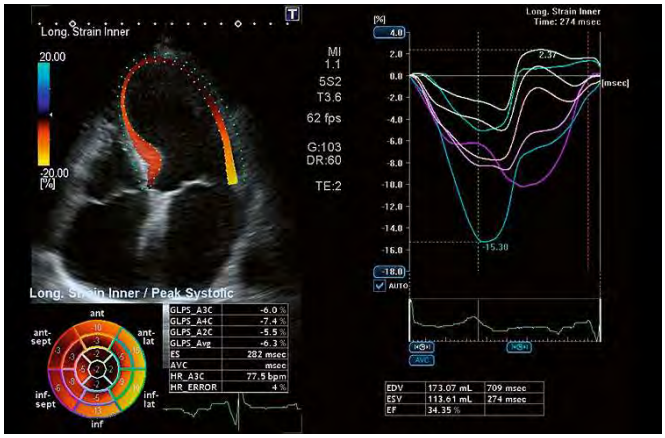



*Figure Global longitudinal strain analysis in paediatric cardiology demonstrating percentage strain in all 19 left ventricular wall segments*

It is a technique which is well researched in adult echocardiography and is in use for the paediatric population (fig 4) but remains in the research phase for foetal echocardiography. Many of the traditional techniques for assessing ventricular function are angle dependant whereas speckle tracking is said to be an angle independent method

relying on the tracking of B-mode grey scale 2D ‘speckles’ or ‘kernels’ from frame to frame which makes this technique perfect for use in foetal echo.

Using the end diastolic dimensions as a surrogate for original length and reference point, these speckles can be tracked as they move in any direction through the cardiac cycle to determine degree of deformation (Lagrangian strain). This means that by calculating the percentage change in deformation, we can interpret the function of the cardiac muscle fibre regardless of the functional mechanism or direction of “strain” and therefore evaluate overall function rather than an individual mechanism.

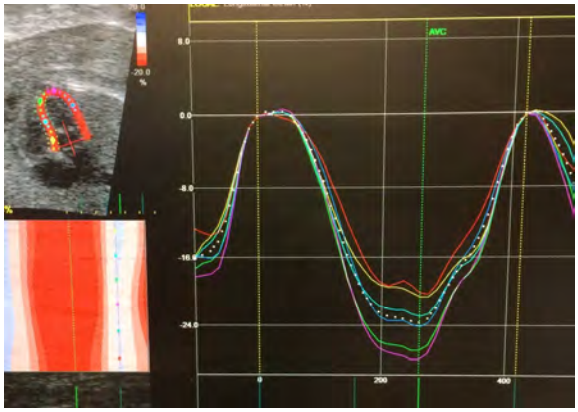


*Figure Example of foetal cardiac strain*

We intend to use 2DSTE to assess the foetal cardiac function (example in fig 5) in all foetuses with an idiopathic ventricular size imbalance and in those with an underlying congenital heart defect to compare myocardial strain variation against a normal control group.

RATIONALE

Presently, the 20-week anomaly scan will identify a ventricular size imbalance as an abnormal 4 chamber view but foetal echocardiography may not provide specific diagnostic evidence to predict whether the imbalance is significant to the post-natal outcome. Whilst the abnormality can be identified, there are limited tools to predict the outcome of a ventricular size imbalance of the foetal heart at 20weeks gestation. Speckle tracking and strain analysis may provide the ability to assess subclinical foetal cardiac function and thus provide vital information to differentiate a ventricular size

imbalance with a compromise in function against this presentation where function is preserved. If this links to clinical outcomes, then it may be a useful tool for antenatal counselling and aid informed patient choice for the management options of their pregnancy

## THEORETICAL FRAMEWORK

Research into myocardial deformation has had a big impact in understanding cardiac function in both physiological and pathological conditions. The literature has a vast array of evidence for the use of strain and strain rate as an indicator of subclinical cardiac dysfunction in the adult and paediatric populations with recommendations being made about clinical management strategies and risk stratification (Miranda et al 2017). This is an exciting area of cardiac mechanics which has been demonstrated as a reliable and reproducible diagnostic tool. However further research into speckle tracking in foetal echocardiography is required before it is utilised into clinical use. Contributing to the research pool will aid this process and support the evidence in implementing strain into routine clinical practice. For families affected by a foetal cardiac diagnosis with unclear or uncertain outcomes, strain and strain rate may have the potential to quantify severity and predict outcome for families faced with a dilemma at mid-pregnancy.

## RESEARCH QUESTION/AIM(S)

### Objectives

To assess the feasibility of assessing foetal cardiac function using speckle tracking and obtaining a percentage of myocardial deformation (Strain).

To compare the strain values obtained between the 2 cohorts with a ventricular size imbalance: those with a confirmed diagnosis of congenital heart defect and those foetuses with a ventricular imbalance of unknown aetiology

To compare cardiac function strain values of affected foetuses with a control group of normal foetal cardiac presentation.

STUDY DESIGN AND METHODS OF DATA COLLECTION AND DATA ANALYSIS

Study design: Retrospective observational study.

Study participants

This retrospective, observational study will review foetal echocardiogram images from pregnancies that have been clinically referred to the Foetal Cardiology Services at St Michael's Hospital over a 3-year period. All the research data obtained will be derived from diagnostic imaging stored for clinical purposes at UHBW between 2017 and 2020. Foetal echocardiograms are stored using maternal identification which is then collated into a service database for standard clinical care.

We are aiming for a maximum of 120 foetal echo studies; of these, there will be 2 sub-groups of those with a confirmed diagnosis of a congenital heart defect (n=20-40) and those with a ventricular imbalance due to unknown aetiology (n=20-40). There will also be a control group of gestational age-matched foetal cardiac echoes diagnosed as normal presentation (n=20-40).

Inclusion criteria

Foetal echo studies with an imbalance between the right and the left ventricles due to.

the presence of a congenital heart defect

unknown aetiology

Foetal echo studies with normal 4 chamber images

Exclusion criteria

Suboptimal quality foetal echo imaging as seen in increased maternal BMI

Extra-cardiac foetal abnormalities which impact normal blood flow distribution

Twin pregnancies

Methods

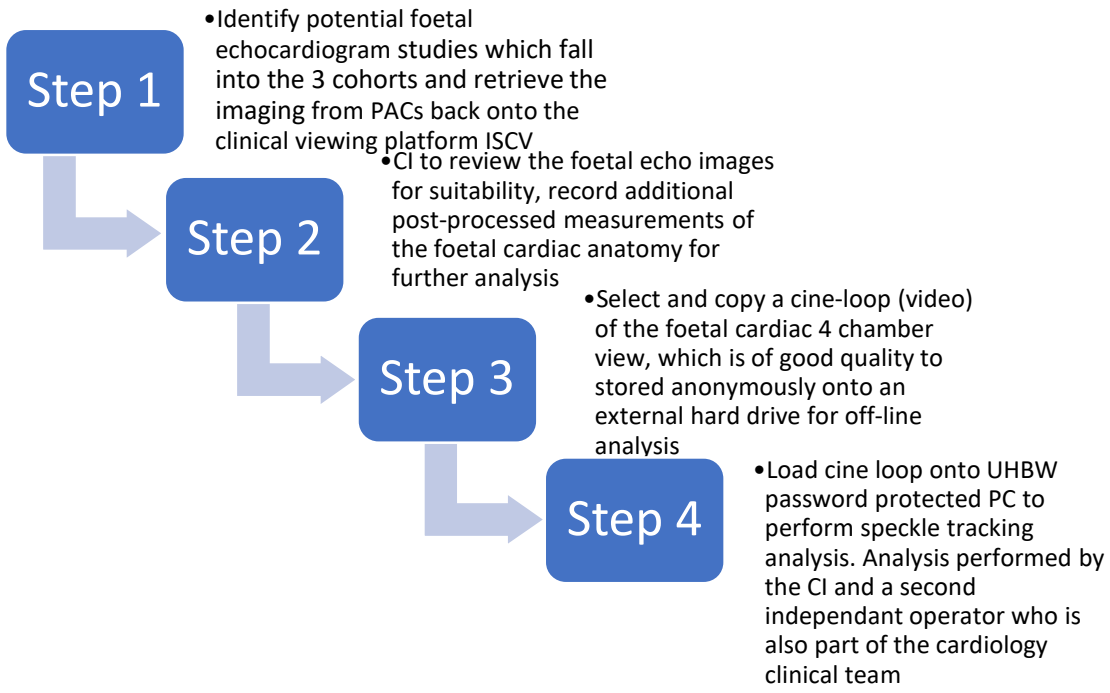
As an authorised member of the clinical team in foetal cardiology, the chief investigator has access to foetal echocardiograms which have been stored as per clinical standard of care.

Standard greyscale 2Dimensional (2D) echocardiography images from a Canon Aplio i800™ are stored on the hospital PACs system via the imaging platform Intellispace Cardiovascular (ISCV). All potential foetal echo studies, both normal and abnormal, will be retrieved from PAC's storage and re-installed ISCV. Foetal echo studies will be reviewed with additional post-processing measurements made of the foetal cardiac anatomy. The maternal hospital number identifying the foetal echocardiogram will be recorded on a password protected Excel spreadsheet. This identification is required to differentiate foetal echo studies into the 3 sub-groups relating to the foetal cardiac presentation.

1) ventricular imbalance of unknown cause,

2) ventricular imbalance due to a diagnosis of a congenital heart defect,

3) normal foetal cardiac anatomy. The four-chamber view of the foetal heart is accomplished by directing the ultrasound beam perpendicular to the foetal chest and storing video clips or cine-loops of between 3-6seconds which is performed as part of the standard foetal echocardiogram protocol. This cine loop, of the standard foetal cardiac 4 chamber view, will be evaluated for suitability and stored onto a password protected external hard drive for off-line speckle tracking analysis. Speckle tracking




software, installed onto a password protected UHBW Trust PC, will be used to obtain a percentage of myocardial deformation, also known as percentage strain. This software also can calculate theoretical volumes of the cardiac chambers based on anatomical calculations from tracking the cardiac muscle wall. A second independent operator from the clinical cardiology team will also be asked to perform the analysis so that inter-observer variability can be observed. Both the CI and the second operator will be blinded to each other's speckle tracking assessment.

Strain Analysis

Global longitudinal systolic strain (%) will be analysed off-line, between patient groups, using two strain analysis packages for comparison between software. Additional parameters of the cardiac dimensions will also be collated including ventricular function via the Myocardial Performance Index (MPI) for comparison of functional assessment. A summary of the routine foetal and paediatric echo protocols and the additional measures can be seen in table 1 and 2 respectively.

Statistical Analysis

This is a feasibility study to investigate whether speckle tracking analysis can demonstrate a difference in the percentage of myocardial deformation between a cohort of normal foetal cardiac anatomy and an “abnormal” foetal cardiac presentation. The numbers of foetal cardiac studies with a ventricular imbalance are limited and, therefore, statistical analysis will focus on any statistically significant difference between the percentages of myocardial deformation in each group. In the gestational age-related normal cohort, the Shapiro-Wilk test will test to confirm normality. The differences in measured % strain between the 2 groups of abnormal foetal cardiac presentation will be analysed using a 2-sample ANOVA, with Bonferroni correction for multiple comparisons.

FOETAL CARDIAC PROTOCOL

Foetal Cardiac Echo Protocol

| Routine foetal echo protocol | Extra post-processed research components |
|---|---|
| Laterality demonstrated via maternal position and the Cordes technique[1]<br><br>Situs – solitus, inversus, ambiguous (isomerism)<br><br>Cardiac position – levo, meso or dextrocardia<br><br>Connections – AV VA concordance<br><br>Aortic and ductal arch side – left or right<br><br>Systemic and pulmonary venous connections<br><br>Axial – four chamber view<br><br>2D Cardiac size<br><br>Colour Doppler – inflow TV and MV<br><br>Pulse Doppler - inflow Doppler of TV and MV and LV inflow/outflow Doppler | Use a 3 beat cine loop of the four-chamber view to perform speckle tracking analysis and longitudinal strain quantification using analysis software<br><br>Measure 2D cardiac dimensions of tricuspid valve annulus (basal RV), mitral valve annulus (basal LV), Right Ventricular and Left Ventricular lengths<br><br>All measured in diastole and referenced to z-scores<br><br>Calculate the Mitral valve: tricuspid valve diameter ratio<br><br>Measure the mechanical AV intervals on an LV inflow/outflow Doppler<br><br>Measurements of IVCT, IVRT and ET on an LV inflow/outflow Doppler to calculate the Myocardial Performance Index<br><br>Perform 2D measurements of Aortic and pulmonary annulus referenced to z-scores |

| | |
|---|---|
| LV outflow view with colour Doppler to assess outflow<br><br>Pulsed wave Doppler to determine aortic velocity<br><br>RV outflow view with colour Doppler to assess outflow<br><br>Pulsed wave Doppler to determine pulmonary artery velocity<br><br>Haemodynamic Doppler assessment<br><br>Foetal heart rate<br><br>Systemic venous Doppler – Inferior Vena Cava / Ductus Venosus<br><br>Pulmonary venous pulse wave Doppler | |

STUDY SETTING

As a member of the clinical team in Foetal Cardiology, the chief investigator has access to all foetal echocardiography images which are securely stored on Trust premises for clinical purposes. Foetal echo images will be retrieved from the internal hospital PACs system and stored directly on the ultrasound machine hard drive in the foetal medicine unit at St Michael's hospital. Additional post-processed measurements will be made within the unit and the relevant clips for analysis will be analysed within the Foetal

Cardiology clinical area at St Michael's hospital. All data will be stored on an encrypted portable hard drive in the Foetal Cardiology Office, which is locked when not in use.

*Consent*

The data is collected as part of the patients' routine clinical care data and will be reviewed by the CI who is a member of the patients' direct clinical care team. The data will be anonymised by the CI prior to analysis.

Therefore, explicit written consent is not required for the purposes of this study. We have applied for HRA approval for this study.

SAFETY REPORTING

This is a retrospective data collection study and no adverse events are anticipated.

AUTHORISATIONS AND RESEARCH GOVERNANCE STATEMENT

Authorisations

The study will be performed subject to favourable opinion/ authorisation/permission or equivalent from all necessary regulatory and other bodies. This includes but is not limited to REC, HRA, and NHS Trusts.

Governance

This study will be conducted in accordance with: The principles of Good Clinical Practice, as set out in the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines and the UK Policy Framework for Health and Social Care Research.

Confidentiality

The chief investigator will ensure that the participants' anonymity is maintained. All study documents will be stored securely and only accessible by authorised NHS

personnel. Electronic patient records will only be accessed by the chief investigator on NHS premises.

All participants will be assigned a unique study identifier. This will be used to pseudonymise all data and scans prior to processing, analysis, and storage.

Images will also be anonymised and will not be recognisable in the database, or in any subsequent publications that will arise from the study.

The study will comply with the Data Protection Act and General Data Protection Regulation which requires data to be anonymised as soon as it is practical to do so.

All data will be deleted from the encrypted portable hard drive at the end of the study

MONITORING AND AUDIT

The study will be monitored in accordance with University Hospitals Bristol's Monitoring SOP. All study related documents will be made available on request for monitoring and audit by UHBW, the relevant Research Ethics Committee and for any other regulatory authorities.

PATIENT & PUBLIC INVOLVEMENT

Patient and public involvement will not be formally performed due to the sensitivity surrounding the outcome of the pregnancy. Informal discussions have occurred with patients affected by a foetal cardiac diagnosis of a ventricular imbalance to gain patient perspective on the counselling and the value of scientific evidence to support prognosis.

DATA MANAGEMENT

Data Handling and Protection

The database and randomisation system will be designed to protect patient information in line with the General Data Protection Regulation. Study staff will ensure that the participants' anonymity is maintained through protective and secure handling and storage of patient information at the study centre in line with the Ethics approval. All documents will be stored securely and only accessible by study staff and authorised personnel. Data will be collected and retained in accordance with the General Data Protection Regulation.

Storage of Records

Stored images will be de-identified, and data will be assigned a specific numeric identifier for use in the results database. The external hard drive and the database of results will be locked away securely in the foetal cardiology office at St Michael's Hospital.

All study data will be entered on a password protected database. At the end of the study all the data and images stored will be deleted form the encrypted portable hard drive.

Archiving

Archiving will be authorised by the Sponsor following submission of the end of trial report. Data will be archived by UH Bristol NHS IT in accordance with local policy. Destruction of essential documents will require authorisation from the Sponsor.

FINANCING, INDEMNITY, AND INSURANCE

Financing

The study has been funded by Health Education England via the Higher Scientific Specialist Training (HSST) programme bursary.

Indemnity

This is an NHS-sponsored research study. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

Insurance

Insurance for the study has been arranged by the sponsor (University Hospitals Bristol and Weston NHS Foundation Trust).

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## APPENDIX 7 - Manchester Metropolitan University Feedback from Project Proposal

# DClinSci C1 Unit Marking Sheet

### Examiner and Student

| | |
|---|---|
| **Name of Examiner:** | Dr M Stout |
| **Name of Student:** | Joanne Jones |
| **Title of Project:** | STE for assessing cardiac ventricular imbalance. |

### Examiners Report and Recommendation

**Examiners are asked to provide a short report on the literature review and research proposal.**

Good literature review. Detailed with good use of sub-sections to thoroughly cover all areas relevant to the project.
Care must be taken to not just describe the literature but to critique in a way relevant to the novelty and clinical impact of the study to be undertaken.
Summary tables are a useful inclusion to provide the reader with simple statements as to the main findings of the pertinent research for each area. This facilitates a more comprehensive critique in the free text.

Nevertheless, a well written review overall which narrows nicely toward the focus of the study. Study aims not presented in the review.

Proposal is also well written and includes all relevant detail except the omission of costings (should still be included even if the project does not require funding). E.g. how much is an echo? How much for your time?
Some questions:

1. More detail is needed on the exclusion criteria (for instance I doubt STE can be performed in all scans, could any other physiology alter results – diabetes?)
2. Consideration of sample size and achieving statistical significance. Perhaps should be labelled a 'pilot, feasibility study'?
3. Although mentioned briefly, there needs to be serious consideration to reproducibility of STE in the fetal heart – given it is only used for limited research use at the moment. This should be both intra and inter-observer and there needs to be a detailed protocol for this aspect of data collection to show robustness and clarity.

**Recommendation**

Pass

**Feedback**

## Feed forward

| Please provide short notes on what the student covered well and what could have been improved. This is to help the student in their research project and also to help the academic and clinical supervisors develop additional support for students. |
|---|
| What the student did well: |
| Useful literature review – covers major important studies in field.<br>Clinically relevant study.<br>Novel research using complex echo data. |
| What could have been improved: |
| Critique of current literature field.<br>Additions to the proposal relevant to detail (see above). |