Brain Growth and Development in Fetuses with Congenital Heart Disease

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Declaration of Originality

I herewith certify that the content of this thesis is my own work and that any material that is not my own work has been properly referenced or acknowledged.

Copyright declaration

Abstract

Introduction and Objectives: In the current era of excellent surgical results for congenital heart disease (CHD), focus has become directed on quality of life for these children. Previous studies have shown that neurodevelopmental outcome in CHD is impaired. The mechanisms are incompletely understood but there is increasing evidence that the origins of this are in fetal life. This thesis aims to describe the in utero brain growth in a cohort of fetuses with CHD and relate this to the circulatory abnormalities and fetal Doppler parameters.

Methods: Pregnant women with a fetus with CHD were prospectively recruited. The congenital heart defect was phenotyped using fetal echocardiography and patients subdivided into three physiological groups on the basis of the anticipated abnormality of cerebral blood flow and oxygen delivery: (1) isolated reduced flow to the brain; 2) reduced oxygen saturation of cerebral blood flow; (3) combination of reduced oxygen and flow. Fetal brain MRI was performed. In addition to standard biometric measurements, snapshot to volume reconstruction (SVR) was used to construct a 3D data set from the oversampled raw data. From these 3D volumes the total brain volume and ventricular volumes were measured by manual segmentation. Serial measurements of fetal growth were also made and umbilical artery and middle cerebral artery Doppler parameters were analysed.

Results: 29 women were included; comparison was made with 83 normal MRI controls. Fetuses with CHD were found to have smaller brain volumes compared to controls when adjusting for advancing gestation ($p<0.01$). This difference becomes more pronounced with advancing gestation, suggesting a slower rate of in utero brain growth. Measurements of growth found that the fetuses with CHD were smaller

throughout gestation with a highly significant difference at the later growth scan. ($p<0.001$). Cerebral and umbilical artery Doppler data showed evidence of reduced cerebrovascular resistance in fetuses with CHD but did not show a difference in the umbilical artery Doppler.

Conclusion: Fetuses with CHD have evidence of impaired brain growth with advancing pregnancy and an increased rate of overall growth restriction. Doppler evidence of cerebral vasodilation supports the mechanism of reduced oxygen delivery as an underlying cause.

Dedication

To Juan, Leo, Sofia and Natalia

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List of Abbreviations

AC	Abdominal circumference
ALARA	As low as reasonably achievable
APOE	Apolipoprotein E
ASQ	Ages and stages questionnaire
BAS	Balloon atrial septostomy
BSID	Bayley scales of infant and toddler development
CHD	Congenital heart disease
CoA	Coarctation of the aorta
CO2	Carbon dioxide
CPB	Cardio-pulmonary bypass
CPR	Cerebro-placental ratio
CVO	Combined ventricular output
DPG	Diphosphoglycerate
EEG	Electroencephalogram
FASP	Fetal anomaly screening programme
FGR	Fetal growth restriction
GMDS	Griffiths mental development score
HC	Head Circumference
HLH(S)	Hypoplastic left heart (syndrome)
HbF	Fetal haemoglobin
IVH	Intraventricular haemorrhage
IVC	Inferior vena cava
ISUOG	International society of obstetrics and gynaecology
MRI	Magnetic Resonance Imaging
MCA	Middle cerebral artery
MI	Mechanical index
NT	Nuchal translucency

PI	Pulsatility index
PDI	Psychomotor development index
SAR	Specific absorption rate
SVR	Snapshot volume reconstruction
TGA	Transposition of the great arteries
TOF	Tetralogy of Fallot
TI	Thermal index
UA	Umbilical artery
WMI	White matter injury

Hypothesis:

Brain growth and development is altered in fetuses with congenital heart disease with restricted cardiac output and/or oxygen delivery.

Aim of Thesis:

This study aims to describe the growth of and blood flow to the brain in a cohort of fetuses with congenital heart disease using MRI, ultrasound and Doppler techniques. By detailed description of the circulatory abnormalities using fetal echocardiography and arterial Doppler, a secondary aim is to examine whether different circulatory patterns in CHD predict different patterns of brain growth.

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Chapter 1: Introduction

1.1 Congenital heart disease in the fetus

Congenital heart disease (CHD) is the most common single structural birth defect, with an incidence of 6-8/1000 live births and a higher incidence if all minor abnormalities are included [1]. Over the past 30 years significant improvements in the diagnosis, surgical and perioperative management of these patients has meant that the majority of children now survive to adulthood. With the improvements in long-term survival, there is now an increased focus on the quality of life[2] for surviving children, in particular the long-term neurodevelopmental outcome [3]. Research into the neurological outcome in these patients has shown high levels of behavioural problems and academic difficulties at school and it is a subject of ongoing research how neurodevelopment in these children should be evaluated and managed[4]. Neuroimaging in children with CHD has found a high prevalence of white matter injury, even before the children undergo cardiac intervention. As a result of this, the focus of research has shifted to look at the in utero environment of fetuses with congenital heart disease to establish the aetiology and timing of these insults, with the ultimate goal of identifying targets for therapeutic intervention that would improve the neurodevelopment and quality of life for this population of children.

1.2 Prenatal screening for congenital heart disease

The ability to image the fetal heart by ultrasound has been greatly developed and refined since its introduction over 30 years ago. Although it is well recognised that

there are some maternal and fetal risk factors that increase the risk of congenital heart disease (see table 1), and which are indications for referral to fetal cardiology, the majority of cases will arise in the low risk population. The UK Fetal anomaly screening programme (FASP) aims to detect these cases in the 18-20 week anomaly scan and from this point the patient would be referred on to a tertiary fetal cardiac unit to accurately define the diagnosis and counsel the patient accordingly as to the future management and prognosis of the condition.

Table 1 – Summary of risk factors for congenital heart disease

Parental	Previous pregnancy with CHD or first degree relative (of fetus) 3.2% in any form with first degree relative affected [5], Some variation with lesion in particular left heart obstructive lesions [6, 7] Lower transmission risk for fathers with CHD Parental consanguinity (2-3 fold increase[8])
Exposure to teratogens (relative risk in brackets)	Anticonvulsants (4.2), Ibuprofen (1.86), sulfasalazine (3.4), Retinoids Many other exposures have been studied but with insufficient evidence to determine risk of CHD. Data taken from American Heart Association scientific statement on non inherited risk factors for CHD[9]
Metabolic	Pregestational Diabetes (5x risk) Phenylketonuria (>6)
Fetal	Raised nuchal translucency at first trimester screening Extracardiac abnormality

1.2.1 Evolution of fetal cardiac screening

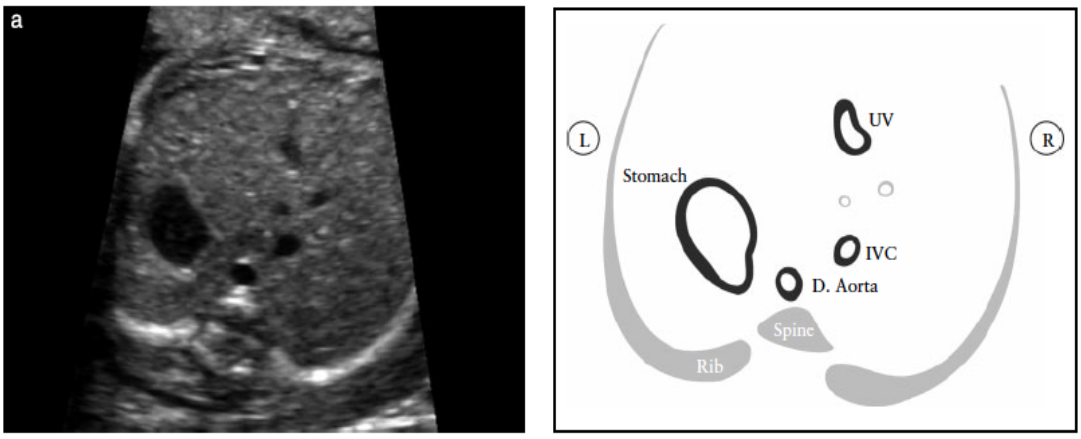
Since the early 1980's it has been possible to visualise the 4 chambers of the heart on fetal ultrasound, with the introduction in France of screening of the 4 chambers of the heart as part of the anatomical survey of the fetus in 1985. Screening of the fetal heart was introduced into the UK screening programme in the mid 1990's and involved identification of a normal appearance of the 4-chamber view of the chambers of the heart [10]. In this early era, the prenatal detection of CHD ranged between 0 and 71%, depending on the local training programmes.

Alongside targeted training of the sonographers who perform the examinations, in 2010 the fetal anomaly screening programme (FASP) guidelines recommended that the views were extended to include examination of the outflow tracts and 3-vessel view (18+0 to 20+6 weeks fetal anomaly scan – National standards and guidance for England 2010. http://fetalanomaly.screening.nhs.uk/standardsandpolicies2010). These changes have lead to an increase in referrals for suspected congenital heart disease in the fetus. The national detection rate for 2014 was 47% compared to 24% in 2003, although the detection rate varies considerably throughout the UK, depending on local training and support (National Institute for Cardiovascular Outcomes Research (NICOR) (www.nicor4.nicor.org.uk)). Practice guidelines were issued from the International Society of Ultrasound in Obstetrics and Gynacology (ISUOG) in 2013 advocating further extension of the views used in screening, to include the 3 vessel and tracheal view [11]. These standard screening views are shown below in figure 1. Along with an active training programme that now runs in

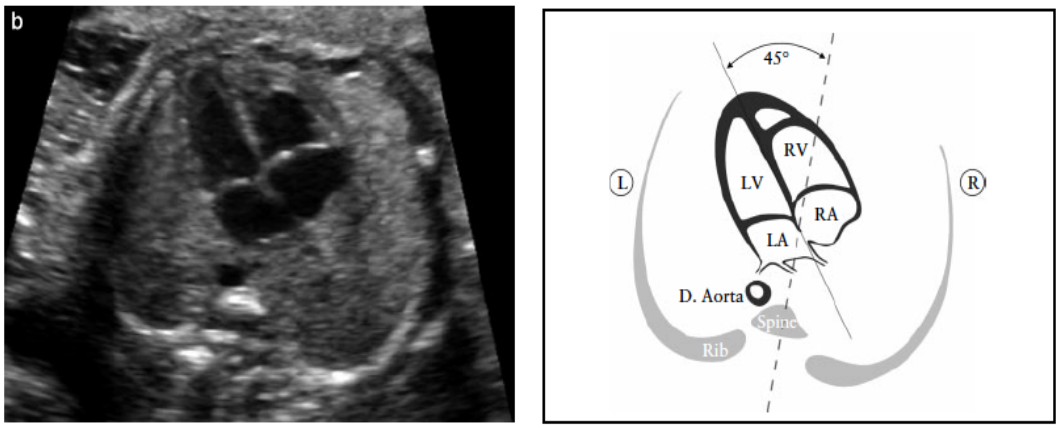
many departments, it is likely that we will see the trend of increased prenatal detection continue nationwide.

Figure 1: Standard Fetal Cardiac Screening views – modified from ISUOG practice guidelines 2013[11]

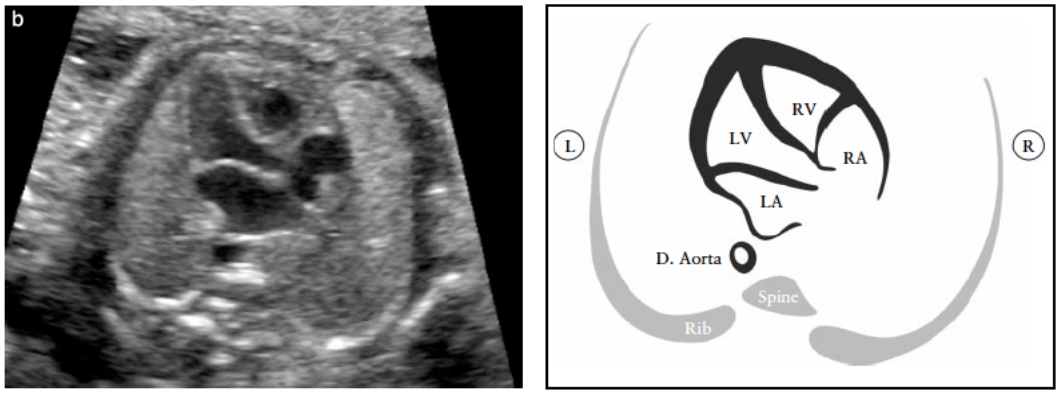
Abdominal situs



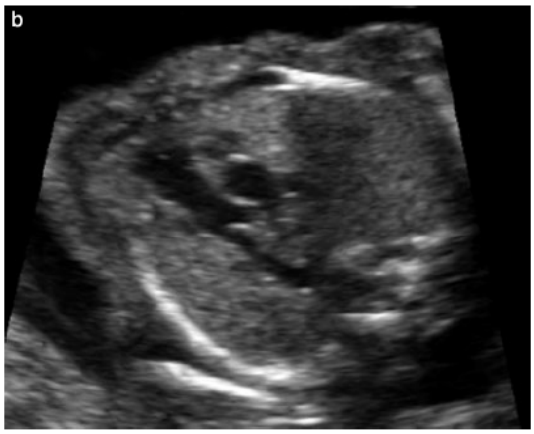
4 Chamber view

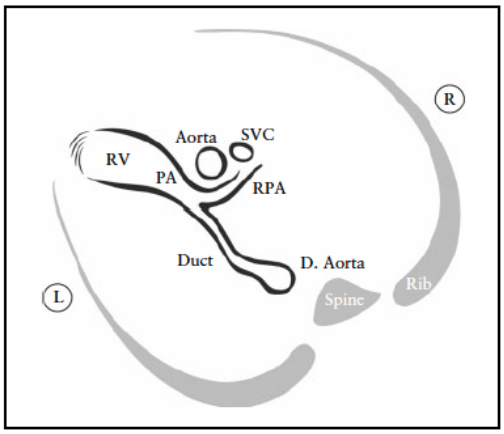


Left ventricular outflow tract

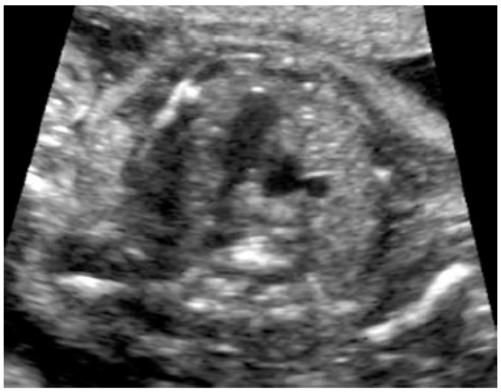


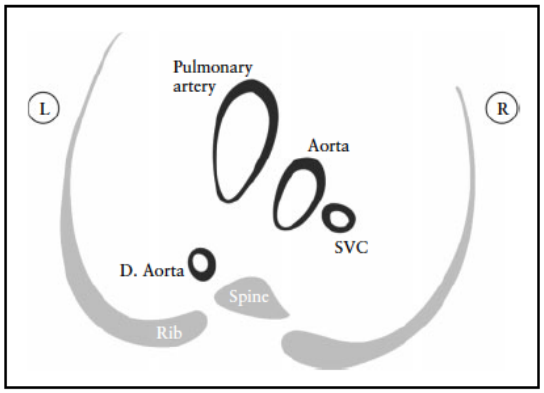
Right ventricular outflow tract



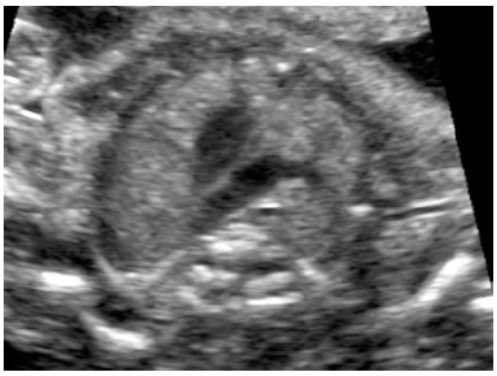


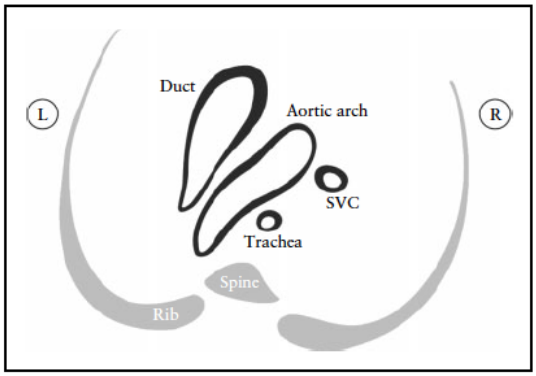
3 Vessel and view





3 Vessel and and tracheal view





1.2.2 The role of prenatal screening for CHD

There are a number of potential benefits of detecting a heart defect prenatally rather than after the child is born. Some cases of major congenital heart disease, in particular those in which the pulmonary or systemic circulation is dependent on patency of the arterial duct, may result in collapse and death of the child in the early days of life. For a number of specific conditions, in particular transposition of the great arteries, an improved morbidity and reduced mortality has been demonstrated for those cases detected before birth[12]. This has also been shown in coarctation of the aorta [13]. In other conditions the impact of prenatal detection of CHD on postnatal morbidity and mortality has not been so clearly demonstrated. Whilst some studies show improved morbidity and mortality [12-14], others have shown improved morbidity but not mortality [15, 16].

Detection of cases antenatally allows a postnatal plan for the child to be made, including the most appropriate site for delivery [17] [18] with the relevant expertise available if early intervention is anticipated.

The second important role is that it allows detailed discussion with the parents about the short and long term prognosis and management of their child.

In some cases parents will choose not to continue with the pregnancy. For families placed in this difficult situation, the quality of life of the child is often a major consideration and the expectation for the child's future neurodevelopment plays an important role in this. It is therefore very important that the counselling is evidence based, particularly when discussing neurodevelopment, as uncertainty in this area has the potential for some parents choosing to terminate a pregnancy which may

generally be considered to have a good outcome. At the current time there is no national consensus on the degree to which these potential problems are discussed.

1.3 The fetal circulation

The fetal circulation differs both anatomically and functionally from the postnatal circulation. Blood is oxygenated in the placenta and is carried to the fetus in the umbilical vein, with umbilical venous blood being around 80% saturated. This vessel separates into the portal sinus and venous duct. The portal sinus joins the portal vein. Although proportions may vary, approximately 50% of the blood volume passes through the venous duct and is directed to the inferior vena cava (IVC), thereby bypassing the liver and avoiding the high oxygen extraction that occurs to the remainder of the blood passing into the portal system of the liver. This oxygenated blood is streamed in the IVC such that when it enters the right atrium it is baffled across the oval fossa towards the left atrium. From the left atrium it passes into the left ventricle and is ejected from the heart to supply the cerebral circulation. In this way, the more oxygenated blood preferentially reaches the cerebral circulation. The lower oxygen venous return preferentially crosses the tricuspid valve and is ejected from the right ventricle. Flow from the right ventricle passes predominantly through the arterial duct to the descending aorta, from where it returns to the placental circulation [19] (see figure 3 and 4).

Figure 2: Illustration of the fetal circulation (www.stanfordchildrens.org)

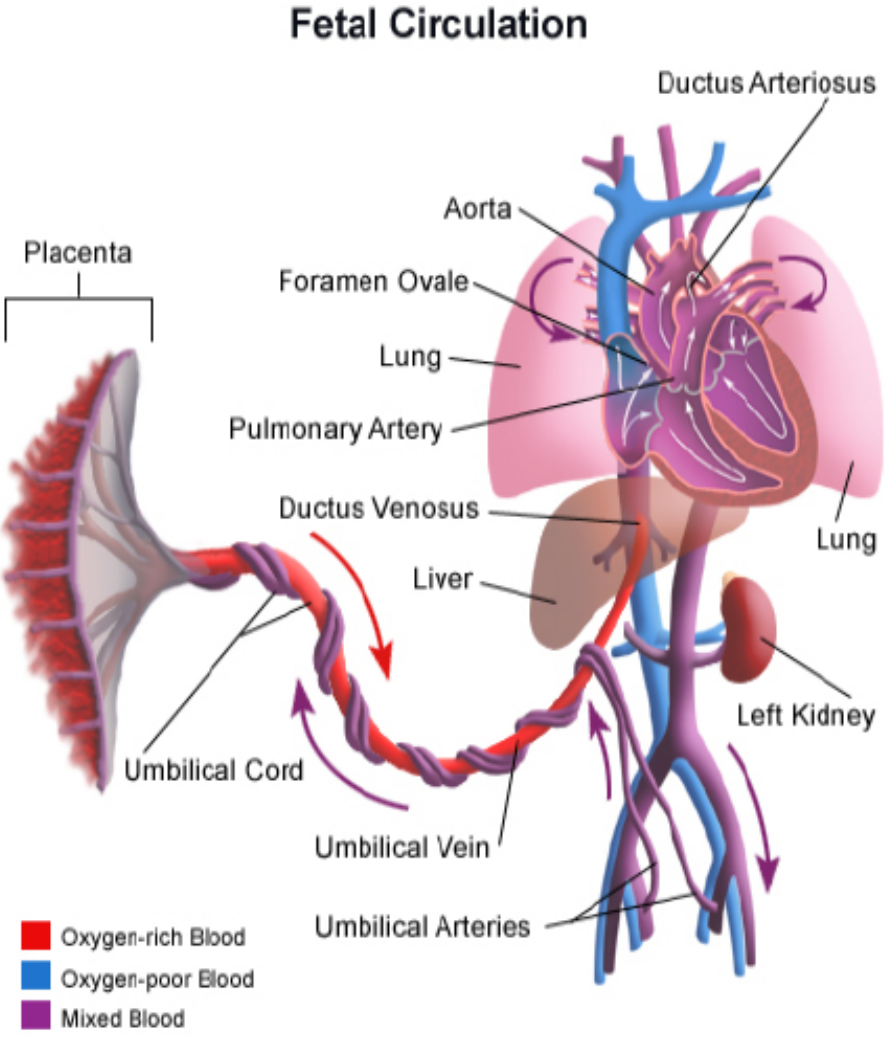
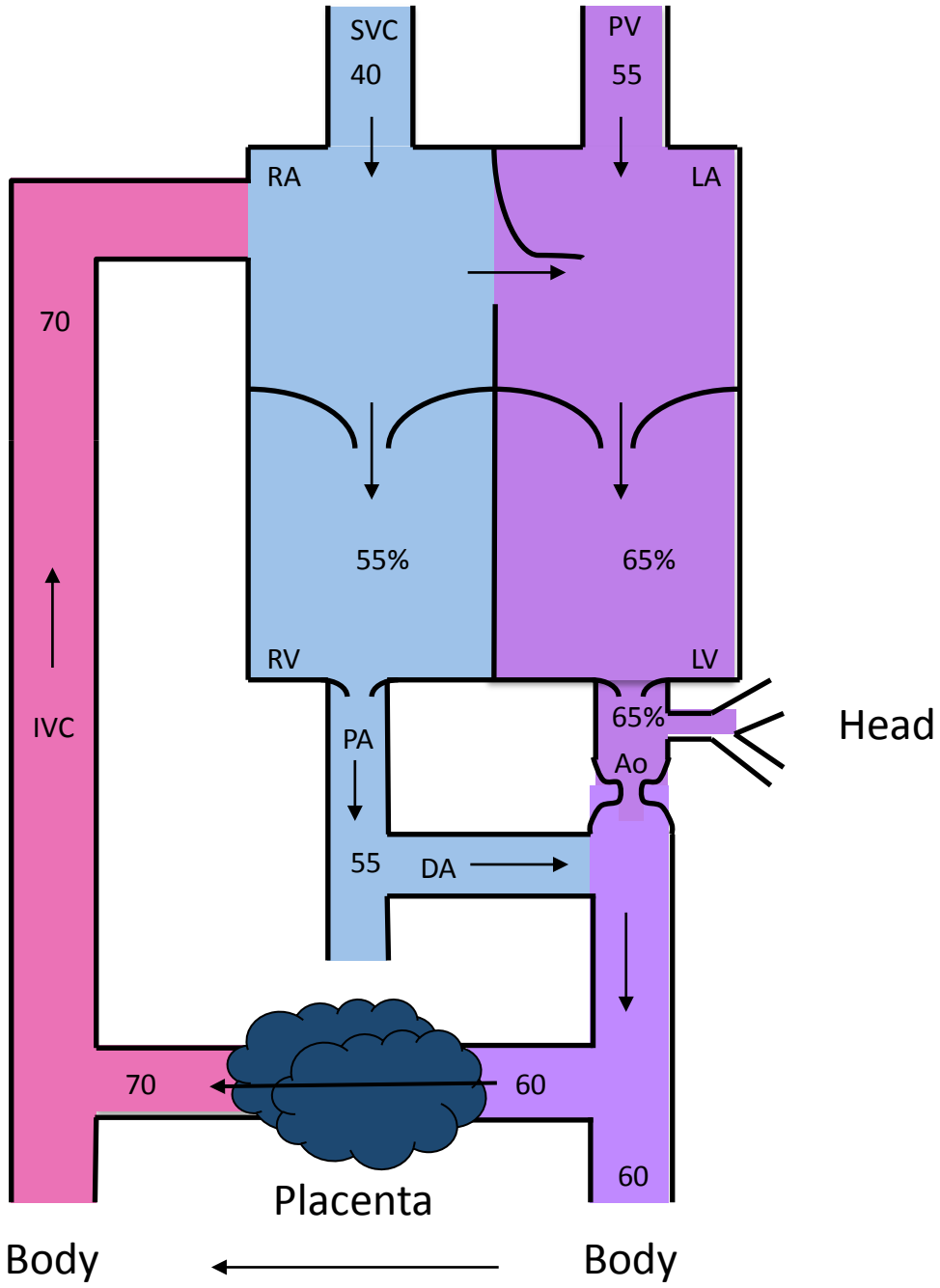


Figure 3 – Fetal circulation in the late gestation lamb

Modified from Rudolph AM: Congenital diseases of the heart. Chicago 1974:1-48.

The numbers indicate the percentage oxygen saturation



1.3.1 Combined ventricular output

The contribution of the left and right heart to the cardiac output in the fetus also differs from the postnatal circulation. In postnatal life, the circulation passes through the left and right heart in series, whereas in the fetus, due to the streaming of blood within the heart and presence of the arterial duct, the ventricles work in parallel. Cardiac output in the fetus is therefore expressed as a combined ventricular output (CVO) rather than that of the left ventricular output postnatally.

1.3.2 Oxygen delivery in the fetus

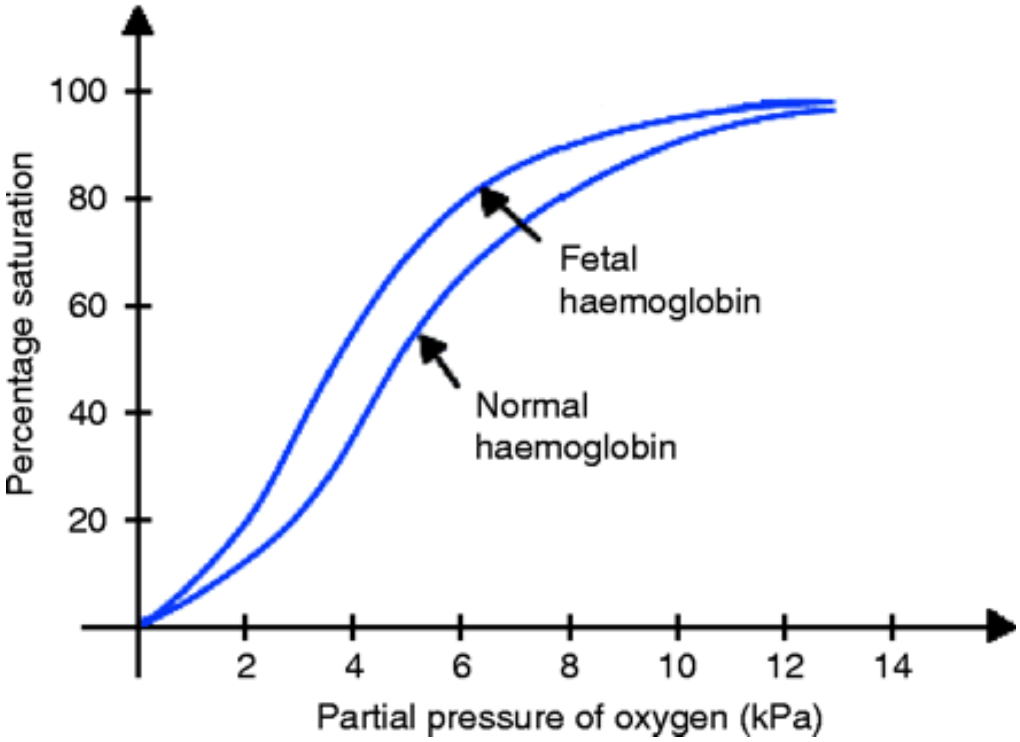
The oxygen delivery to the fetal tissue is affected by both the combined ventricular output and the oxygen content of the fetal blood. The oxygen content of the blood is predominantly dictated by the level of haemoglobin and oxygen saturation of the haemoglobin (with a smaller contribution from dissolved oxygen). The circulation functions to promote oxygen passing from the maternal to fetal circulation and the fetus is specifically adapted to the lower oxygen environment in a number of ways.

The first is that the fetus has a relatively high haemoglobin concentration of around 16g/dL at term. Secondly, there is a high percentage of Fetal Haemoglobin (HbF). Fetal haemoglobin differs structurally from adult haemoglobin in that it has a higher affinity for oxygen. The reason for this is that is binds more weakly to 2,3-diphosphoglycerate (2,3DPG) in red blood cells than adult haemoglobin. This causes a rightward shift for the adult haemoglobin allowing more oxygen to be released to the fetal haemoglobin which is unaffected by the 2,3DPG. As a result of this, oxygen

passes from the mother's circulation to that of the fetus. The oxygen dissociation curves for maternal and fetal cells are illustrated below in figure 4.

Figure 4: Oxygen dissociation curve for adult and fetal haemoglobin:

This shows the percentage saturation of haemoglobin at various partial pressures of oxygen in fetal red cells and maternal red cells.



1.3.3 The Fetal circulation in congenital heart disease

The presence of congenital heart disease may lead to alterations in brain substrate delivery by a number of mechanisms which alter the oxygen content of blood reaching the fetal circulation or the pattern of flow[20].

Due to the streaming of blood in the fetal circulation, whereby the relatively oxygen rich blood from the placenta is directed across the foramen ovale, the left ventricle normally has a higher oxygen saturation of around 65% compared to the right ventricle, which is approximately 50%. The presence of a structural heart abnormality may alter this streaming. Other abnormalities, such as ventricular septal defects, particularly in the presence of outflow tract obstruction that changes the pressure in the ventricles, may result in mixing of oxygen rich and poor blood within the heart, lowering the oxygen concentration of blood delivered to the brain. An example of this is tricuspid atresia, in which the systemic venous return is unable to pass through the right side of the heart. It crosses the foramen ovale, resulting in complete mixing of blood in the left atrium. Due to this mixing of venous return, the blood ejected from the left ventricle will have a lower oxygen concentration than normal.

Secondly, due the variations in anatomy, the amount of the combined ventricular output from a cardiac chamber may be altered with redistribution of flow.

Finally, there may be a combination of altered oxygen concentration and altered flow patterns.

Advances in fetal MRI, with the application of techniques that are widely used in postnatal imaging, such as phased contrast MRI, now offer the potential to study the effect of fetal haemodynamics on brain growth and development. Oxygen saturation and blood flow to the fetal brain can be quantified, providing further evidence for a link between reduced oxygen delivery to the brain and impaired brain growth[21].

The most studied conditions are transposition of the great arteries (TGA) and hypoplastic left heart syndrome (HLH). In TGA, the aorta arises from the right

ventricle and the pulmonary artery from the left ventricle. As shown in the figure 5 below, this results in the less oxygenated blood from the right ventricle (which would normally be preferentially shunted through the ductus arteriosus towards the placenta for reoxygenation) instead being directed to the cerebral circulation. In HLH, due to underdevelopment of the left ventricle, there is mixing of systemic and pulmonary venous return caused by reversed shunting across the foramen ovale. In addition to the lower oxygen content of the blood ejected from the heart, the course of the blood flow is also abnormal due to obstruction to the left ventricular outflow. The cerebral circulation is therefore supplied in a retrograde direction from the duct.

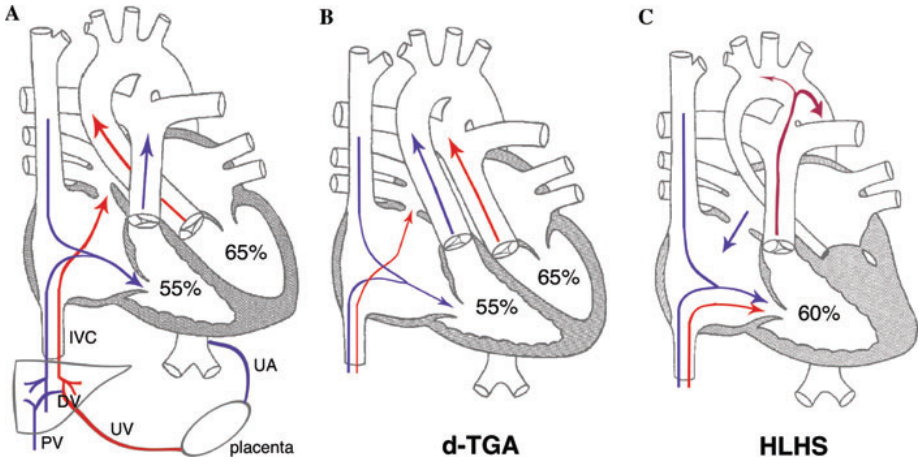
Figure 5 shows the normal fetal circulation and changes with congenital heart disease (McQuillen et al [3])

The course of blood flow is shown in a late gestation fetus with:

(A) Normal heart anatomy

(B) Transposition of the great arteries and

(C) Hypoplastic left heart syndrome due to aortic atresia



Blood flows to and from the placenta are shown only for the normal circulation (A). The course of relatively oxygenated (red) and deoxygenated blood (blue) is shown by arrows. Estimated haemoglobin oxygen saturation in percent is shown for each ventricle

1.4 Growth in congenital heart disease

1.4.1 Prevalence of growth restriction in fetuses with CHD

Early population based studies observed that there was a high prevalence of congenital heart defects in those infants with a low birth weight. Following this, the association between congenital heart disease and low birth weight [22-24] and impaired fetal growth has been well described [22, 24, 25]. Importantly, this increased prevalence of fetal growth restriction in fetuses with CHD occurs in the absence of chromosomal abnormalities[26].

1.4.2 Abnormalities of growth in later childhood and adulthood in congenital heart disease

Abnormalities of growth persist into childhood. Initial failure to thrive as a result of the cardiac abnormality is common, but body size at 2 years has also been shown to be affected by cardiac diagnosis[27]. The longer-term impact into late childhood and adult life is less well studied, but there are some data suggesting that that they remain shorter and lighter than their peers[28].

1.4.3 Theories of origin of growth disturbance

Mechanisms which have been considered in the aetiology of growth restriction in CHD have focused on 2 theories:

Growth disturbance of embryologic origin

The first is that there is an embryologic origin of growth restriction and that those with abnormalities of growth are at increased risk of abnormal cardiogenesis. A number of studies have demonstrated the importance of oxygen levels in fetal programming and normal heart development, both structural and functional. Animal studies have shown that fetal development occurs in a relatively hypoxic environment and that oxygen levels are tightly regulated at various stages of cardiac development, including modelling of the outflow tracts. It is recognised that people living at high altitude are at increased risk of pregnancies with growth restriction. Animal studies have shown that hypoxia can cause abnormal development of the heart, with an increase in ventricular septal defects in hypoxic rats[29].

It is possible that gene expression affecting both fetal growth and fetal heart development are altered by epigenetic mechanisms that are, as yet, not well understood.

Growth disturbance secondary to haemodynamic abnormality

The second and more widely considered theory is that the growth disturbance is secondary to the haemodynamic abnormality that results from the congenital heart defect.

This theory is supported by the findings of Rosenthal et al., who investigated the relationship between different subtypes of CHD categorised on the basis of the circulatory abnormality and the pattern of growth abnormality. The findings of this study were that the different flow abnormalities predicted different patterns of fetal growth. Table 2 summarises the findings of this paper. Four conditions were examined: Hypoplastic left heart (HLH), Transposition of the great arteries (TGA), Tetralogy of Fallot (TOF), and coarctation of the aorta (CoA). Conditions in which the

predicted oxygen content of the cerebral circulation were reduced were associated with smaller head size, those in which oxygen delivery to the body was reduced were associated with reduced somatic growth[30]. Fetal biometry has also shown a reduction in femur length and estimated fetal weight compared to controls [31].

Table 2: Patterns of growth in neonates with CHD in different congenital heart lesions modified from Rosenthal et al.[30]

	Hypoplastic left heart	**Transposition of the great arteries**	**Tetralogy of Fallot**	**Coarctation of the aorta**
Head Circumference	Very Reduced	Reduced	Reduced	Increased
Birth Weight	Reduced	Normal	Reduced	Reduced
Length	Reduced	Normal	Reduced	Reduced

Some insight into this process is gained from comparison with growth restriction in fetuses with placental insufficiency that has been extensively studied. In this situation, as a result of hypoxia, the fetus responds by redirecting blood flow away from peripheral tissue to vital organs. In this way an adaptive response is activated whereby the blood flow to the brain and heart is conserved or increased at the expense of peripheral blood flow. Head growth is therefore maintained relative to the growth of the rest of the fetus, resulting in asymmetrical fetal growth restriction

(FGR), the so-called ‘brain-sparing’ phenomenon. Outcome data suggest that this protective mechanism may be incomplete as there is evidence of reduced brain volumes[32] and neurodevelopmental delay in childhood[33].

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1.4.4 Brain growth in fetuses with CHD

Studies of fetuses with CHD have demonstrated that, similar to placental insufficiency, there is also evidence of the brain-sparing phenomenon. With advances in fetal MRI, it has been possible to quantify brain size and growth as a whole and to look in more detail at individual structures within the brain. It has been found that the volume of intracranial structures are reduced in fetuses with CHD[34].

In the ex-preterm population, the neurodevelopmental consequences of reduced brain growth have been studied: lower brain volume has been correlated with lower cognitive and perceptual function[35, 36]. This has also been examined in children with congenital heart disease, where a number of studies have found evidence of a reduction in brain volumes and a link between lower brain volume and performance in neurodevelopmental testing[37, 38].

1.5 Ultrasound and Doppler

1.5.1 The history of ultrasound imaging

Ultrasound imaging (US) is firmly embedded as the standard screening and monitoring tool in both routine obstetric practice and the management of high risk and complex pregnancies.

The use of US a diagnostic tool evolved from the growing understanding from the work of physicists in the 1800's into the principles of sound vibrations. This work was the basis for the development of underwater sonar and in the early 1900's in the development by Langevin and Chilowsky of the first high frequency ultrasonic echo-sound device known as the hydrophone, which was used in the surveillance of German U-Boats.

Another important development was based on the ideas of a Soviet scientist Sergei Sokolov that lead to the development of ultrasonic metal detectors that were used to detect flaws in the metal structure of ships and tanks. Whilst early methods attempted to look at the energy transmitted across the metal, this was subsequently refined to use a reflection method, comparable with today's ultrasound, whereby the returning echos of short pulses of ultrasound were detected to measure the propagation time.

The first descriptions of the use of ultrasound in medicine date back to the 1920's. Initially, therapeutic uses were explored. It became a neurosurgical tool used in craniotomies and was used for therapeutic destruction of regions of the vestibular nerve in Meniere'e disease and basal ganglia for Parkinson's disease [39].

In the late 40's, its use as a diagnostic imaging modality was researched. Austrian neurologist Karl Dussik explored the use of ultrasound in differentiating soft tissue structures and used this to locate brain tumours and the cerebral ventricles. Following on from this was the work of Professor Ian Donald in Glasgow, in the late 1950's, who pioneered the use of ultrasound as the basis for fetal head measurement, a technique which became the standard for fetal growth measurement for many years [40].

1.5.2 Physics of ultrasound

Ultrasound waves are sound waves with a frequency above 20kHz. This is the highest frequency that can be detected by the human ear. The waves are generated by a piezo electrical crystal as it deforms under an electrical field. The velocity of the sound wave that is generated then depends on the constitution of the medium through which it travels, waves travelling faster through solid mediums and slower in liquid and air. As the ultrasound wave travels through the tissue it undergoes a number of interactions with the medium:

Reflection: This occurs to ultrasound waves at the boundary with a new medium with a different composition and this wave returns to the transducer. The image is formed from the returning waves.

Attenuation: This is the loss of energy from the wave as it travels through the medium due to conversion to heat or reflection and scattering of the beam. More attenuation occurs with higher frequency so that a lower frequency transducer would be used if better penetration of tissue is needed.

Refraction: When the wave encounters a medium through which the wave travels at a different speed there is the change in beam direction known as refraction. The amount of refraction depends on the angle at which the beam hits the interface and the resistance at the interface.

Scattering: Depending on the size, targets either reflect directly back to the transmitter or, in the case of smaller targets, scatter the ultrasound in multiple directions. Targets that are small relative to the transmitted ultrasound wavelength result in scatter.

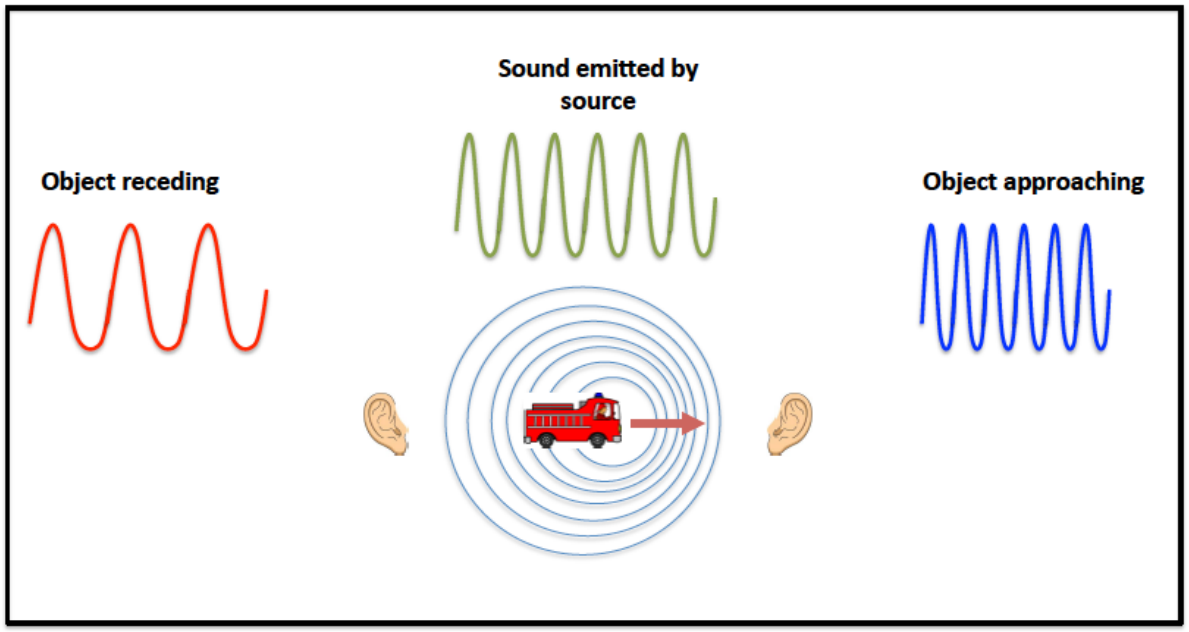
For two structures to be distinguished then at least one wavelength needs to separate them, so higher frequency transducers provide better spatial resolution but at the expense of tissue penetration.

The reflected echoes generated by each pulse from the crystal return to the probe at time intervals which reflect the depths of structures that have reflected the beam. The signal is then processed and converted into an image. The time is along the x-axis and the line of information on the vertical axis. The grey scale displayed for each tissue is determined by the intensity of the returning echos; this is the basis for the standard B-Mode (Brightness modulation) form of ultrasound imaging.

1.5.3 The Doppler shift effect

The Doppler shift effect was first described in 1842 by Christian Doppler. It describes the change in frequency of a wave for an observer moving relative to the source of the waves. This is most clearly demonstrated by observing the change in frequency of a siren as it approaches and passes, as illustrated in figure 6 below. The frequency is higher than the emitted frequency on the approach, identical at the point of passing and lower than the emitted frequency as the siren moves away from the observer.

Figure 6 – Illustration of the Doppler shift effect



This effect can be exploited in clinical ultrasound by measuring and detecting the interaction of the ultrasound beam with the moving red blood cells within a vessel.

The Doppler shift that occurs depends on:

1. The velocity and the direction of the blood flow

2. The angle between the beam and the flow (angle of insonation)

3. The velocity of sound within the tissues

This relationship is expressed in the Doppler formula as follows:

$\Delta f = (2fov \cos\theta)/c$

ΔF is the observed shift in frequency, Fo is the transmitted frequency, V is the velocity of the flow of blood and c is the velocity of sound in human tissue (1540m/s).

The shifts in frequency can be detected as audible signals that are then processed and displayed graphically as a spectrum. These are then converted by a mathematical method (fast Fourier transformation) into a spectral display with time on the horizontal axis and velocity on the vertical axis. Signals moving towards the transducer are displayed above the baseline and those away below the baseline. Once the Doppler shift is known, the equation can be rearranged so that the velocity can be calculated. This can be used to produce an accurate assessment of the direction of blood flow and the velocity of blood flow at a given point.

1.5.4 Safety of ultrasound

Ultrasound has been widely used in obstetrics for over 20 years and the use of B mode and M mode, in particular, are widely considered to be safe across the range of gestation due to the low energy output of these modalities. Doppler methods have an increased potential for biological effects due to the higher energy output but are considered safe when used by trained health professionals adhering to safety guidelines.

There are two mechanisms by which there is the potential for ultrasound to induce bioeffects:

Heating the tissues: The potential for ultrasound to heat tissue is well established and factors which could contribute to an increased risk of a biological effect have been studied[41]. The amount of heating of a tissue depends on the amount of energy that the tissue absorbs. The absorption of energy in bone is many times that of soft tissue and therefore bone, and the soft tissue at the interface with bone, is at

the highest risk of heating and heat induced damage [42]. In the fetus this risk increases from the second trimester as the bones become ossified. The temperature rise is also enhanced when using colour Doppler, which uses higher energy levels and with a stationary beam focused on a small region of interest.

Cavitation: Ultrasound acts on pre-existing gas nuclei, or microbubbles, in the tissue to produce small pockets of gas in body fluids or tissues. The ultrasound wave can cause expansion of the bubble to a critical threshold where it can no longer maintain its expansion. At this point surrounding fluid rushes into the bubble, causing rapid collapse. A sudden burst of heat and pressure occurs in a restricted space as the energy content of the bubble is released, with potential damage to surrounding tissue.

The vast majority of epidemiological studies have shown antenatal ultrasound to be safe[43, 44]. There are, however, a few reports of thermally induced teratogenesis in animal studies and a small number of reports on limited showing effects on humans including growth restriction[45], delayed speech[46] and an increase in left handedness in [47]. Effects appear to be enhanced in hyperthermic conditions raising the possibility of an increased risk in febrile patients[48]

The energy output of ultrasound machines is displayed with two indices[49]:

The thermal index (TI) - this is the potential for rise in temperature at the focal point of the ultrasound; and the mechanical index (MI) - this is a measure of the potential to cause cavitation.

When performing Doppler imaging, the displayed thermal index should be ≤ 1 and exposure time kept as short as possible required to gain the information needed, following the ALARA (As Low As Reasonably Achievable) principle[50]. This would usually be no longer than 5-10 minutes and <1 hour[51]. Guidelines are available from the British Medical Ultrasound Society and American Institute of Ultrasound in Medicine.

1.6 Cerebral blood flow

1.6.1 Auto regulation of cerebral blood flow

In the normal fetus, cerebral blood flow is regulated to maintain adequate oxygen and substrate delivery. Metabolic, neural and chemical mediators act to vasodilate or vasoconstrict cerebral vessels, thereby controlling flow and delivery of oxygen and glucose.

With advancing gestational age, a fall is seen in cerebral vascular resistance, allowing an increase in blood flow to meet the increasing metabolic demands of the developing brain[52, 53].

1.6.2 Cerebral blood flow adaptation in fetal growth restriction

This autoregulatory process has been extensively studied in fetuses with placental dysfunction and fetal growth restriction (FGR) who demonstrate altered cerebrovascular resistance with vasodilation of the cerebrovascular bed [54]. This vasodilation results in an increase in forward flow to the brain by reducing

downstream resistance. This auto regulation of cerebral blood flow, known as brain sparing, improves [55] cerebral perfusion by redistributing blood flow to the brain at the expense of other organs that have a lower metabolic requirement[56]

1.6.3 Middle cerebral artery (MCA) Doppler

Brain sparing is detected and monitored in obstetric practice by measurement of middle cerebral artery (MCA) flow. As the cerebral vasculature dilates in response to hypoxia, there is a reduction in downstream resistance, seen on Doppler as a reduction in the pulsatility index. The pulsatility index from the MCA is calculated as the peak systolic velocity-end diastolic velocity / time averaged velocity [57]. Comparison can be made with normal reference ranges [58-60]. Examples of MCA Doppler are shown in figure 7a and b.

1.6.4 Umbilical artery Doppler and Cerebro placental ratio

Within the umbilical cord there are 2 arteries and one vein linking the fetus and placenta. The umbilical artery carries blood from the fetus back to the placenta, where oxygenation occurs in the placental vascular bed. The flow in the umbilical artery is dependent on both the fetal cardiac output and on the placental vascular resistance. Doppler of this vessel can therefore be used to gain important information about the resistance downstream in the placental circulation. It is widely used for this purpose in obstetric practice to identifying and monitor fetuses with growth restriction secondary to placental pathology[53]. UA flow can be quantified by calculation of the pulsatility index (peak systolic velocity- end diastolic velocity/ time averaged mean velocity) and provides a useful estimation of placental resistance. In

fetuses with placental pathology, an elevated resistance is seen within the UA, which is measured as a reduction in the pulsatility index on Pulsed wave Doppler. A progressive pattern of abnormality is seen in fetuses with growth restriction, with an initial reduction in diastolic flow followed by absent and then reversed end diastolic flow.

A ratio comparing the MCA-PI and UA-PI reflects the relative resistance of the cerebral and placental vascular beds and can be used to quantify redistribution of cardiac output to the cerebral circulation. This is known as the cerebro-placental (or cerebro-umbilical) ratio (CPR) [61-65].

Figure 7: Middle cerebral artery Doppler showing reduced pulsatility index < 5th centile (7a) and normal pulsatility index (7b)

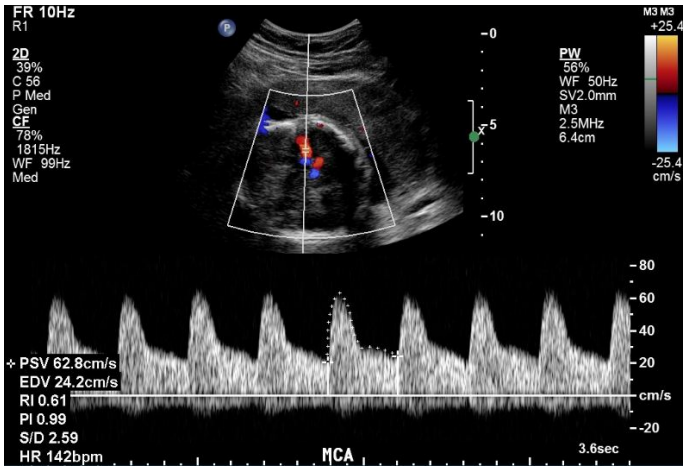


Figure 7a

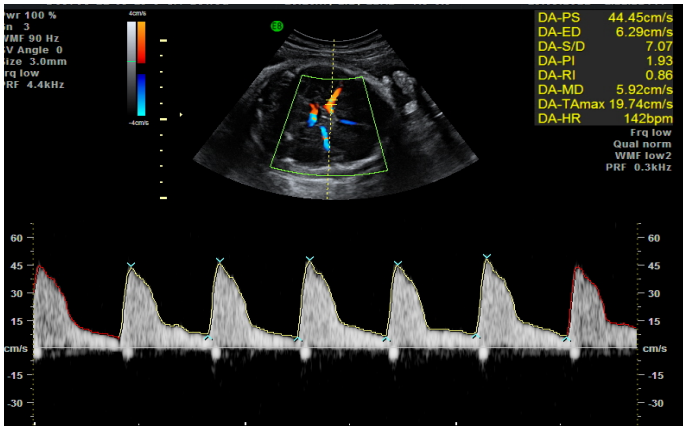


Figure 7b

1.6.5 Cerebral blood flow in fetuses with CHD

In fetuses with congenital heart disease, in addition to the lower oxygen content of the blood delivered to the cerebral circulation that may result from intracardiac mixing of oxygenated and deoxygenated blood alterations, there may also be alternations in the normal patterns of blood flow.

This may be the result of interference of the normal streaming (as described above), whereby the more oxygenated blood returning from the placenta is directed to the brain but, added to this, the presence of outflow tract obstruction may result in reduced pulsatility and volume of flow delivered to the brain. The most extreme end of the spectrum is aortic atresia, where the aortic valve is completely obstructed. In this situation flow reaches the cerebral vessels in a retrograde manner by reversal of flow through the arterial duct.

A number of studies have examined Doppler indices of cerebral blood flow in fetuses with congenital heart disease. Donofrio et al. hypothesised that cerebral auto regulation occurred in fetuses with CHD. They measured the cerebral: placental resistance ratio (cerebral/umbilical resistance ratio which is normally >1) and found that cerebral resistance was reduced in CHD, but this differed across different types of CHD, being particularly severe in HLH[66]. Berg et al. measured the pulsatility and resistance indices of the middle cerebral artery and the cerebral placental resistance ratio in fetuses with congenital heart disease and found that, in fetuses with HLH, the MCA pulsatility and resistance index was significantly lower compared to controls, but that this was not the case in the other subtypes of congenital heart disease that they studied[67]. Kaltman et al. examined 28 fetuses with HLH, 13 fetuses with left heart obstruction and 14 with right heart obstruction. Those with

HLH had lower middle cerebral artery pulsatility indices and those with right heart obstruction had higher pulsatility indices compared to normal controls. Those with left heart obstruction without HLH did not differ significantly from normal controls[68]. The findings suggest active auto regulation of cerebral blood flow in HLH, shown by a decrease in cerebral vascular resistance as a compensatory mechanism for the reduced oxygen delivery to the brain in this condition.

In addition, in fetuses with left heart obstruction and reversed arch flow, there was a positive correlation between MCA-PI and head growth[69].

Szwast et al. compared MCA pulsatility in fetuses with single ventricle anatomy on those with aortic obstruction and those with pulmonary obstruction and found the MCA pulsatility index was decreased in those with aortic obstruction and increased in those with pulmonary obstruction[70].

Masoller et al. found that MCA Doppler and CPR<1 were independent predictors of abnormal brain development at birth[71]

More recently, the association between middle cerebral artery Doppler and neurodevelopmental outcome has been studied. In a multivariate analysis of a cohort of 72 fetuses with single ventricle lesions, the findings were that lower cerebrovascular resistance (measured by MCA pulsatility index *Z*-score) was associated with higher neurodevelopmental scores at 14 months, supporting the theory that there is some physiological compensation for the cardiac abnormality[72].

1.7 Neurodevelopmental outcome in children with congenital heart disease

There is a now a compelling body of evidence that children with congenital heart disease have impaired neurodevelopment[73, 74]. The aetiology of this impairment is incompletely understood and likely to be multifactorial.

1.7.1 Deficits observed in children with CHD

The nature of the neurodevelopmental impairment observed in children with CHD crosses a broad range of function. This includes speech and language, impairment of visual- spatial and visual-motor skills, attention deficit hyperactivity disorder, motor delay and learning difficulties[75]. A significant proportion of children will require remedial help, including tutoring, special education, physiotherapy, occupational and speech therapy [75].

Recognition of the importance of not simply survival but quality of life has taken centre stage over the past few years [76, 77]. Although quality of life is a complex subjective measure, it is clear that, along with physical status, the neurodevelopmental outcome and psychosocial function are key components of this.

Impairments in executive function, which is a combination of skills that help an individual achieve what they set out to achieve, are prominent in children and young adults with CHD[78, 79] and can have a major impact on the quality of life of individuals.

Social cognition may also be impaired [80]. Characteristically, the features are of difficulty understanding the reactions of others and this can affect the social development of these children and integration into their peer group. Moving towards adult life, these difficulties may be contributory factors in the increased rates of anxiety and depression seen in this group in later years. [81]

1.7.2 Factors that influence Neurodevelopmental outcome

1. Birth weight and gestation

Preoperative clinical condition and postoperative care play an important role in early postoperative neurological events. These include lower Apgar scores; low birth weight and gestational age; and abnormal brain imaging prior to surgery[82, 83]. A recent study by Goff et al. found that infants born at 39 and 40 weeks had improved performance for cognition, language, executive function, social skills visual and fine motor skills compared with those born “near” term (36-39 weeks)[84].

2. Genetic Factors

Although it is clear that children may acquire neurological injury at the time of cardiac catheter or surgery, this does not provide a full explanation. A study by Limperopoulos et al. found that 50% of infants with CHD show neurological abnormalities such as poor suck, hypotonia and jitteriness, prior to cardiac surgery[85]. This suggests other factors are involved in this process. The explanation for this may be in part due to common genetic factors involved in both brain and heart development. In one study of neurodevelopmental outcome in CHD[86], in which patients with identified chromosomal abnormalities or genetic syndromes at

birth were excluded, further examination by a medical geneticist at 1 year of age revealed clinical evidence of a genetic syndrome or abnormal genetic tests in 28%, suggesting that many more of these children may have genetic abnormalities than previously thought. With more widespread use prenatally of comparative genomic hybridisation microarray (CGH microarray), which is a cytogenetic technique that is able to detect variations in copy number in the genome, more of these abnormalities are likely to be picked up in pregnancy or early childhood.

In addition, there may be factors that alter an individual's susceptibility to neurological insult. For example, the apolipoprotein E (APOE) genotype appears to be an important predictor of impaired neurodevelopment. It may be that apoE protein (which is involved in low density lipoprotein (LDL) receptors) may be important in maintaining the integrity of neurons and, therefore, different alleles may confer increased resilience to neurological insults.

3. Subtype of congenital heart defect

At the most severe end of the spectrum is the neurodevelopmental impairment seen in HLH. In early studies from the mid 1990's, major disability was seen in approximately 60% of survivors[87, 88]. Some improvements were shown in studies a decade later[89], with a study from Gaynor et al. in 2008 reporting 48% of infants with HLH had a psychomotor development index (PDI) <70[83]. Further data published by the same group in 2010 again showed a further reduction, with significant impairments identified in 18% of patients with HLH. Even in the current era, with the improvements in cardiopulmonary bypass and postoperative care, the Single Ventricle Reconstruction Trial reported a high prevalence of neurodevelopmental abnormalities of nearly 50% at 14 months[90]. Furthermore,

there is evidence that this persists throughout childhood, with Davidson et al. reporting educational concerns in 41% of patients with HLH surviving beyond 10 years[91].

In studies not restricted to particular subgroups of congenital heart disease, lesions in which palliative surgery is performed in the first year of life were associated with a worse neurodevelopmental outcome at 1 year of age[92]. There may be some improvement in neurodevelopment[93, 94], however, it is clear that in many children, in the medium and long term, deficits persist. In a cohort of children who had had either repair of Tetralogy of Fallot or closure of ventricular septal defect, rates of mild neurological dysfunction were increased compared to normal children. This included motor function, IQ, academic achievement and expressive and receptive language[95].

4. Perioperative Seizures

Factors relating to neurological outcome in some of these studies have found significant neuromorbidity in the postoperative period, including seizures and abnormalities of tone and alertness[82, 96]. The Boston Circulatory Arrest Trial detected perioperative seizures on EEG monitoring in 20% of children, although only 1/3 of these were manifest clinically. The presence of seizures was significantly associated with a reduction in mean PDI scores on testing at 1 year of age and an increased risk of late imaging abnormalities. When the cohort were examined at 16 years of age, perioperative seizures were the variable that was most consistently related to worse neuropsychological and neuroimaging outcomes. These findings have been confirmed in other studies.

5. Surgical and cardiopulmonary bypass techniques

A number of studies have focused on the operative management of this group of children [82, 96-99]. Increased number of surgical procedures has been shown be associated with poorer neurodevelopmental outcomes[100]. Studies which have performed neuroimaging before and after intervention have shown a high incidence of new lesions after cardiac surgery of up to 67% [82, 101]. One of the largest studies – The Boston Circulatory Arrest Trial, looked at a cohort of babies with transposition of the great arteries undergoing the arterial switch operation. It prospectively randomised babies to receive either deep hypothermia with total circulatory arrest or deep hypothermia with low flow bypass at the time of their operation. This study showed that circulatory arrest was associated with decreased psychomotor development scores and increased risk of neurological abnormalities[102]. The neurodevelopment of this cohort has been assessed at serial intervals, most recently at 16 years of age. This has shown that both strategies result in lower scores in IQ, expressive language, visual-motor integration, motor function and oromotor control. The circulatory arrest group had a higher degree of functional deficits than the low flow bypass group, suggesting a beneficial effect on cerebral metabolism of maintaining cerebral blood flow.

5. Social factors

Social factors may also impact significantly in the neurodevelopmental outcome, as illustrated by the finding that maternal education was associated with better neurodevelopmental outcome in a cohort of Australian children following surgery for CHD[103]. This cohort also suggested that language may be improved by intervention that targets social and emotional competence.

1.7.3 Detection of neurodevelopmental impairment and intervention

Although the prevalence of neurodevelopmental abnormalities has been found to be high, as impairments may be at the less severe end of the spectrum, problems may go undetected without targeted screening. In 2012 the American Heart Association released a scientific statement on the evaluation and management of neurodevelopment in children with CHD[4]. This aims to identify patients that are at high risk of neurodevelopmental problems (summarised in table 3 below). According to the level of risk, patients are stratified to whether they have routine surveillance or periodic neurodevelopmental assessment to allow early intervention if problems are identified.

Table 3 shows categories of patients at high risk of neurodevelopmental problems [4].

1.	**Neonates or infants requiring open heart surgery**
2.	**Children with other cyanotic lesions not requiring neonatal surgery**
3.	**CHD in combination with any of the following:** Prematurity Developmental delay recognised in early infancy Suspected genetic abnormality History of mechanical support Heart transplantation CPR Prolonged length of hospitalisation>2wks Perioperative seizures Neuroimaging abnormality or microcephaly
4	**Other condition at the discretion of healthcare provider**

1.7.3 Neurodevelopmental assessment

Increasingly it is recognised that we should be attempting to identify neurodevelopmental problems at an earlier stage, to allow the opportunity for therapeutic intervention. There is a wide range of validated tests that can be used to assess neurodevelopment and identify problems in specific domains. Two of the most commonly used methods of assessment in infants are toddlers are those that were used in this study - the Bayley Scales of infant and toddler development (BSIDIII) and the Griffiths Mental Development Scales (GMDS 0-2), which are described in the methods section.

1.8 MRI abnormalities in congenital heart disease

1.8.1 Structural brain abnormalities in CHD

There is a high incidence of structural brain abnormalities in patients with CHD[104-107]. In Hypoplastic left heart syndrome, a post-mortem study identified major or minor central nervous system abnormalities in 29%. This included agenesis of the corpus callosum and holoprosencephaly. Microcephaly was seen in 27% and an immature cortical mantle in 21%[104].

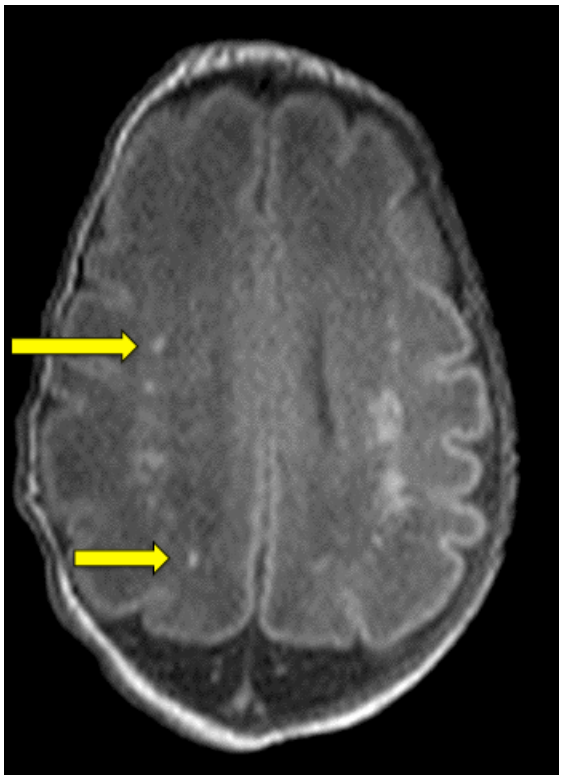
Two recent studies of fetuses with CHD found that brain abnormalities were present in 23% and 39% of cases of CHD respectively[107, 108]. Brossard-Racine's findings did not show any significant association with the cardiac physiology, suggesting that these abnormalities are not limited to those with more severe CHD. Although cases with extracardiac abnormalities identified on fetal ultrasound, congenital infection or documented chromosomal abnormalities were excluded, it is recognised that not all

abnormalities can be excluded antenatally and there is variation in different settings in how extensive genetic investigations are.

1.8.2 Stroke and White matter injury in CHD

Neuroimaging studies have demonstrated a high incidence of clinically silent lesions that have been acquired prior to, or at the time of cardiac surgery. Some studies show a predominance of stroke and others white matter injury as the main pathology. In part this may reflect a differing classification of lesions. Stroke can be defined as ‘a focal area of diffusion restriction in an arterial territory’ and white matter injury as’ punctate periventricular lesions associated with T1 hyper intensity with or without restriction of water diffusion’[109]. The terminology of lesions in the published literature is, however, often confused as stroke also represents an injury to the white matter. An alternative distinction would be between arterial and non-arterial lesions, although this may leave some lesions difficult to classify, including strokes secondary to venous infarction. In addition, punctate lesions may be venous or secondary to vasculitis.

Figure 8 shows an example of the appearance of white matter lesions in a premature infant.



Magnetic resonance spectroscopy, which gives in vivo insight into brain metabolism, has also been examined in many of these studies, which have suggested that these patients demonstrate altered brain metabolism.

The first study to use MRI to examine preoperative brain injury in babies with CHD was performed by Mahle et al. in 2002[110]. This study identified that 25% of patients had evidence of white matter injury or stroke prior to cardiac surgery. Following surgery, lesions were present in over 50% of cases. This high incidence of preoperative brain injury, in addition to that seen postoperatively, has been

confirmed in a number of other subsequent studies [111-115]. The findings of these studies are summarised in table 4.

Markers of brain maturation were investigated in one study by Childs et al. They devised a maturational score for assessing the cortex, germinal matrix, white matter and myelin and suggested there was a one month delay in the maturation markers of brains of babies with congenital heart disease compared to normal controls[116].

In infants with transposition of the great arteries a significant proportion of infants will require an initial palliative procedure (balloon atrial septostomy - BAS) to improve mixing of oxygenated and deoxygenated blood prior to corrective surgery. There has been some controversy as to whether this procedure increases the incidence of brain injury demonstrated on MRI scan. This has been investigated by a number of centres with conflicting results. A study of 29 infants with TGA by McQuillen et al. in 2006 found that BAS was an independent predictor of brain injury. However, another report in 2009 by Beca et al. in New Zealand, which included 64 infants with TGA, found a preoperative brain injury in 30% of fetuses but no relationship with balloon atrial septostomy[113].

Preoperative factors that have been shown to influence the pattern of acquired brain injury include the oxygen saturation and time to surgery[117]. Other potential factors include the management of delivery, including the site of delivery, induction of labour and mode of delivery, although these have not yet been fully investigated. Risk factors for injury acquired at the time of surgery include CPB with regional cerebral perfusion and lower intra operative cerebral oxygen saturation. Post-operative white matter injury has been associated with a low mean blood pressure during the first post-operative day[101].

Block et al. looked at existing injuries to examine the impact of cardiac surgery on these and found that pre-existing MRI abnormalities were not exacerbated by cardiac surgery[118]. These findings were supported by a further study of 26 babies with TGA from the USA[117].

One study has looked at cerebral blood flow on MRI in relation to white matter lesions [119]. Pulsed arterial spin labelling was used to quantify cerebral blood flow non-invasively on MRI. The findings were that periventricular leukomalacia was associated with decreased baseline cerebral blood flow and a smaller change with hypercarbia.

The presence of MRI lesions prior to intervention in neonates with CHD has provoked interest in determining the timing of onset of these lesions and ascertaining if abnormalities in the brains of fetuses with congenital heart disease can be detected prenatally. In 2010, Limperopoulos et al. published a series of 55 fetuses with CHD who had undergone fetal brain MRI imaging[120]. Their findings were that some subgroups of CHD, particularly HLH and TGA, and those without forward flow in the transverse aortic arch, had smaller brain volumes and altered brain metabolism compared to controls.

Table 4: Summary of studies that have shown postnatal brain MRI abnormalities before intervention.

Author, Date Journal	Methods	Main findings
Mahle et al., 2002 Circulation	24 infants CHD 13 single vent (8 HLH) Prospective study	Pre-operative MRI: WMI 16%, infarct 8%. On post operative MRI: new or worsening of lesions in 67% (WMI 48%, stroke 19%, haemorrhage 33%) Elevated lactate on MRI spectroscopy
Licht et al., 2004 JTCS	25 widely varying CHD CO2 reactivity	Microcephaly 24% WMI 28% 5 patients CBF< 10ml/100g/min
McQuillen et al., 2006 Circulation	29 infants TGA	41% Brain injury BAS risk factor for pre op brain injury
Miller et al., 2007 NEJM	41infants CHD: 29 TGA,12 single ventricle Prospective study	32% WMI, MRI spectroscopy (lower NAA to choline ratio and raised lactate to choline ratio) Abnormal diffusion tensor imaging
McQuillen et al.,2007 Stroke	62 infants CHD Prospective study	Brain injury 39%, Stroke most common pre operatively, WMI post operatively
Beca et al., 2009 JACC	64 infants CHD TGA 44, HLH 13 PAT 7 Prospective study	Brain injury 30%: (WMI 27%, stroke 5%) No association between BAS and brain injury.
Petit et al,. 2009 Circulation	26 TGA Retrospective study	36% WMI, No stroke Risk factors: lower O2 saturations delay in surgery, No association with BAS
Licht et al., 2009 JTCS	29 HLH, 13 TGA	Mean total maturation score significantly lower than reported normal controls, HC 1SD below normal.
Awate et al., 2009 Neuroimage	Statistical framework for analysis of folding	Subtle differences between folding patterns in HLH and TGA
Block et al., 2010 JTCS	92 infants CHD (62TGA, 30 single ventricle)	Brain injury 40% (stroke 23/WMI 21/IVH7) no progression of lesions with surgery

Shedeed et al., 2011 Paediatric Cardiology	38 CHD	Abnormal MRI spectroscopy in CHD (reduced NAA to choline ratio, elevated lactate to choline ratio) Abnormal diffusion tensor imaging
Raheen et al., 2012 Ann Paed Cardiol	52 infants CHD Subdivided cyanotic and acyanotic	Abnormal MRI spectroscopy in CHD (reduced NAA to choline ratio, elevated lactate to choline ratio)
Ortinau et al., 2012 JTCS	67 infants pre op MRI	42% WMI Regional alterations in growth
Von Rhein et al., 2015 J Paediatr	19 preop CHD patients	Reduction of brain volumes on MRI compared to controls
Nagaraj et al., 2015 J Paediatr[121]	43 CHD newborns 58 controls	Cerebral perfusion using MRI arterial spin labelling significantly lower in newborns with single ventricle
Peyvandiet al., 2016 JAMA Pediatr	152 Newborns CHD	Higher rate of brain injury in those with a postnatal diagnosis
Lim et al., 2016 J Thorac Cardiovasc Surg	32 Newborns CHD 31 Controls	Lower cerebral oxygen delivery in cases 10 cases high white matter signal intensity 2 white matter injury 5 high single and white matter injury
Brossard-Racine et al,. 2016 AJNR	103 fetuses with CHD postnatal MRI pre surgery	Brain abnormalities in 16% fetuses and 32% of neonates Fetal MRI sensitivity for postnatal MRI abnormality 27%, specificity 89%

TGA – transposition of the great arteries, CHD congenital heart disease, CO2 – Carbon dioxide, HLH – hypoplastic left heart, RO I- Region of interest, MRI Magnetic resonance imaging, WMI- white matter injury, PVL –BAS – balloon atrial septostomy, WMI white matter injury, IVH intra ventricular haemorrhage. DTI – diffusion tensor imaging, Diff – Diffusivity, FI – fractional anisotropy

1.9 Fetal MRI

MRI imaging is well established and widely used as a cross sectional imaging modality. It offers excellent contrast resolution and, unlike other imaging modalities, does not involve the use of ionising radiation, making it an attractive option for fetal imaging. Although the mainstay of fetal imaging remains, and is likely to remain, ultrasound, there is increasing use and need for an imaging modality that can offer information beyond that provided by ultrasound examination. Fetal MRI offers improved resolution and soft tissue contrast and a larger field of view. It is less dependent on fetal position and more tolerant of mothers with an increased body mass index. It is superior to ultrasound in the assessment of posterior fossa abnormalities, neuronal migration, gyral formation and myelination[122, 123]. In addition, magnetic resonance spectroscopy provides an insight into brain function that is not possible with ultrasound.

1.9.1 Physics of MRI

Magnetisation

Each atom consists of protons and neutrons in the nucleus and electrons that orbit the nucleus. The positively charged protons within the nucleus spin about their own axis and, as they do so, create a small magnetic field. If there are an even number of protons and neutrons these cancel each other out and there is no net spin. However, if there is an odd number of protons (such as in a hydrogen atom), a magnetic force (moment) is created.

Excitation

Protons in living tissue are able to accept electromagnetic energy given as a radiofrequency pulse. This results in excitation of the protons to a higher energy level. When this energy is released, it creates a small energy signal that becomes lost within the ‘noise’ created by the surrounding protons. However, when protons are placed in a magnetic field, they align with the magnetic field or at 180 degrees to it, with a slight predominance of protons aligning. This creates a net magnetic field that can be manipulated. The protons can then be excited by a radiofrequency pulse and the signal generated by relaxation detected.

Precession

Each proton spins (precesses) around its own axis. If a radiofrequency pulse is applied, these protons all precess in the same way and in this way a much stronger signal can be generated

Relaxation

From this high energy state of excitation with the protons aligned with the magnetic field and spinning in unison, the protons undergo two types of relaxation:

T1 is a time constant that relates to the time taken for the longitudinal magnetization to go back to its original value.

T2 Is the time constant for the transversal (or spin spin) relaxation

Image acquisition

The excess energy that is released as the protons return to the state of equilibrium is detected by the receiver coil placed at 90 degrees to the magnetic field.

1.9.2 Challenges of Fetal MRI

The most significant challenge of fetal MRI is to gain high quality images in the presence of fetal movement. Most fetal imaging uses T2 weighted imaging, as the more rapid return to equilibrium allows for faster image acquisition. In particular, the most common sequence used in fetal imaging is T2 single shot fast spin echo (SSFE). These sequences have a high signal to noise ratio and good contrast. It is possible to obtain one slice at a time. Each slice is acquired individually in less than a second, so that corruption of each slice by fetal motion is much less likely. However, individual slices are not parallel, so if there is any movement of the fetus, even with overlapping of slices, this would give incomplete coverage. This is overcome by obtaining several acquisitions. T1-weighted imaging has a more limited use, as it is more compromised by fetal motion due to the long scan time and cannot be acquired in single slices.

1.9.3 MRI Volumetry

Due to the widespread use of ultrasound, there are reference ranges based on large series of patients which are used in everyday clinical practice[124]. The measurement of brain structures on MRI has some advantages over ultrasound. It allows true measurements of the brain tissue without inclusion of the skull and CSF. A recent large study looking at MRI biometry has shown high interobserver reproducibility and good agreement with data from ultrasound studies[125].

Further quantification of brain structures and growth by 3D volumetry is clearly desirable; however, early MR imaging was not of sufficient quality to allow accurate

measurement. Initial attempts to measure fetal brain volumes used a systematic sampling method to obtain an estimation of volume [126]. With advances in acquisition and processing techniques, the quality of data is now sufficient to allow 3D reconstruction of the fetal brain from SSFE sequences and, from this, volumetric measurements can be generated. This and similar approaches are increasingly being used in normal fetuses to establish normal reference values[127-129] and as a research tool to examine the effect of brain growth in pathological states[130-133]. Methods of measuring structures from 3D data sets that have so far been published predominantly involve manual segmentation of structures. This time-consuming method currently limits the use of volumetric data in clinical practice. However, studies using automated segmentation techniques are now being reported[127]. This may make the use of volumetric measurements more widespread.

In the Robert Steiner MRI Unit, we use a method called snapshot volume reconstruction (SVR), that is detailed in the methods section. This involves acquiring dynamic single shot scans in orthogonal planes with multiple overlapping slices. Images are then registered into a 3D matrix that allows slicing of the 3D volume in any plane. Measurement of structures is then performed by manual segmentation.

1.9.4 Safety of fetal MRI

There are a number of potential risks for MRI imaging in pregnancy which have been investigated. These include the effect of the static magnetic field, the effect of the gradient fields and the exposure to radiofrequency energy. Studies have addressed these issues and have not identified findings to suggest harmful effects on the developing human fetus[134]. Kanal et al. studied the effect of the static magnetic

field on MR healthcare professionals and did not find any negative effect on reproductive outcome[135] .

Radiofrequency energy may result in tissue heating during the MRI procedure[136]. It is recognised that the developing human embryo and fetus is vulnerable to increases in temperature [137]. International standards have therefore been developed whereby manufacturers must ensure that each pulse sequence must not cause a rise in body temperature of more than 0.5-1°C (IEC60601-2-33, 2002). Scanners have different specific absorption rates (SAR) settings that can be manually set and, for fetal imaging, the low SAR settings are used. These settings are, however, not calculated specifically for the fetus. Some fetal modelling on heat absorption has been done to allowing calculation of how much heat deposition occurs[138], which helps to inform practice.

A further potential hazard is the high levels of noise generated by the radiofrequency pulses and the potential impact this may have on the hearing of the developing fetus. Reeves et al.[139] performed neonatal hearing tests on 96 infants who had undergone MRI imaging in the second and third trimester. They did not find an increased risk of substantial hearing loss in the study cohort. In clinical practice, noise levels are limited in all fetal sequences and the noise is attenuated by maternal tissue and amniotic fluid; however, further research is needed into the possible impact on hearing of different sequences used in MRI.

Guidelines on performing MR in the fetus are from The National Radiological Protection board (NRPB 1991), The International Non-Ionising Radiation committee of the International Radiation Protection Association (ICNIRP) 2004 and from an American College of Radiology white paper on MRI safety[140, 141].

Chapter 2: Methods

2.1 Summary of my involvement in the research project

Together with Prof. M Rutherford and Dr H Gardiner, I developed the initial plan for the research project. I was responsible for the application to the ethics committee and attending the research ethics committee meeting to discuss the project with the panel. I was responsible for recruiting patients and carried out the clinical echocardiographic evaluation of the heart defect. I also made the Doppler measurements of cerebral and umbilical flow. Growth scan measurements were made by a specialist in fetal medicine. Brain MRI scans were performed at the Robert Steiner MRI unit. I consented the study patients and provided medical supervision for the duration of the scan. I performed the registration and reconstruction of images and made the measurements of brain volumes. MRI controls were recruited over a longer period for a number of on-going research studies and I was also involved in the consent and supervision of scans for a proportion of these patients. Neurodevelopmental assessments were performed by either a general paediatrician or clinical psychologist. I performed all the analyses presented in the thesis.

2.2 Ethical approval

The study received full ethical approval REC number 11/LO/0394 (see appendix 1).

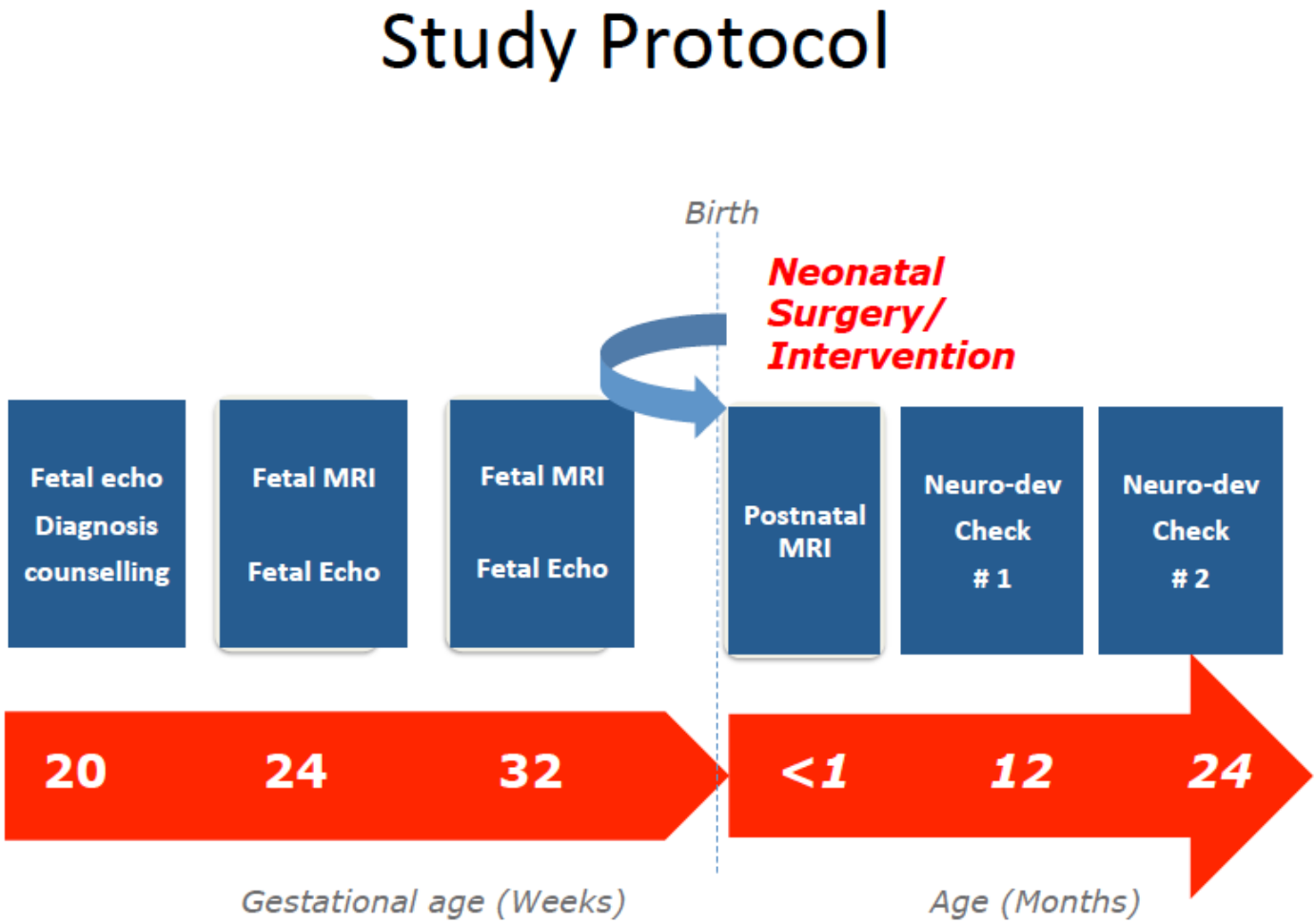
Consent form, patient information sheet and advertising material can be found in appendix 2-4.

2.3 Study Design

The study protocol is summarised in the flow diagram below (figure 9). Pregnant women recruited initially had a full echocardiographic evaluation of the congenital heart defect as part of their clinical care. From this anatomic and Doppler information, they were subdivided into the anticipated abnormality of cerebral oxygen delivery and cerebral blood flow of congenital heart defect. Women then underwent fetal brain MRI, ideally twice during pregnancy, and with a plan to perform a scan in the early postnatal period before undergoing any intervention. Ultrasound information on fetal biometry, the cardiac abnormality and Doppler assessment of cerebral and umbilical artery blood flow was also gained at the time of their MRI appointment.

Neurodevelopmental assessment was offered at 1 and 2 years of age.

Figure 9 – Flow diagram summarising the study protocol



2.3.1 Power Calculation

Brain volume constituted the primary outcome. The range of volumes expected in groups were from published data by Gong et al.[126]. The mean brain volume from 18 normal fetuses was 255.4ml+/-65ml, with a daily increase of 2.7ml/day in the third trimester. To detect a significant difference in brain volume between CHD fetuses and controls with a statistical power of 82% and an alpha error of 5%, a sample size of 30 was required. This number was in keeping with the number required in a previous research study in our department comparing brain volumes in fetuses with intrauterine growth retardation compared to controls.

Online software was used for sample size and power calculations (Lenth, R.V. (2006-9). Java Applets for Power and Sample size. http://www.stat.uiowa.edu/-rlenth/Power.)

2.3.2 Inclusion and exclusion criteria

Women were recruited prospectively from the fetal cardiology clinic in the Centre for Fetal Care, Queen Charlotte's and Chelsea hospital.

Inclusion criteria were women with a fetus affected by congenital heart disease that were competent to consent to involvement in the study and did not have any contraindication to MRI scan. We did not limit the gestation for the study.

Exclusion criteria for the primary study were fetuses with known karyotype or structural brain abnormalities and monochorionic twin pregnancies.

Informed consent was gained on all participants.

2.3.3 Statistical analysis

Statistical analysis was performed using SPSS version 23 for mac (SPSS, Chicago, Ill). Normality of data was determined using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed data are expressed as means (+/- standard deviation). Non-normally distributed data are expressed as median (interquartile range). Pearson's correlation coefficient was used to assess the linear relationship between normally related continuous variables; Spearman's correlation coefficient was used to assess the relationship between non-linearly related variables. Categorical data are expressed as n (%). Medians between groups were compared using the Kruskal-Wallis test.

The effect of advancing gestational age on brain growth was adjusted for by the use of linear regression. Analysis of the variance (ANOVA) was then used to assess differences between groups following adjustment for gestational age.

2.4 Fetal ultrasound, echocardiography and Doppler

Fetal echocardiograms were performed on a GE Voluson E8 (BT08) using RAB 4-8-L 3D hybrid abdominal Probe (General electric, Chicago. Ill). A full sequential segmental description of the congenital heart defect was made, including both anatomic and Doppler parameters. In addition, the following biometric information was gained:

2.4.1 Fetal femur length, head circumference and abdominal circumference

These were recorded at 3 separate time points. Estimated fetal weight was calculated from this using Hadlock formula[142]. The growth of each fetus was monitored throughout pregnancy using gestation related growth centiles.

2.4.2 Middle cerebral artery and umbilical artery flow

Pulsed wave Doppler was used to acquire waveforms from the middle cerebral artery (MCA) and umbilical artery (UA), using published protocols.

Middle cerebral artery Doppler: In an axial section of the brain, the thalami and sphenoid bone wings were identified. The circle of Willis was identified. Doppler measurements were taken from the proximal third of the MCA.

Umbilical artery Doppler: Measurements were obtained from insonation of a free loop of umbilical cord.

All pulsed wave Doppler measurements were obtained in the absence of fetal movements with maternal breath hold when necessary. The targeted blood vessel was initially identified by colour flow mapping. The cursor was aligned as near as possible with blood flow and always with an angle of insonation of < 20 degrees. A minimum of 4 cardiac cycles was displayed. The pulsatility index (PI) was calculated for the MCA and UA using the equation (peak systolic velocity-end diastolic velocity / time averaged velocity). This information was used as a measure of the diastolic impedance of the vessel. To account for differences in gestational age, MCA-PI Z-scores were calculated from published normal reference ranges [52]. The cerebro-

placental ratio (CPR) was calculated (MCA-PI divided by the UI-PI) to look for evidence of redistribution of blood flow.

2.5 Patient Grouping

Subjects were then subdivided into the following diagnostic groups on the basis of the flow and oxygen delivery to the fetal brain that would be anticipated in each heart defect.

The first group included cases in which a reduction in volume of flow to the brain would be anticipated without a reduction in the oxygen level in the blood;

The second group consisted of those in which there would be an anticipated reduction in the oxygen saturation blood to the brain;

The third group was composed of those in which it would be expected that there would be both a reduction in blood flow to the brain and a reduction in the oxygen saturation of blood delivered to the brain.

To ensure accuracy in the designation of groups, two other experts in paediatric cardiology were also asked to assign patients to subgroups. (Expert 1 – A senior consultant paediatric cardiologist with 40 years experience in the management of congenital heart disease. A world expert in his field who lectures internationally and has published many papers and book chapters in the field; Expert 2 - A consultant fetal cardiologist with over 20 years experience in the field. Widely published and an internationally recognised expert in the field of fetal cardiology). The two experts were given the sequential segmental diagnosis of the heart defect and asked to designate which of the three physiological subgroups the patient should be in. They

had no other identifiable information on the patient, such as name or age, and no knowledge of the brain MRI findings. If there was disagreement in the designation of group then the patient was categorised under the decision of the majority.

2.6 Fetal MRI

Fetal MRI studies were performed using a 1.5-T scanner (Philips Acheiva, Philips medical systems, The Netherlands) using a SENSE wrap around 32 channel cardiac coil.

2.6.1 Safety

The pregnant woman was positioned in the left or right lateral tilt position to avoid compression of the inferior vena cava. No sedation was used and scanning time was limited to one hour. Maternal temperature was recorded before and after the examination. Specific absorption rates were adhered to. Mothers with a temperature of > 37.5 degrees before scanning commenced had the scan rearranged to ensure compliance with safety guidelines.

2.6.2 Volumetric data

Anatomical data were acquired with a combination of single shot T2 weighted and T1 weighted SNAPIR sequences in coronal, axial and sagittal views according to the departmental protocol.

T2 weighted single shot turbo spin echo sequences were acquired in three orthogonal planes to ensure overlapping of data sets. This allows complete brain coverage even in the presence of significant fetal motion. 4 loops were acquired in

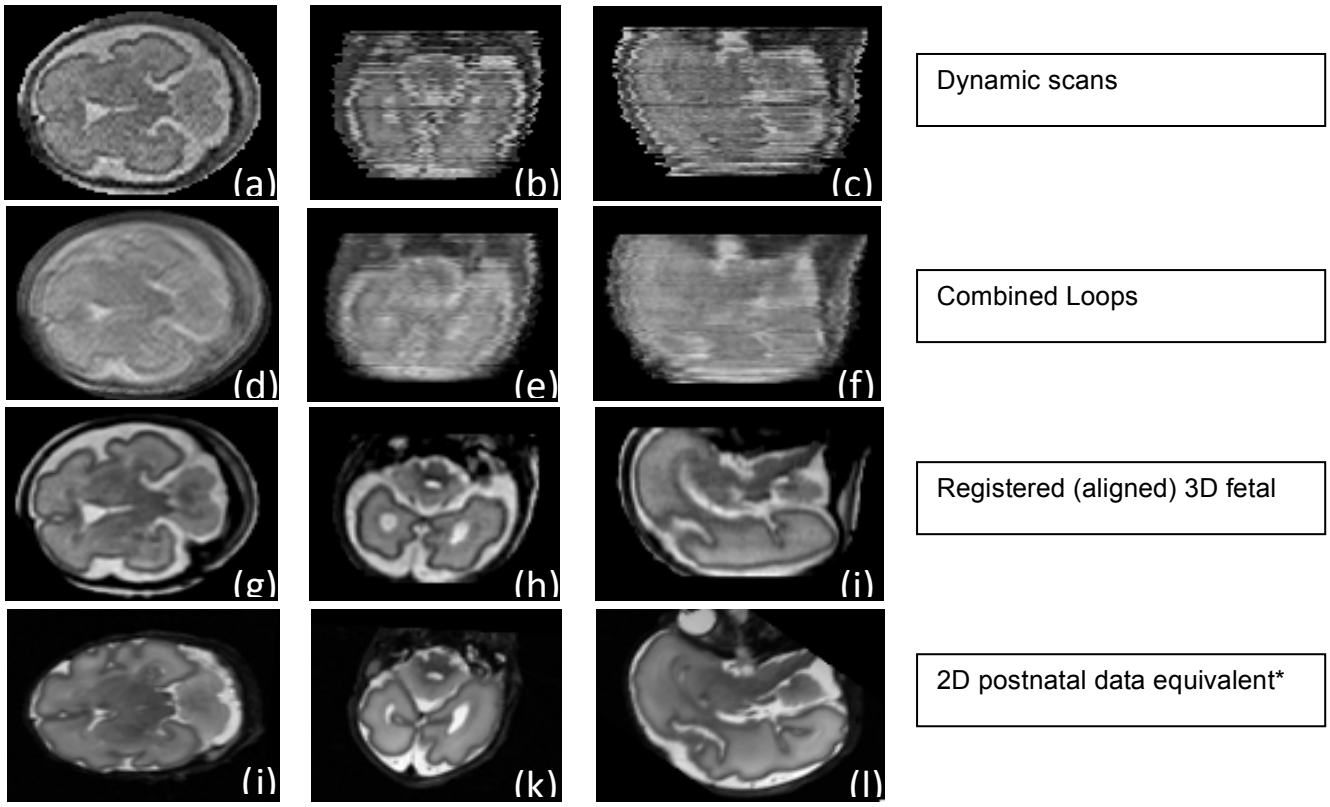
the axial plane and 2 in the coronal and sagittal planes. (TE:110, TR 1500, NSA, 1, Matrix 256×272, FOV 430×353×88, slice thickness 2.5mm, slice gap:-1.25mm, acquisition time:4min, slices 64-80 (appendix 6 Fetal MRI guideline).

2.6.3 SVR (snapshot to volume reconstruction)

This method is used to reconstruct a 3D volume from the oversampled raw data, following removal of any motion-corrupted slices. This process provides high signal-to-noise, high resolution 3-Dimensional volumetric datasets of the brain in the presence of fetal motion [143]. The process involves removal of maternal tissue from the image data and selection of a 'best' loop to act as a target for registration and reconstruction. The datasets are then aligned into a 3D coordinate system and scattered interpolation used to reconstruct the fetal brain. Rotation of the brain is corrected and volumes quantified according to the number of voxels in each region of interest. An example of this with comparison of the quality of images in a registered and aligned 3D fetal data set and postnatal image is seen in figure 10.

Figure 10: Examples of quality of images from SVR where dynamic scans are combined to produce registered 3D volumes Jiang et al 2008[143]

***postnatal acquisition at same post menstrual age**



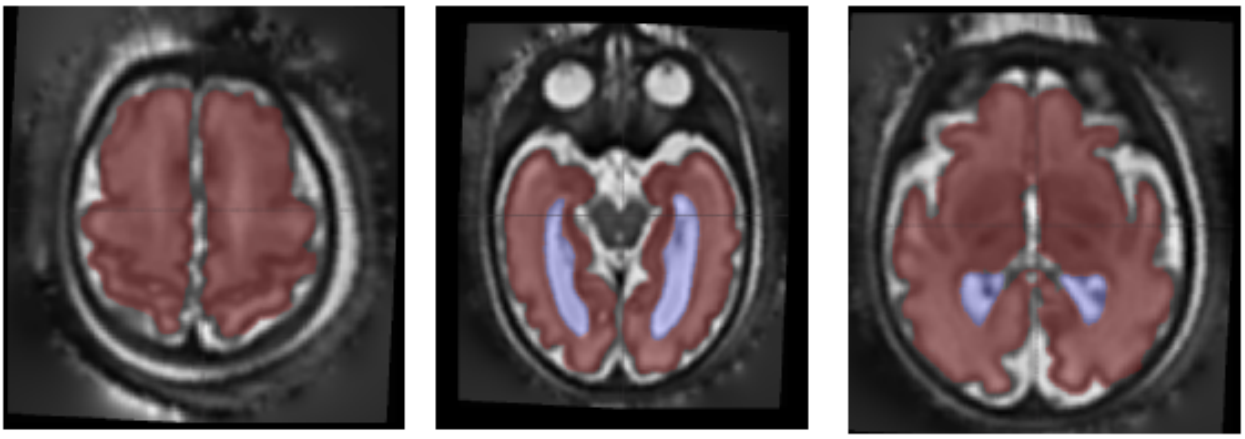
Following creation of a 3D dataset, manual segmentation was performed using itk-SNAP[144]. The segmentation of the whole brain was performed in the transverse plane by drawing around the outer margin of the cerebral tissue. This was then visually checked in the sagittal and coronal planes. The midbrain, brain stem and cerebellum were excluded.

The ventricular system was segmented in the transverse plane. The lateral ventricles were included; the third ventricle and cavum septum pellucidum was excluded.

Figure 11: Examples of manual segmentation of the fetal brain in different transverse planes

The segmentation was performed in the transverse plane and checked visually in the sagittal and coronal planes.

(Red – Cortical gray and white matter, deep gray matter. Brain stem excluded. Blue- Ventricular system)



2.7 Postnatal follow up

2.7.1 Postnatal outcome parameters

The following parameters were recorded for each baby:

Mode of delivery

Birth weight

Gestational age

Apgar scores and need for neonatal resuscitation

Postnatal diagnosis and any new postnatal findings, such as dysmorphic features or abnormal karyotype.

2.7.2 Neurodevelopmental assessments

The original study plan was to perform at neurodevelopmental assessment at 1 and 2 years of age. Patients were contacted and neurodevelopmental assessment was offered to all surviving patients, although contact was poor. Tests were performed either by a paediatrician trained in developmental examination (AC, MM) or by a clinical psychologist (BB) .The clinician was aware of the diagnosis of congenital heart disease but was blinded to the prenatal MRI volumetric and spectroscopy findings. The assessment was scored according to the Griffiths Mental Development Scales (GMDS 0-2) or Bayley Scales of infant and toddler development (3rd edition) BSID III. The GMDS 0-2 test was preferentially used at the one year assessment as the test is shorter, allowing for better cooperation.

Griffiths Mental Development Scales (GMDS 0-2) (1996 Huntley and Griffiths)

These are validated for use between birth and 2 years. There are 5 developmental domains assessed in the test: Locomotor, Personal–Social, Hearing and Language, Eye and Hand Co-ordination and performance. A kit of standard equipment is required. Each task in the test is given a score of 0 for failure or 1 for success. The scores in each sub-scale are then added to provide a raw score. This raw score can then be converted to a standard score in one of three ways: age equivalent, sub or general quotients or percentile equivalents. The scoring scales are only supplied to health professionals that have successfully completed an accredited training course.

Bayley Scales of infant and toddler development (BSIDIII) (Bayley 2006)

This was developed in the USA and has more recently come into widespread use in the UK. It is used to assess the development in infants and toddlers up to 4 years of age. There are 5 developmental domains that are assessed: Cognitive, Language, Motor, Social-Emotional and Adaptive behaviour. Each task is marked as 0 (failure) or 1 (success). Raw scores are calculated for each subtest, which are then converted to scale scores and composite scores. These are then used to compare the child's performance to normally developing children of their age. In this study, the children were assessed in Cognitive , Language and Motor domains and an informal assessment of Social and emotional behaviour was made at the time of the test.

Ages and stages questionnaires – III (ASQ-3)

Ages and stages questionnaires were offered to parents that had declined to attend formal neurodevelopmental assessment. These are parent-completed questionnaires comprising 30 questions relating to their child's development in a range of developmental domains. They are designed to detect children who may be in need of further assessment. These questionnaires have been compared against formal ND testing (Suires and Bricker 2009) with an agreement of 86% (http://www.brookespublishing.com).

2.8 Study Population and clinical characteristics

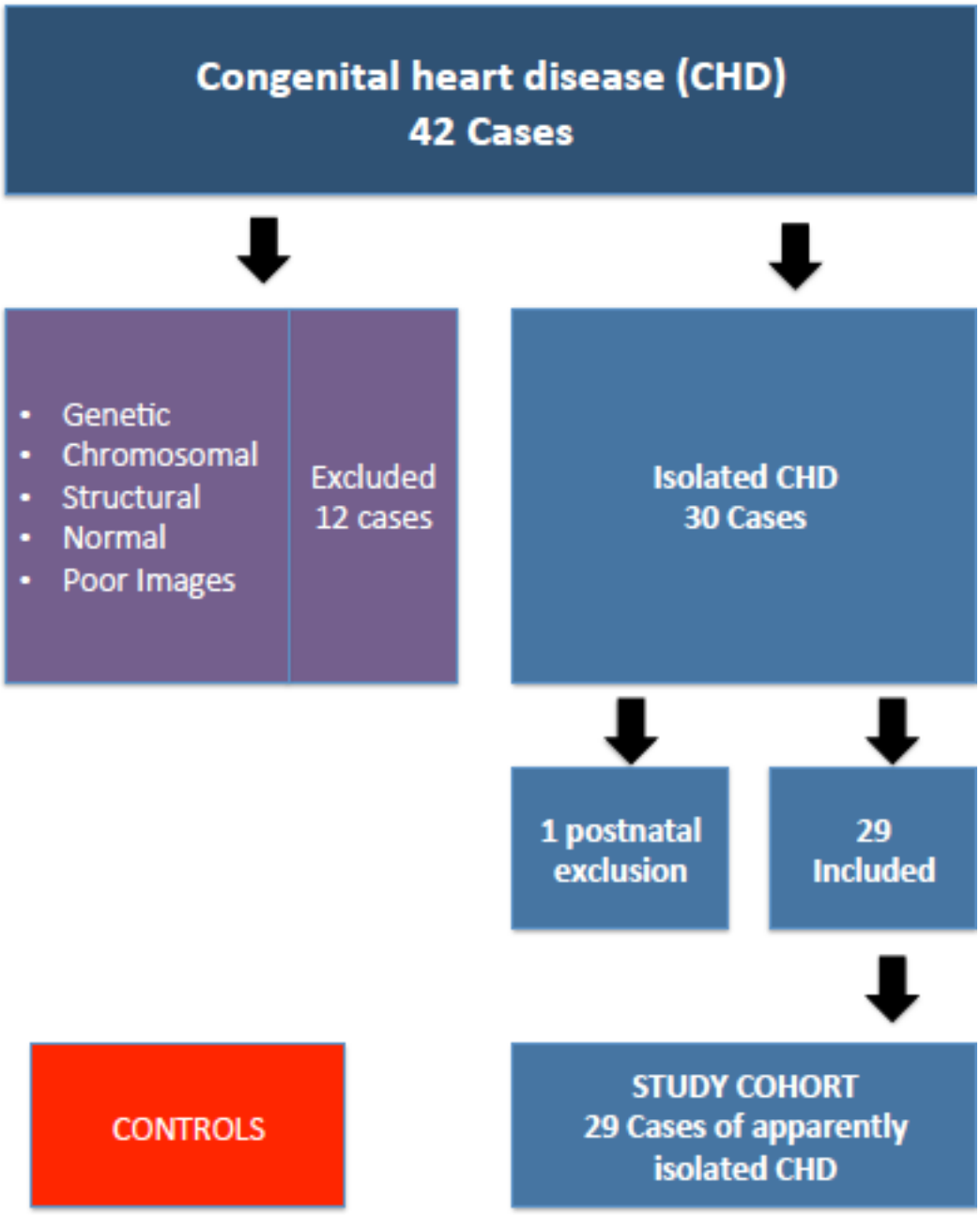
2.8.1 study population

During the study recruitment period from 1st July 2010 to 31st December 2012, participation in the study was offered to all on-going pregnancies with a diagnosis of congenital heart disease. 42 patients agreed to be included in the study. Of these, 12 patients were excluded (11 patients were excluded prenatally and one postnatally). The reason for exclusion is detailed in table 5. Prior to commencement of recruitment for this project, 18 additional fetuses with congenital heart defects had undergone fetal brain MRI for another study. Three of these fetuses had isolated congenital heart disease and were therefore also included in this project. A summary flow chart of the study cohort is shown in figure 12.

Table 5: Prenatal cases excluded from study with reason for exclusion

Apparently isolated CHD One excluded postnatally for metabolic abnormality	1
Chromosomal abnormality Trisomy 13 – 2, Trisomy 21 – 1 22q11 deletion - 1	4
Genetic abnormality CHARGE syndrome - 2	2
Antenatal detection major structural abnormality absent lung - 1, bowel obstruction -1	2
Normal cardiac physiology	2
Inadequate images for reconstruction of MRI	1

Figure 12: Flow chart of study cohort



2.8.2 Control data

MRI controls

For analysis of MRI data, comparison was made with 83 patients. In patients that had had 2 scans in pregnancy, the first scan was used. The majority of these women were healthy volunteers; 14 women with a previous child with a neurological abnormality were included where imaging was normal and the neurodevelopmental assessment was normal at 1 year. Healthy volunteers were excluded if a structural abnormality was identified on brain MRI imaging or if neurological abnormality (for example seizures) developed after birth.

Ultrasound Controls

Patients were identified from the fetal cardiology database, which records all patients scanned, and the study indication. Scans performed for a family history of congenital heart disease were selected during the study period from July 2010. They were included if they had:

1. Normal fetal cardiac scan
2. No extracardiac abnormalities.

These were then cross-checked on the ultrasound database (Astraia software gmbh, Munich, Germany) and included if a second growth scan was performed. 48 consecutive patients were identified that had had a growth scan at a later stage in pregnancy. A summary flow chart of the controls is shown below in figure 13.

Figure 13: Summary of controls

2.8.3 Subgrouping of cases by presumed underlying physiology of congenital heart defect

Methods

It is widely speculated that the abnormalities in brain development and, in the longer term, neurodevelopment in CHD, may be secondary to reduced oxygen and substrate delivery to the developing brain. There are two potential haemodynamic factors that may result in reduced oxygen delivery to the brain in CHD. The first is a reduction in the oxygen level of the blood reaching the brain, the second a reduction in the volume of flow and therefore oxygen delivery to the cerebral circulation. When considering different congenital heart lesions, some lesions primarily affect flow, some oxygen delivery and some a combination of both. It might therefore be anticipated that those with a combination of both reduced oxygen delivery and reduced flow would display the most significant deviation from controls.

For the purposes of analysis, cases were therefore subdivided as in table 6 below, on the basis of the abnormality of flow and/or oxygen delivery to the fetal brain that would be anticipated for the particular heart defect.

For validation, 2 additional independent observers with expert knowledge of congenital heart disease were also asked to subdivide the patients along with the category that I had chosen for each patient. If all observers did not agree on the same subgroup, this case was sub grouped according to the majority.

Table 6 - Summary of subgrouping of cases

Group 1	Decreased volume of cerebral blood flow
Group 2	Decreased oxygen saturation of cerebral blood flow
Group 3	Combination of decreased volume of cerebral flow and decreased oxygen saturation of cerebral blood

Group 1 - Lesions in which there is an isolated abnormality of flow without any significant impact on saturations expected.

This would include lesions such as coarctation of the aorta, where more blood might be expected to pass through the arterial duct due narrowing at the aortic isthmus, or aortic stenosis.

There were 2 cases in this group. In one case of isolated coarctation one observer's decision was that there would be no change in cerebral blood flow and oxygenation. Both cases had coarctation of the aorta, where it would be anticipated that, due to a degree of narrowing or obstruction of the aortic arch at the level of the isthmus, there is preferential flow through the ductus arteriosus. The mechanism for this may be that the distal obstruction in the arch would reduce the normal right to left streaming of blood across the atrial septum therefore resulting in increased flow through the tricuspid valve, though the right ventricular outflow tract into the duct.

Group 2 - Lesions in which there is an anticipated reduction in oxygen saturation of cerebral blood flow.

This includes cardiac abnormalities with intracardiac shunts and those that disrupt the normal streaming patterns in the fetal circulation, whereby the more oxygenated blood preferentially crosses the foramen ovale and enters the left ventricle and ascending aorta, with relatively deoxygenated blood returning to the heart from the SVC directed through the tricuspid valve into the right ventricle and then passes through the arterial duct towards the placenta.

The majority of cases (22/29) were categorised as having reduced oxygen delivery to the fetal brain. There was agreement between the three observers in 19/22 cases. These cases include those in which the predominant abnormality is intracardiac mixing of oxygenated and deoxygenated blood in the absence of any significant left ventricular outflow tract obstruction. In one case it was uncertain whether any decrease in oxygen saturation would be expected. Where unanimous consensus was not reached, the two fetal cardiologists were in agreement with the paediatric cardiologist differing.

Group 3 - This group consists of those lesions in which there would be expected to be a combination of abnormal flow and abnormal oxygen content of the cerebral blood flow. This would include lesions such as hypoplastic left heart and critical aortic stenosis, where there is flow reversal in the transverse aorta and the cerebral circulation is supplied in a retrograde manner from the arterial duct.

5/29 cases were subdivided on the basis that they had a combination of reduced volume and oxygen content of cerebral blood flow. This group consists of cases of left heart obstructive lesions of sufficient severity that the reduction of forward flow is supported by flow reversal in the transverse arch, which can be demonstrated on colour flow Doppler on the fetal echocardiogram.

Table 7: allocation of individual cases to sub groups

Case	Diagnosis	Sub Group
1	Coarctation	1
5	SS, AV VA discordance, VSD, Coarctation	1
2	Dextrocardia, Probable LAI (recent MRI ?SS) Biventricular AV connection LA to LV connection, VA discordance, Dyplastic pulmonary valve, small VSD, juxtaposed atrial appendages	2
3	SS, DORV sub pulmonary VSD, aorta anterior rightward, sub PS	2
6	Common arterial trunk - type 2	2
7	Dextrocardia, RAI, Double inlet univentricular connection to single (probably morphological right) ventricle, common AVV, Hypoplastic LV, Single outlet heart with aorta from RV, Pulmonary atresia, TAPVC to LSVC, IVC to RA, LSVC to left sided RA, small RSVC, Left arch, AV valve regurgitation.	2
8	Tricuspid valve hypoplasia, VSD, Severe PS progressing to pulmonary atresia	2
9	Tetralogy of Fallot	2
11	Levocardia RAI ?SS, Biventricular AV connection with R hand topology, Unbalanced AVSD with dominant RV, DORV with right anterior aorta, PS, LSVC to coronary sinus, Apical VSD	2
12	SS, AV VA concordance, superior dominant LV, Inferior hypoplastic RV (Criss Cross Heart), large inlet VSD, right sided SVC IVC, common atrium left arch ARSA	2
13	Pulmonary atresia intact ventricular septum hypoplastic RV	2
14	Situs solitus, AV concordance, DORV, Sub Aortic VSD, normally related great arteries, Bilateral infundibuli with mild muscular sub PS	2
15	Partial AVSD	2
16	SS, DILV hypoplastic morphological RV on the left, VA discordance with aorta from hypoplastic left sided right ventricle, Pulmonary atresia (from dominant LV)	2
18	Levocardia, LAI, Interrupted IVC, azygous to right RSVC, DILV with hypoplastic morphological RV anteriorly on left VA discordance with aorta from left sided anterior rudimentary RV, Severe stenosis of left AV valve, Non restrictive VSD, Pulmonary veins to left sided LA, Restrictive PFO with channel from LA to RA, (no PS, No coarctation)	2
19	Valvular PS, dilated MPA	2
20	RAI, biventricular AV connection via complete AVSD, Moderate atrial and vent components, DORV non committed VSD with aorta anterior to right	2
21	TGA	2
22	TGA	2
25	Tetralogy of Fallot, Left arch, ARSA, mild PS	2
26	Situs solitus, double inlet right ventricle, straddling and overriding MV rudimentary LV, single outlet heart PAT, Ao from RV, Bil SVC left SVC to coronary sinus	2
27	Simple TGA	2
28	AVSD with 2 valve orifices primum AVSD, LAVV regurgitation	2
29	TGA VSD	2
4	Coarctation, bicuspid aortic valve moderate stenosis (Prenatal reversed flow in arch)	3
10	Borderline left heart, Coarctation developed at 7wks (Prenatal reversed flow in arch)	3
17	SS, AV VA concordance, Mitral stenosis, bicuspid aortic valve, Mild Sub aortic stenosis, Long segment coarctation of the aorta, borderline left ventricle (Prenatal reversed flow in arch)	3
23	VSD, interrupted aortic arch (type B)	3
24	Critical aortic stenosis	3

KEY LAI (left atrial isomerism), AV (atrioventricular), SS (Situs solitus), LA (left atrium), LV (left ventricle), VA (ventriculararterial), VSD (ventricular septal defect), DORV (Double outlet right ventricle), RAI (right atrial isomerism),TAPVC (total anomalous pulmonary venous connection), LSVC (left superior vena cava), IVC (inferior vena cava), RA (right atrium), ASD (atrial septal defect), RV (right ventricle), PS (pulmonary stenosis), AVSD (atrio ventricular septal defect), ARSA (aberrant right subclavian artery), PFO (patent foramen ovale), TGA (transposition of the great arteries)

Chapter 3: Ultrasound Biometry in Fetuses with CHD

3.1 Introduction

Fetal growth and ultimately birth weight are determined by the interaction of multiple factors, including genetic and developmental factors in the fetus, maternal factors and external environmental factors. There is a clearly described association between CHD and abnormal birth weight, supported by studies of abnormal in utero growth [22, 23] [24, 25], although there is potential difficulty in interpreting this finding due to the high rate of extra-cardiac and chromosomal abnormalities that may also impact on growth in fetuses with CHD. A number of studies have, however, shown that this difference remains even when these confounding factors are excluded.

There are two theories that could potentially explain the increased observation of growth abnormalities in CHD. The first is that the presence of CHD, due to abnormal blood flow and or oxgen delivery to fetal tissues, results in suboptimal growth. The second theory is that there is a common underlying aetiology that predisposes the developing embryo to the increased risk of CHD and growth restriction.

3.2 Aims

This chapter aimed to characterise the in utero growth of the cohort of fetuses with CHD with the use of serial ultrasound scans

3.3 Methods

3.3.1 Participants

Cases: Each of the 29 patients underwent serial growth scans in line with our departmental policy of on-going growth surveillance for fetuses diagnosed with major congenital heart disease. This involves 4-6 weekly growth scans throughout pregnancy, including measurement of head circumference and abdominal circumference in addition to middle cerebral artery and umbilical artery Doppler measures.

Controls: For comparison, a population of normal controls was used. This included sequential cases of subjects that were referred during the study period for fetal cardiac screening due to a family history of congenital heart disease. All had a normal fetal echocardiogram. These patients were identified retrospectively from the departmental database and included if they had, in addition to a normal fetal echocardiogram, undergone at least 2 fetal growths scans during the course of the pregnancy. Patients were excluded if there was an identified extracardiac abnormalitiy (including raised nuchal translucency) or if there was an identified maternal factor that could impact on normal fetal growth, such as maternal diabetes or autoimmune disease.

3.3.2 Techniques

A specialist in fetal medicine performed ultrasound measurements of fetal biometric parameters using a GE Voluson E8 using 4-8-D RAB Probe (GE Healthcare). The number of growth scans in pregnancy varied depending on the gestation at the time of diagnosis and maternal and fetal indications for increased frequency of growth scans. For analysis, the first and the final growth scan for each patient were used.

Parameters recorded at each scan follow below. Measurements were made in accordance with the guidelines in the fetal anomaly screening programme (FASP) (Loughna 2009).

Gestation – This was calculated from the measurement of the crown rump length at the initial dating scan;

Head circumference (HC) – A cross sectional view at the level of the lateral ventricles was obtained and an elliptical measurement around the outer edge of the skull;

Abdominal circumference (AC) – This is measured on a transverse view through the fetal abdomen. A circular view of the abdomen is obtained with the stomach bubble and umbilical vein visible;

Femur length (FL) – this is visualised lying as close as possible to the horizontal plane, making sure all the bone is visualised and measurements made from metaphysis to metaphysis excluding epiphysis

[145].

Z-scores were calculated for measurement of head circumference and abdominal circumference. Head Circumference: Abdominal Circumference ratios (HC:AC ratio) were also calculated (the normal ratio below 36 weeks gestation is 1:1). A higher ratio, indicating a larger head size relative to abdominal circumference, can be indicative of brain sparing.

Data were recorded in a specialist obstetric datatabase (Astraia software gmbh, Munich, Germany).

3.3.3 Statistical analysis

Normality of data was determined using Kolmogorov-Smirnov and Shapiro-Wilk tests. The data were not normally distributed data and are therefore expressed as median (interquartile range). Medians between groups were compared using the Kruskal- Wallis test. The Mann Whitney U test was used for the diagnostic subgroups comparing HC Z-score at the final growth scan, for difference between medians. Z-scores were calculated for each recorded measurement of the abdominal circumference and head circumference using reference data from Chitty et al. 1997[146]. The Z-score was calculated using the formula:

(Observed value - mean value for gestational age) / standard deviation

3.4 Results

3.4.1 Gestation at scan - Fetuses with congenital heart defect

The gestation at the initial scan ranged from 20 to 33 weeks gestation with a median of 22 (IQR 21-24.1) weeks gestation. The gestation at the final scan ranged between 30 and 40 weeks gestation with a median of 36 (IQR 35-37.7) weeks gestation.

3.4.2 AC Z-scores

At the initial growth scan the spread of data points for AC Z-score suggest that there is a tendency for cases and controls towards positive Z-scores compared to population standards. The timing of the growth scan for the controls is clustered

around 20-22 weeks gestation, which differed from the more even spread of the gestation of the cases (see figure 14).

The final growth scan Z-score for controls shows a non significant trend towards scores greater than 0, in contrast to cases with CHD, which appear to have lower Z-scores compared to controls (see table 10).

Figure 14: Scatter graph showing Z-score of abdominal circumference (AC) at initial growth in cases and controls.

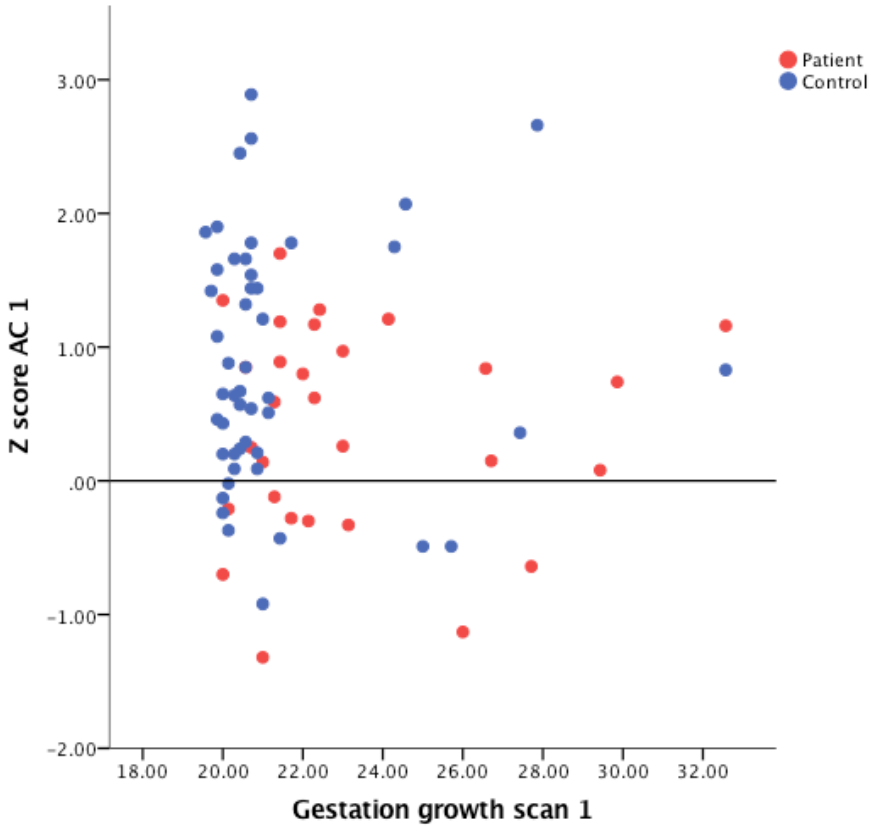
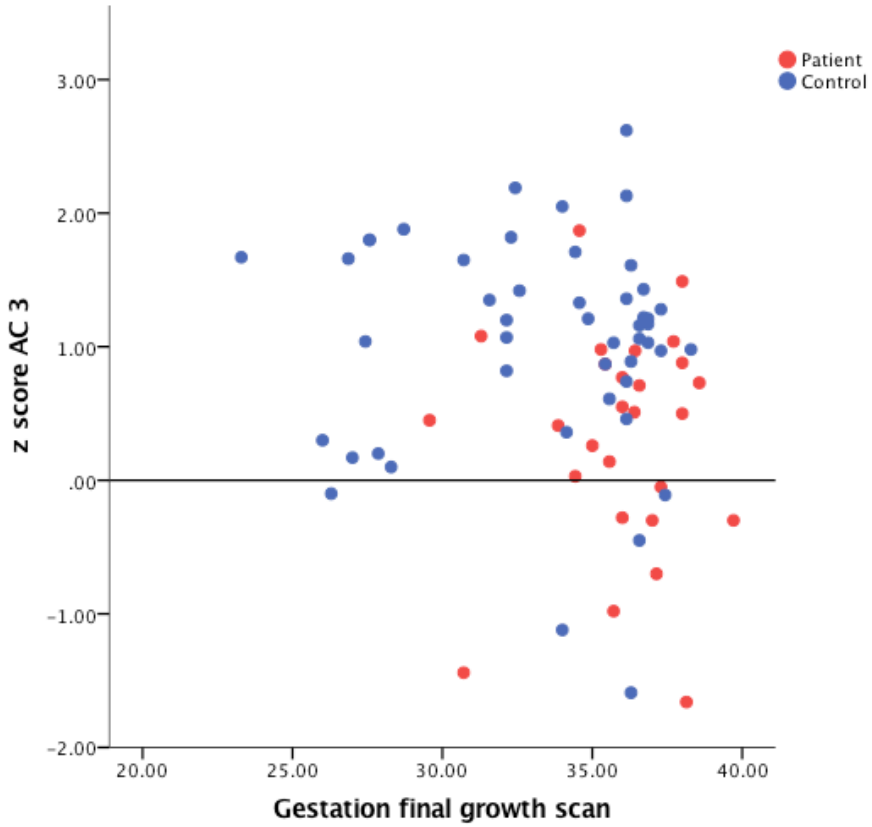


Figure 15: Scatter graph showing Z-score of abdominal circumference (AC) at final growth in cases and controls.



3.4.3 HC Z-scores

At the initial growth scan the head circumference Z-score appears spread relatively evenly throughout the normal population Z-score range. However, at the second scan there is a significantly lower HC Z-score for cases (figures 16 and 17; table 8). As with the AC Z-score data, the timing of the initial growth scan for controls is clustered around 20 weeks.

Figure 16: Scatter graph showing Z-score of head circumference (HC) at initial growth in cases and controls

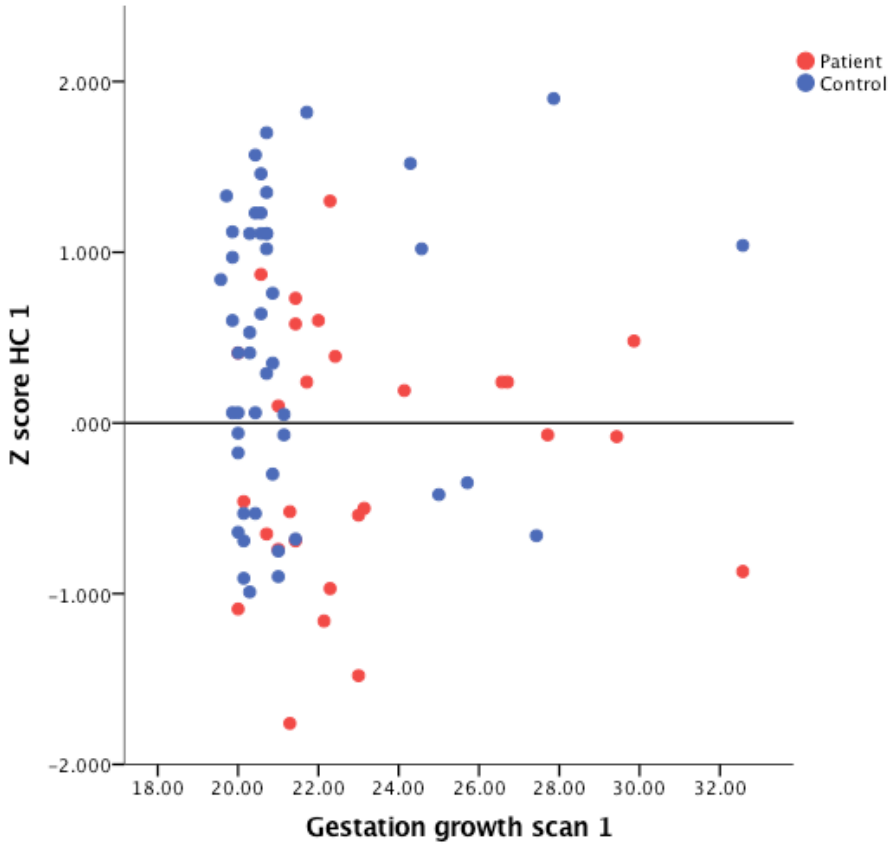
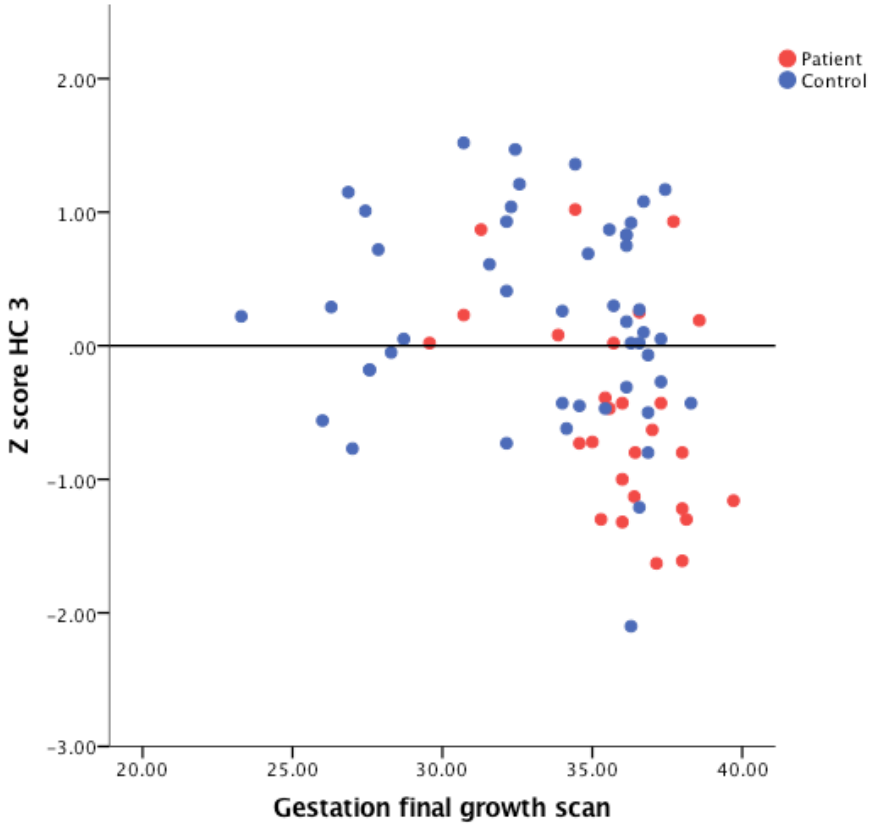


Figure 17 - Scatter graph showing Z-score of head circumference (HC) at final growth in cases and controls



3.4.4 HC and AC Z-scores at first and final growth scan

Table 8 shows the head and abdominal circumferences at initial and final scans for cases and controls. Fetuses with congenital heart disease were found to be significantly smaller than controls in terms of both their head and abdominal circumference at both the initial and final growth scans. The difference was highly significant at the final scan.

Comparison of the HC:AC ratio at the first and final scan showed a significantly lower values at the final scan, suggesting that the head circumference is relatively smaller at this stage ($p<0.01$).

Table 8: Median and interquartile range (IQR) of head circumference (HC) and abdominal circumference (AC) Z score at initial and final growth scans in fetuses with CHD

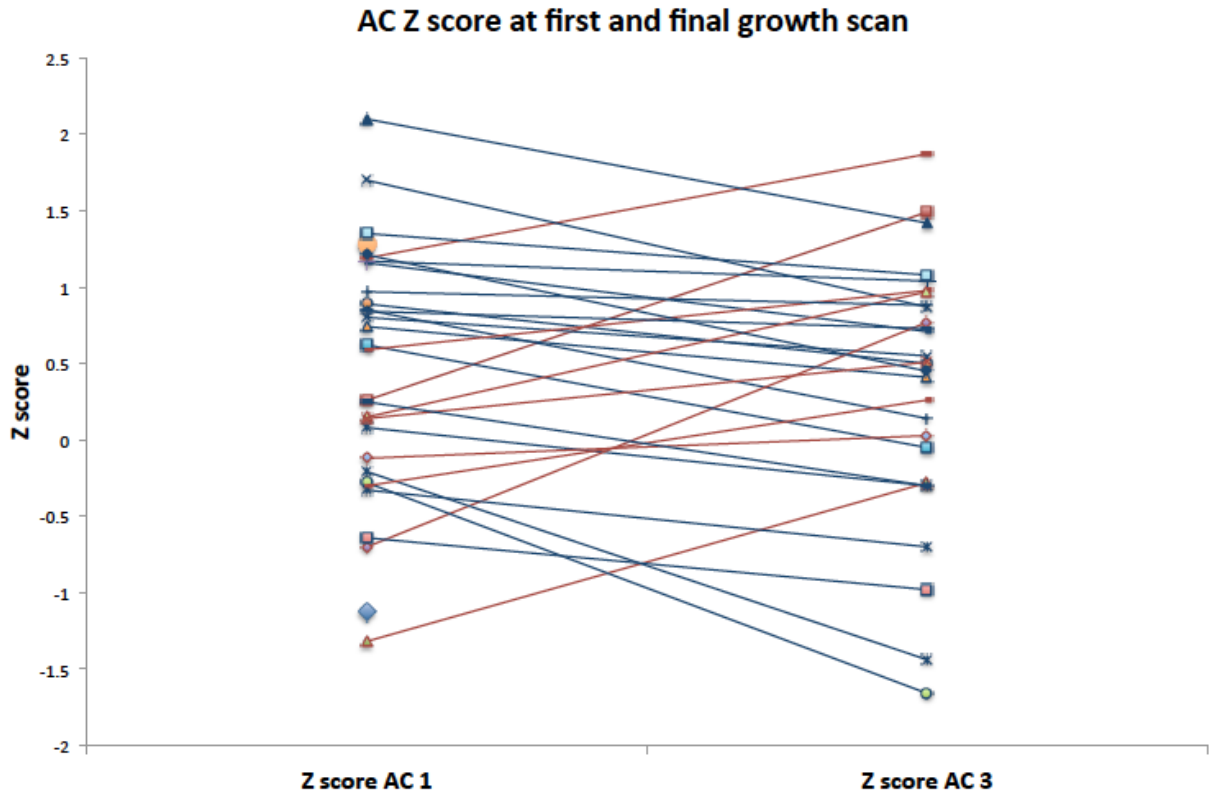
	CHD	Controls	P value
Initial growth scan HC Median z score (IQR)	-0.08 (-0.7-+0.41)	0.47 (-0.34-1.11)	0.004*
Initial growth scan AC Median z score (IQR)	0.61(-0.19-1.11)	0.66 (0.2-1.64)	0.042*
Median HC:AC ratio initial scan	1.14 (1.1-1.18)	1.15 (1.12-1.18)	0.327
Final growth scan HC Median z score (IQR)	-0.63 (-1.16-+0.08)	0.2 (-0.4-0.86)	<0.001*
Final growth scan AC Median z score (IQR)	0.5 (-0.28-+0.88)	1.17 (0.64-1.64)	<0.001*
Median HC:AC ratio final scan	1.03 (1-1.07)	1.03 (0.98-1.08)	0.843

*p<0.05 statistically significant

3.4.5 Comparison of median AC Z-score at first and last scan in fetuses with CHD.

19/28 (68%) of cases showed a decrease in AC Z-score from the first to the last scan, whereas 9/28 (32%) patients demonstrated an increase in the AC Z-score from the first to the last scan. One patient only had one measurement of the AC (see figure 18). AC Z-scores for the first and final scan were compared, finding no significant difference between first and final scan (P=0.421).

Figure 18: AC Z-score at first and final growth scan

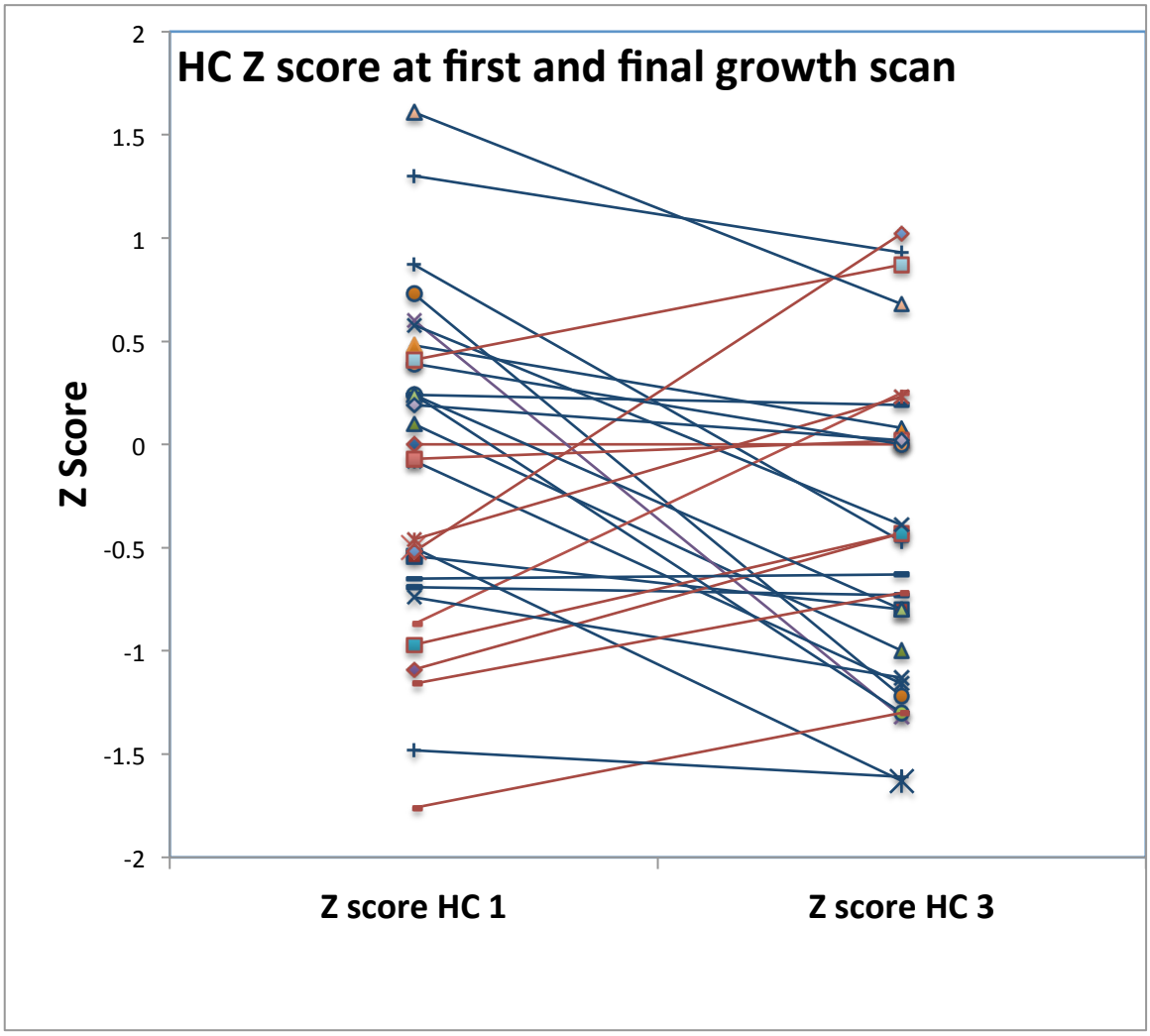


3.4.6 Comparison of median HC Z-score at first scan and last scan in cases

19/29 patients (66%) showed a decrease in HC Z-score from the first to the last scan; 10 cases (34%) showed an increase (figure 19).

Head circumference Z-scores for the first and final scan were compared. No significant difference was demonstrated between first and final scan (p=0.167).

Figure 19: HC Z-score at first and final growth scan



3.4.7 Comparison of diagnostic subgroups

Comparison of median HC Z-score at final scan for each of the 3 diagnostic subgroups compared to the controls.

Table 9: comparison of HC in the three diagnostic subgroups

Group 1 compared to controls (n=1; 1 patient had single growth scan)	P=0.12
Gp 2 compared to controls (n=22)	P=0.002*
Gp 3 compared to controls (n=5, 4 valid)	P=0.046*

3.5 Discussion

This chapter has assessed the growth of fetuses with CHD compared to normal controls using the parameters of head and abdominal circumference. The measurements of both HC and AC in fetuses with CHD were smaller at both the initial and final growth scans, with a highly significant difference at the final scan suggesting a slower rate of growth as pregnancy advances.

These findings are in keeping with the majority of studies that have also demonstrated impaired growth in utero of fetuses with CHD[24-26, 31, 147, 148].

One contrasting study is a case control study by Perez-Delboy that found that CHD fetuses did not demonstrate a pattern of reduced growth once patients with chromosomal and structural abnormalities were excluded. In the present study, patients with known chromosomal and extracardiac abnormalities were excluded although (as described in the outcomes chapter), 3 cases in the cohort had extracardiac abnormalities detected postnatally.

Comparison of initial and final scan showed a trend towards smaller HC at the final scan (although not reaching statistical significance). This finding, if confirmed in larger studies, would support the theory of the congenital heart defect leading to the disturbance in growth, as it would be anticipated in this circumstance that, with advancing gestation, the effect on growth would become more pronounced with time. One recent study has addressed the timing of onset of growth abnormalities and found that in 95 fetuses studied, a high proportion of fetuses with CHD have a smaller head and increased brain perfusion in the second trimester [149]. This is not dissimilar to our finding of smaller HC and AC at the first growth scan in addition to the later scan.

In this study, in contrast to other published studies, which grouped the cases by lesions, cases were sub-grouped by the anticipated flow and substrate delivery to the brain. This grouping aimed to differentiate how these factors contribute to the abnormalities of growth, whilst avoiding having a large number of small sub-groups with low statistical power. The basis for this subgrouping is the presumed mechanism, whereby differences in tissue oxygen delivery occur due to disruption of streaming of blood by the presence of CHD. In a normal fetus, the oxygen rich blood returning from the placenta in the umbilical vein preferentially passes across the foramen ovale to the left atrium, through the mitral valve to the left ventricle and is

ejected into the aorta supplying both the coronary arteries and head and neck vessels. In this way, oxygen rich blood reaches the brain and oxygen poor blood is returned to the placenta. However, despite this sub division, when analysing the subgroups in this study it was not possible to draw clear conclusions, due to the small numbers, particularly in the more severe abnormalities that had a lower anticipated oxygen delivery and abnormal pattern of flow to the cerebral circulation. Other studies have had similar difficulties in the analysis of subgroups due to the small numbers. Further larger studies are required to elucidate the mechanism more clearly.

3.6 Conclusion and clinical implications

The findings in this chapter are consistent with previous findings of abnormal in utero growth of fetuses with CHD. From a clinical perspective, the findings support the serial monitoring of the growth in pregnancies affected by CHD. Current practice is to apply the same guidelines for bringing forward the timing of delivery to those applied to growth restricted babies of other aetiologies. Further research may provide additional insight into whether more specific guidelines are needed in this group of patients, particularly as there is evidence that early delivery is detrimental to the neurodevelopmental outcome of children with CHD[84]. In addition, the abnormalities of growth seen give some clues as to the potential impact of the heart defect in utero on the developing brain and other organs, and provide a rationale for further study in detailed effects of congenital heart defects on the both overall and brain growth.

3.7 Limitations

The gestation of cases was widely spread at the initial growth scan compared to the gestation of controls, that were clustered towards earlier gestation. The reason for this is that the CHD patients would have had a scan performed at the time of referral for the cardiac defect. This time varies depending on the time of detection of the abnormality and additional time between detection of the abnormality and referral to the tertiary cardiac unit. In contrast, the initial growth data for controls were from the time of the anomaly scan, which in the cast majority of cases is between 19 and 22 weeks.

In the selection of controls, an inevitable bias may be introduced by excluding any patient with maternal illness; therefore this study, in common with others, does not truly represent a normal population.

The study is limited by the small number of cases with CHD and analysis of a larger cohort would be recommended.

Chapter 4: Arterial Doppler in Fetuses with Congenital heart disease.

4.1 Introduction

In addition to the observation that head growth may be impaired in fetuses with CHD, it has also been suggested that fetuses with heart defects may demonstrate abnormalities of cerebral blood flow. In the normal fetus, the oxygen delivery to the brain is controlled through a range of autoregulatory physiological changes[150, 151]. Pulsed wave Doppler techniques to measure flow in the middle cerebral artery can be used to quantify changes.

4.1.1 Middle Cerebral Artery (MCA) Doppler

Measurement of the cerebral artery Doppler provides a useful estimate of cerebrovascular resistance. The MCA Pulsatility index (peak systolic velocity-end diastolic velocity / time averaged velocity) is used as a tool to assess the downstream resistance of the cerebral vasculature. A number of investigators have shown that fetuses with congenital heart disease display abnormal patterns of cerebral blood flow[66-68].

4.1.2 Umbilical Artery Doppler

The Umbilical artery Doppler is a widely used tool in monitoring fetal well-being. The pulsatility index is used, in a similar manner to the MCA-PI, but in this case to measure the downstream placental resistance.

4.1.3 Cerebro placental ratio

This ratio comparing the MCA-PI and UA-PI reflects the relative resistance of the cerebral and placental vascular beds. It is used to quantify redistribution of cardiac output to the cerebral circulation. In a fetus with normal heamodynamics, the resistance in the cerebral vasculature is higher than the resistance of the placental vessels. With a fall in cerebrovascular resistance that occurs with hypoxia, this ratio changes such that the resistance in the cerebral circulation falls. This is reflected in a cerebro-placental (cerebro-umbilical) ratio changing from greater than one (normal) to less than 1 (suggestive of redistribution ‘brain sparing’).

4.2 Methods

4.2.1 Participants

25 of the 29 Patients in the CHD study group had umbilical artery pulsed wave Doppler analysis. 19 of these 29 patients in the congenital heart disease study group had middle cerebral artery pulsed wave Doppler analysis. In those that had both, a cerebro-placental ratio was calculated.

4.2.2 Techniques

Fetal echocardiograms and measurement of cerebral and umbilical blood flow were performed on Voluson E8 (BT08) system with RAB 4-8L 3D hybrid abdominal probe (GE, Chicago, Ill).

Pulsed wave Doppler was used to acquire waveforms from the middle cerebral artery (MCA) and umbilical artery (UA) using published guidelines from the International Society of Obstetrics and Gynaecology (ISUOG)[59].

All pulsed wave Doppler measurements were obtained in the absence of fetal movements with maternal breath hold when necessary.

Measurement of MCA Doppler

In an axial section of the brain, the thalami and sphenoid bone wings were identified. The circle of Willis was then identified. The blood vessel was initially identified by colour flow mapping. The cursor was aligned as near as possible with blood flow and always with an angle of insonation of < 20 degrees. A minimum of 4 cardiac cycles

was displayed. Doppler measurements were taken from the proximal third of the MCA with an angel of insonation as close to zero as possible and < 20 degrees.

To account for differences in gestational age, MCA-PI z scores were calculated from published normal reference ranges[52].

Measurement of umbilical artery Doppler

Measurements were made from a free loop of umbilical cord. The pulse Doppler gate was aligned as near as possible with blood flow and always with an angle of insonation of < 20 degrees. A minimum of 4 cardiac cycles was displayed. The pulsatility index (peak systolic velocity- end diastolic velocity/ time averaged mean velocity) was recorded in each patient.

The cerebro-placental ratio (MCA-PI divided by the UA-PI) was calculated for each patient.

4.2.3 Statistics

Normally distributed continuous data were expressed as mean (SD). Non-normally distributed data were expressed as median (IQR). Categorical variables are expressed as proportions (%). MCA PI Z-scores were calculated using previously published normal data from Parra-Cordero et al [52].

4.3 Results

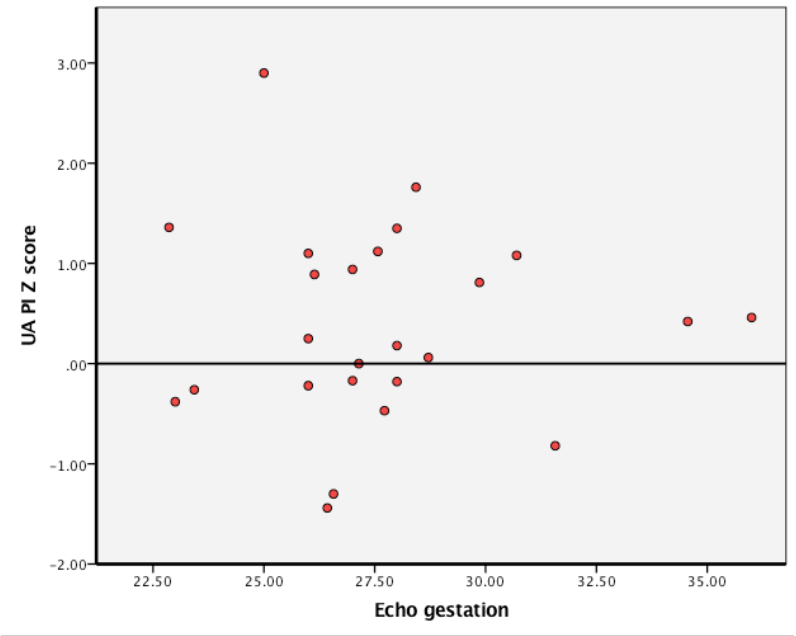
4.3.1 Umbilical artery Doppler

25 of the 29 cases in the CHD study group had umbilical artery pulsed wave Doppler analysis. The mean gestational age was 27.9 weeks (SD 3.26 weeks) and ranged between 22.9 and 36weeks.

The umbilical artery PI Z-scores were analysed as a whole and in the three diagnostic subgroups. In group 1 there were 2 cases with UAPI Z-scores of 1.76 and 1.1; in group 2 there were 20 patients with a median umbilical artery Z-score of 0.34 (IQR -0.34-1.05); in group 3 there were 3 cases with UAPI Z-scores of 0.0, 1.2, -0.17.

Figure 20 below shows the umbilical artery PI Z-score for all patients plotted against the gestation at the time of scan, demonstrating a relatively even spread of data throughout the range of normal population Z-scores.

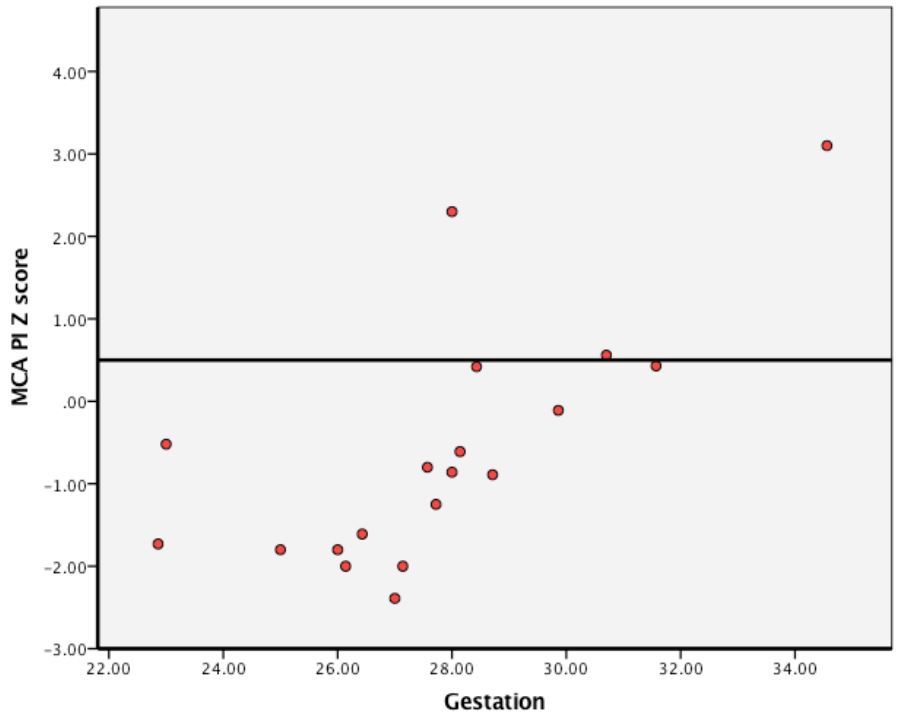
Figure 20: Scatter graph of umbilical artery PI Z score for cases with CHD



4.3.2 MCA Doppler analysis

19 of the 29 cases in the congenital heart disease study group had middle cerebral artery pulsed wave Doppler analysis. The mean gestational age at the time of measurement was 27.73 weeks (SD 2.77weeks), range 22.86-34.56 weeks. The median MCA-PI Z score of the group was -0.86 (IQR –1.8- 0.42); range -2.39-3.1. The MCA PI Z score and the gestation at the time of measurement are shown in figure 21 below. 16/19 (84%) cases had a negative Z-score compared to 3 with positive Z-scores. MCA PI Z scores for subgroups was not possible as out of the 19 patients there was only one case in each of groups 1 (MCA PI Z score 0.42) and 3 (MCA PI -2).

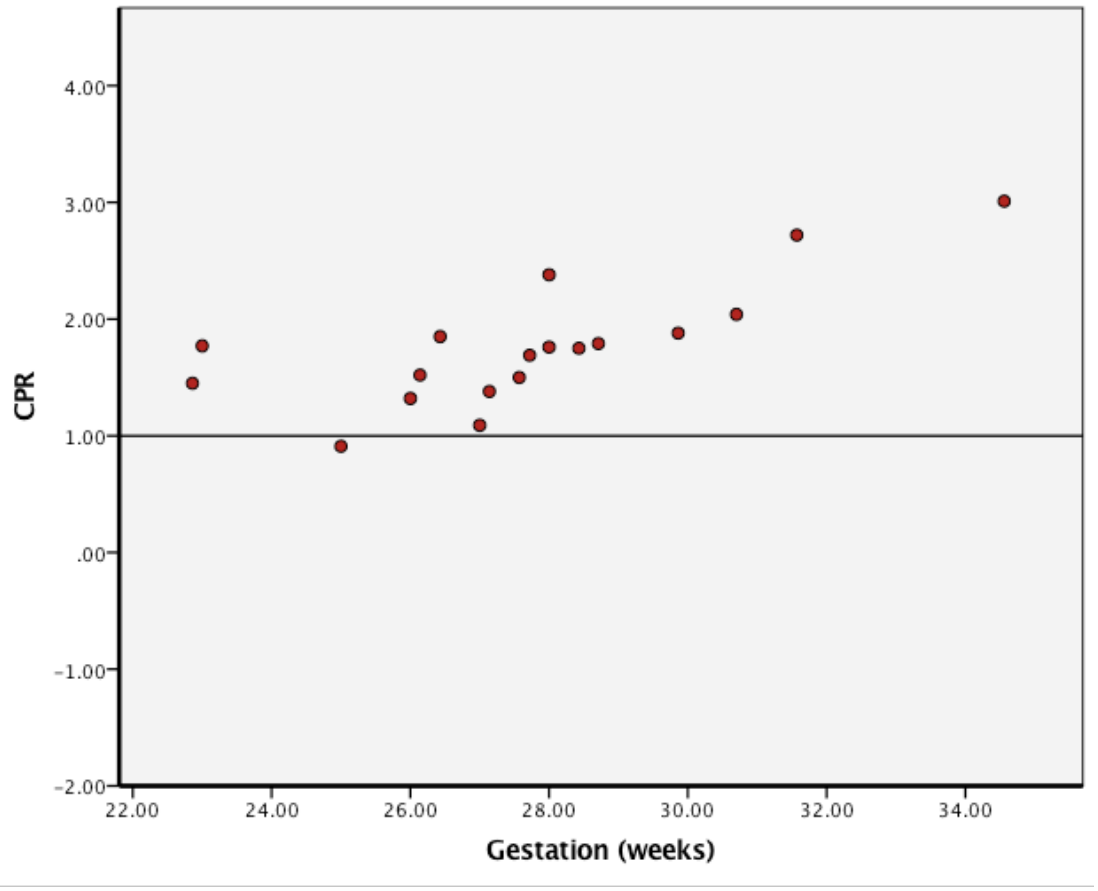
Figure 21: Scatter graph of middle cerebral artery PI Z scores for cases with CHD



4.3.3 Cerebro-placental ratio (CPR)

19 patients had a cerebral placental ratio (CPR) calculated. 18 cases had a CPR of greater than one; only one patient had a CPR of less than one [152].

Figure 22: scatter graph of cerebro placental ratio in cases with CHD



4.4 Discussion

The findings of this chapter show that, although study patients demonstrated lower MCA PI Z-scores, which are suggestive of reduced cerebral vascular resistance, no difference was observed in umbilical artery Z-scores and when the cerebro-placental ratio was calculated, all except one value was in the normal range.

4.4.1 Mechanisms of abnormal MCA Doppler

In a healthy fetus, most of the flow occurs in systole with a very small amount in diastole. In the setting of hypoxia, the cerebral arteries dilate resulting in an increase in diastolic flow through the cerebral vasculature. The increase in diastolic flow results in a decreased pulsatility index (peak systolic velocity-end diastolic velocity / time averaged velocity). The fetuses in this study demonstrated lower MCA PI Z-score, suggesting that there is cerebral vasodilation in response to the lower oxygen content of cerebral blood flow in the study group.

The finding of normal umbilical artery PI Z-scores differs from that seen in fetuses with placental insufficiency and growth restriction. In this setting, along with the reduced resistance in the cerebral vascular bed (reflected as a low MCA-PI), these fetuses also characteristically have an increased resistance in the placental vascular bed (reflected as a high UA-PI). The umbilical arteries carry blood from the fetus back to the placenta. The blood flow in these vessels is therefore dependent on two factors, the first is cardiac output of the fetus and secondly the resistance in the placental vascular bed. Contrary to the situation in IUGR, there is not generally abnormal placentation in CHD so, unless the cardiac defect causes a reduction in

cardiac output, the UA-PI would be expected to be normal. Some studies in CHD have observed changes in the UA-PI and resultant changes in the CPR suggestive of brain sparing. One possible explanation for this is that this is seen in cases at the most severe end of the spectrum of congenital heart disease where the cardiac output may be reduced. This is supported by recent data from fetal cardiac MRI showing a 30% reduction in combined ventricular output in fetuses with single ventricle heart lesions compared to those with bi ventricular heart defects[21].

4.4.2 Doppler abnormalities in subgroups of CHD

Previous studies that have addressed the question of whether fetuses with CHD show evidence of brain sparing have reported some inconsistent results. Donofrio et al. showed a reduction in cerebral vascular resistance similar to that seen in fetuses with growth restriction, whereby a reduction in cerebrovascular resistance results in an increase in flow to the brain relative to the systemic circulation. One of the larger studies, by Berg et al., which was well controlled in terms of excluding fetuses with potential confounders such as extracardiac abnormalities and fetal growth restriction (FGR), showed abnormalities were only detectable in fetuses with HLHS. In this current study, analysis by subgroups was limited by the small numbers in individual groups; however, the patients in group 2 (with anticipated lower oxygen delivery to the brain) did demonstrate a lower MCA PI Z-score compared to controls, suggesting a degree of cerebrovascular dilation in response to the lower oxygen content of cerebral blood in lesions other than HLHS. Kaltman and colleagues showed that the pattern of abnormality appears to be influenced by the type of congenital heart defect, with a decreased MCA PI in left sided heart lesions and increased MCA PI in

right sided lesions. In this current study, the patients were grouped differently on the basis of the anticipated flow and substrate delivery to the brain, which may be more relevant in terms of explaining the haemodynamic effects on brain growth.

In this study, group 2 was disproportionately large compared to the other groups. In many heart defects the systemic oxygen saturation of blood is lower due to mixing within the heart of oxygenated and deoxygenated blood. In these lesions there is conflicting evidence from studies as to whether the cerebral vascular resistance is lower[69, 70, 153]. Those lesions which display the most significant abnormalities appear to be those in which, in addition to mixing, there is obstruction to the outflow of the left sided of the heart. In these cases, the blood flow in fetal life reaches the cerebral circulation in a retrograde direction supplied by arterial duct with flow reversal in the transverse aortic arch.

Changes over the course of pregnancy appear to be progressive with the lowest MCA-PI in the third trimester [70]. One study has linked these changes in cerebrovascular resistance to neurodevelopmental outcome, with those fetuses with lower cerebrovascular resistance displaying higher outcome scores on neurodevelopmental testing. This is an interesting finding, as it is the opposite to that observed in other disease states such as IUGR, where low resistance confers an increased risk of ischaemic brain damage suggesting a degree of compensation for the reduced cerebral oxygen delivery in single ventricle heart defects [72].

4.5 Clinical implications

Further information is needed on how lower cerebrovascular resistance manifests in terms of outcome. It is not clear whether this response reflects better adaptation in some fetuses that could translate to improved outcome or if it is indicative of a more severe reduction to brain oxygen and substrate delivery.

4.6 Limitations

One of the main limitations was the small size of the study group and, in particular, that it was not possible to draw any meaningful analysis from the sub groups due to small numbers in some groups. This has been a major limitation of many of the published studies, as to determine cause and effect of the cardiac lesion on the cerebral haemodynamics requires a large number of patients with very similar lesions. In practice this is difficult to achieve due to the variable anatomy of congenital heart defects and relatively low prevalence.

Serial measurements would have been useful to determine potential differences in significance of changes in cerebral blood flow depending on the time at which it is observed.

Chapter 5: MRI Brain volumes in fetuses with CHD

5.1 Introduction

The interest in MRI examination of the fetal brain in CHD evolved from the observation of high rates of development impairment and concern over the potential for neurological damage resulting form cardiac surgery and intervention. However, when MRI studies were performed on these children pre and post operatively, it was found that non only did they have neuroimaging abnormalities after surgery, but there was also high prevalence of MRI abnormalities present on brain imaging pre-operatively, showing that, in a significant number of cases, abnormalities were present pre-intervention[110, 113]. This challenged the assumption that lesions were the result of the intervention and surgery that these children undergo. In attempting to understand the aetiology and elucidate the mechanisms that may lead to brain abnormalities and impaired neurodevelopment in these children, the question that perhaps the origins lie in fetal life was raised and the interest in imaging the fetal brain in this group developed. However, our understanding of brain development in congenital heart disease is incomplete and the subgroups in which this has been described are limited.

5.2 Aims

In this chapter the aim was to describe the fetal brain in a cohort of fetuses with CHD using MRI techniques to establish whether fetuses with CHD demonstrated abnormalities of brain development and correlate this with the anticipated alteration in cerebral oxygen delivery and flow.

5.3 Hypothesis

Fetuses with congenital heart disease will display smaller brain volume on MRI and this can be predicted by the pattern of oxygen delivery based on the category of abnormal flow.

5.4 Methods

5.4.1 Participants

29 cases of isolated CHD were included for analysis of fetal brain volumes. (One patient was excluded from analysis as in the early neonatal period they developed severe brain abnormalities and died as a result of a postnatally-diagnosed inborn error of metabolism).

17/29 patients had a single prenatal MRI scan and 12/29 patients had scans at 2 time-points in pregnancy.

5.4.2 Controls

83 normal controls, within the same gestational range as cases, were used to compare brain biometry, supratentorial brain volumes and ventricular volumes. In addition, a larger cohort of 127 fetuses, which includes the above 83 controls was used to calculate brain volume Z-scores (Dr V Kyriakopoulou, Prof M Rutherford,

data submitted for publication).

5.4.3 Imaging Protocols

Imaging was undertaken according to the protocol described in the methods section (Chapter 2). In addition to volumetric analysis for research purposes, all scans were formally reported by a specialist in perinatal brain imaging (MR). A standard reporting scheme was used which systematically assessed the following (see appendix 5):

Extracerebral - shape and size of the skull and brain, BPD (measured from the outer to inner edge of the bone)

Ventricular system – shape and size of lateral third and fourth ventricles, appearance of germinal matrix, size of cavum septum pellucidum

Cortex configuration

Corpus callosum

Basal ganglia and thalami

Internal capsule

Cerebellum – shape size signal intensity, TCD and vermis height

Brain stem – shape size signal intensity, AP pons diameter, pituitary, optic nerve and lenses.

5.4.4 Statistical analysis

General statistical analyses were performed as described as in the Methods (chapter 2). Correlation between variables was measured using Spearman's rank coefficient. Linear regression models were created for cases and controls to adjust for the effect

of increasing gestation. The variation of data points from the calculated regression line were expressed as R2 values (correlation coefficient). Regression coefficients (the slope of the regression line) for cases versus controls were compared using analysis of the variance (ANOVA). A p value of < 0.05 was considered significant in all cases.

5.5 Results

5.5.1 Baseline characteristics

The median gestational age of fetuses with CHD at the time of the initial MRI scan was 27.3 weeks (IQR 25-27.9). This compared to the median gestational age in controls of 28.3 weeks (IQR 25.3-32); p=0.033.

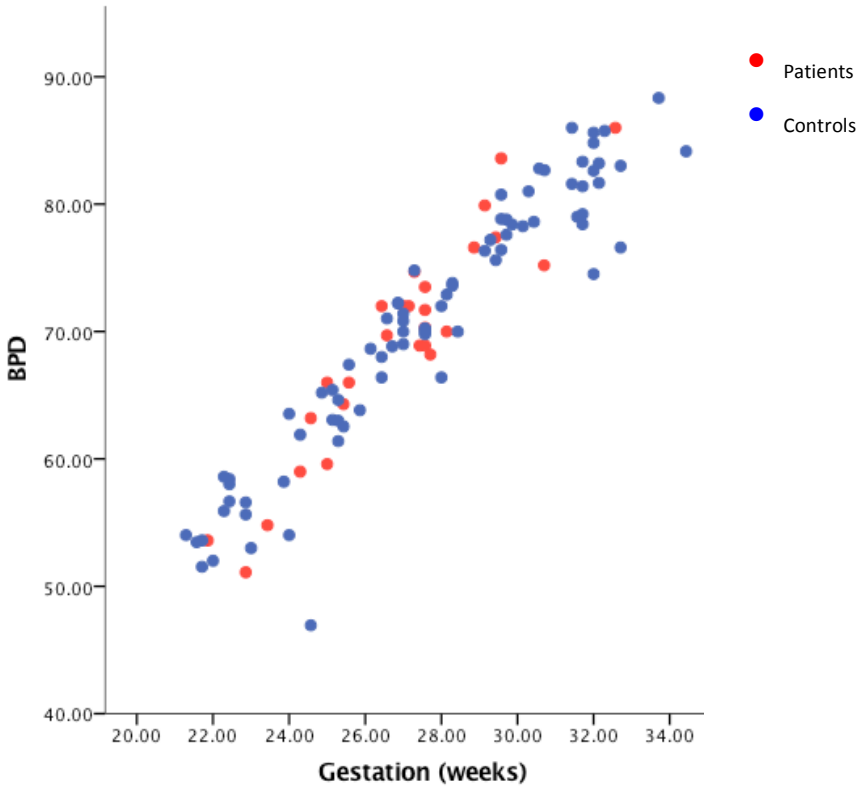
5.5.2 Structural brain abnormalities

No major structural abnormalities were identified (in keeping with the exclusion criteria of any patient with major structural abnormality identified on ultrasound imaging being excluded from the outset). In 28 patients (97%) the MRI was reported as showing no structural abnormality. In one case (case 16) the lateral ventricles and 4th ventricle were slightly prominent, although measuring within normal range, and mild cerebellar vermis rotation was noted.

5.5.3 Biometry brain structures - Biparietal diameter

There is a linear increase in BPD with gestational age in both cases (R2=0.871, p<0.01) and controls (R2=0.970, p< 0.01). Figure 23 shows the spread of BPD values in fetuses with CHD and controls.

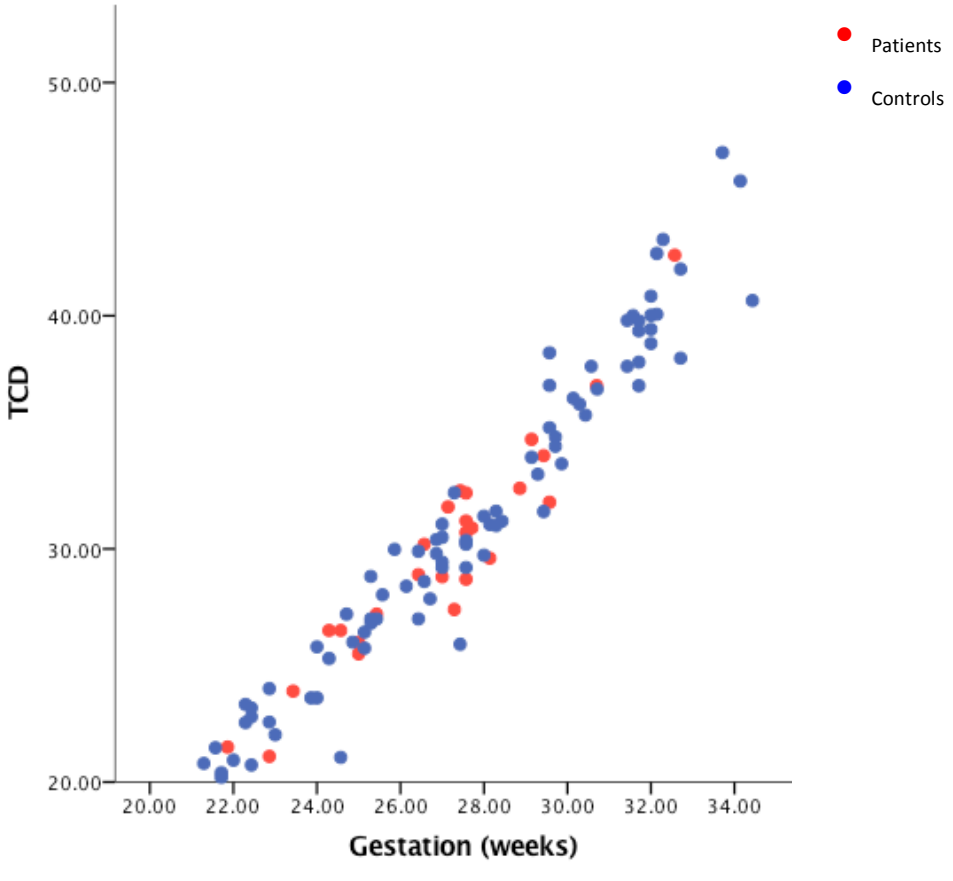
Figure 23: Change in biparietal diameter (BPD) in fetuses with CHD and controls



5.5.4 Biometry of brain structures - Transcerebellar diameter (TCD)

There was a linear increase in TCD with gestational age in both cases (TCD R2= 0.901 ($p<0.01$)) and controls (R2=0.982 ($p<0.01$)). Figure 24 shows the spread of TCD values in fetuses with CHD and controls.

Figure 24: Change in trans cerebellar diameter (TCD) with gestation in fetuses with congenital heart disease and controls

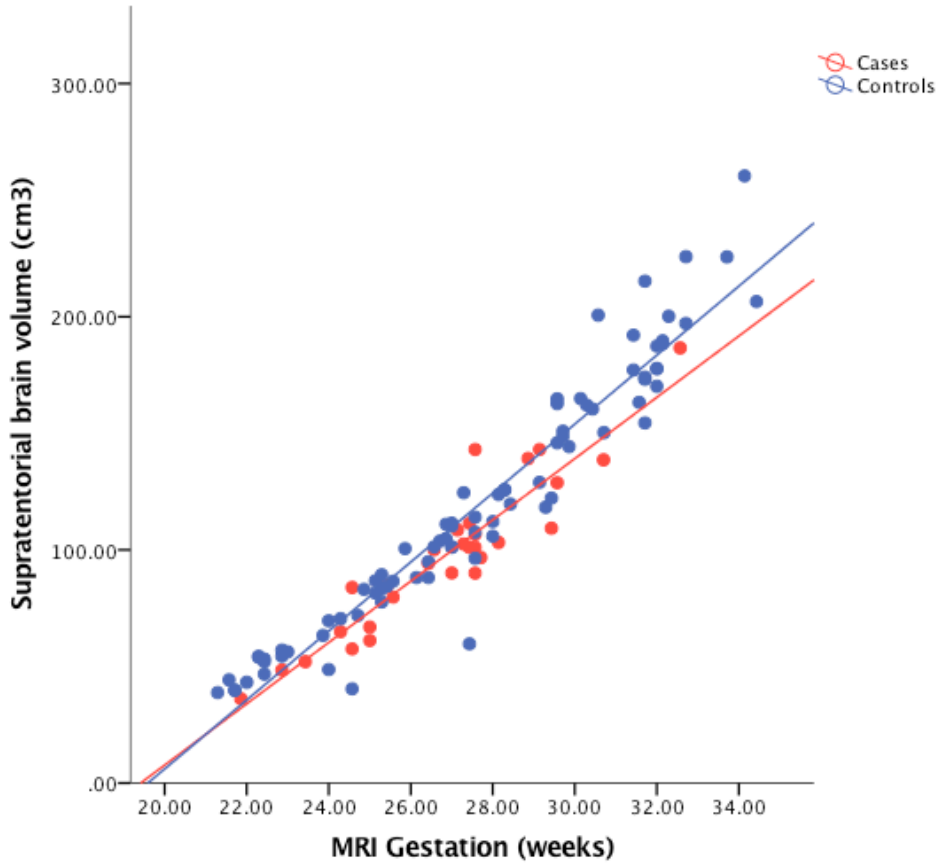


5.5.5 Volumetric measurements of brain - Supratentorial brain volume (SBV)

The SBV increases linearly with advancing gestational age in both cases (R2 = 0.897 p<0.01) and controls (R2 = 0.981 p<0.01).

The regression coefficient (slope of the regression line) for cases with CHD was significantly lower than controls (P<0.01).

Figure 25: Grouped linear regression comparing whole brain volume with increasing gestation in fetuses with CHD and controls.



5.5.6 Volumetric measurements of brain - Ventricular volumes

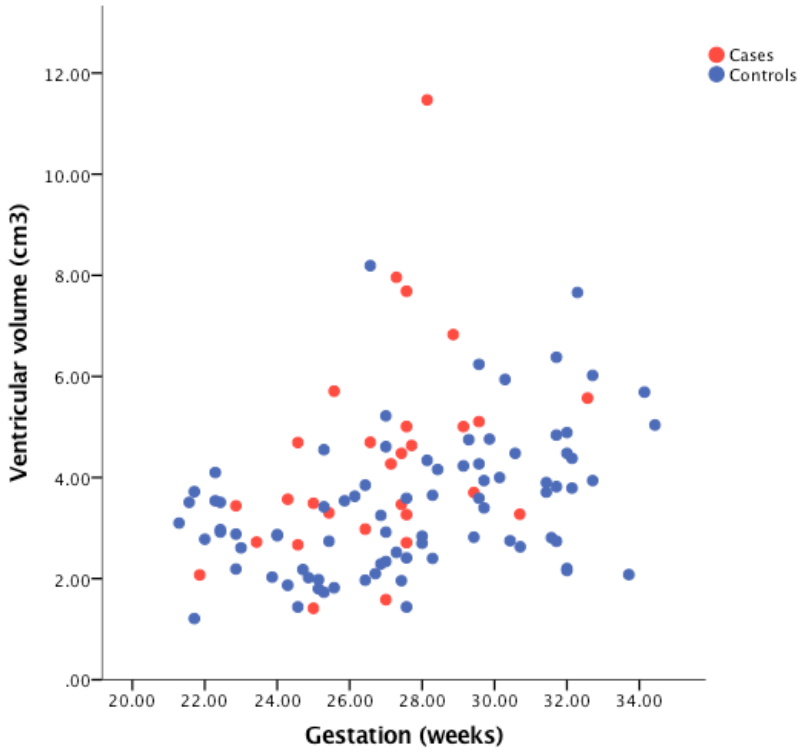
There was a weak correlation between ventricular volumes and gestation in both groups (cases R2 = 0.489, p<0.01; controls R2 = 0.502 ,p<0.01).

The median ventricular volume in cases with CHD was 3.70cm3 (IQR 3.12-5.06)

In controls the median ventricular volume was 3.54 cm3 (IQR 2.59-4.48)

The ventricular volumes in cases and controls are shown in the scatter graph below (figure 26)

Figure 26: Scatter graph comparing cerebral ventricular volume with increasing gestation in fetuses with CHD and controls.



In comparing ventricular volume (figure 26), there was one patient (case 16) that was an outlier, with ventricular measurements at the upper end of normal on biometry but ventriculomegaly on volumetric analysis. There had been concern about an underlying genetic syndrome prenatally but due to normal karyotype and normal postnatal examination the patient has been included in the study.

5.5.7 Subgroup analysis

The whole brain volume (excluding ventricular volume) was plotted for the different subgroups of CHD in the study. Due to small numbers in each subgroup, a valid statistical analysis was not possible at this stage. The graph showing the brain volumes in each of the subgroups is shown below.

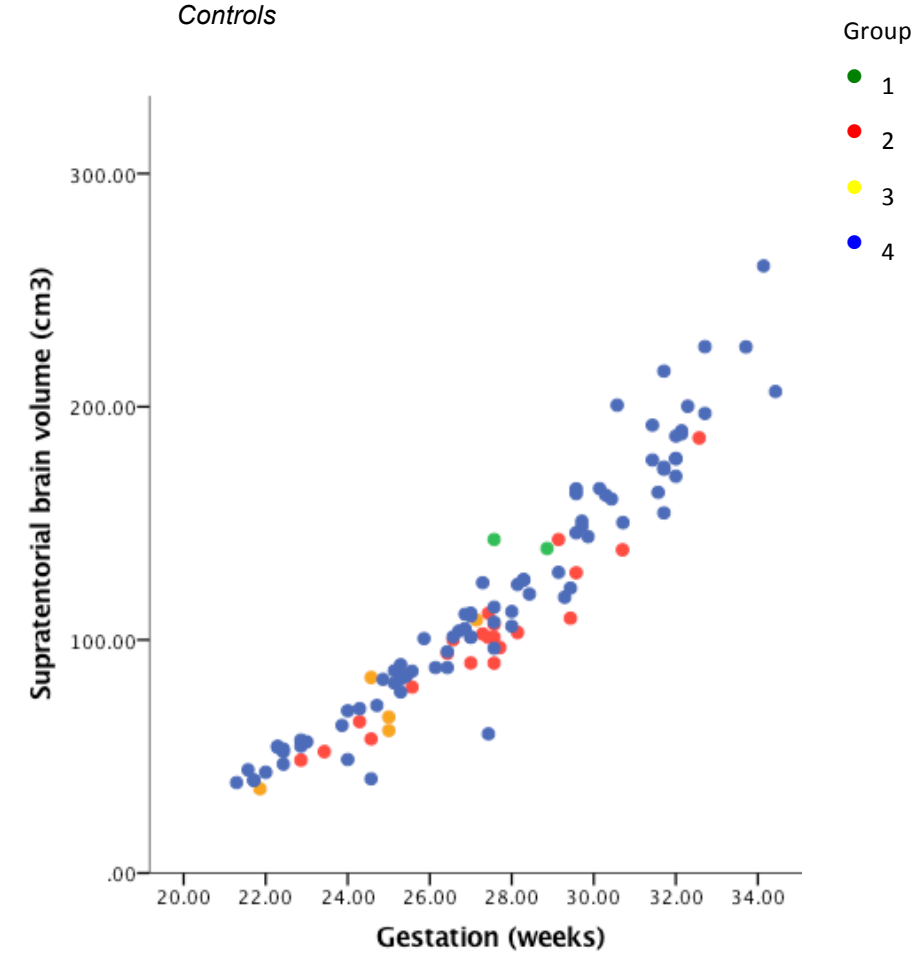
Figure 27– Whole brain volume-ventricular volume showing subgroups according to type of congenital heart defect.

Group 1 *Decreased volume of cerebral blood flow*

Group 2 *Decreased oxygen saturation of cerebral blood flow*

Group 3 *Combination of decreased volume of cerebral flow and decreased oxygen saturation of cerebral blood*

Group 4 *Controls*



5.5.8 Comparison of Z-scores across gestational range

The mean Z-score for cases at initial scan was -0.53 (SD 1.31). 14/22 mothers agreed to have a second MRI scan in pregnancy. In two cases the examination had to be abandoned due to maternal discomfort at advanced gestation. For the 12 patients that had a second scan in pregnancy the mean Z-score was -2.10 (SD 2.31). Comparison of Z-scores at first and final scans using paired t-test (p=0.05).

5.5.9 Z-scores in diagnostic groups

Mean Z-scores were calculated for each diagnostic group and are shown in table 10 below:

Table 10 Mean Z-scores for diagnostic subgroups

	Number of cases	Mean Z score (SD)
Group 1	2 cases	not calculated
Group 2	22 cases	-0.61 (SD 0.75)
Group 3	5 cases	-1.33 (SD 1.98)

Comparison between group 2 and 3 P=0.182

Z-scores were calculated in groups of 3 weeks gestation and are shown in Table 11 (first and second scans included).

Table 11: Median Z scores in 3 weekly intervals

Gestational range (weeks)	Median Z score
21-23+6 weeks	-1.46
24-26+6 weeks	-0.66
27-29+6 weeks	-0.08
30-32+6 weeks	-0.38
33-36 weeks	-1.45

5.6 Discussion

5.6.1 Summary of findings

The main finding of this chapter is that brain volumes were smaller in fetuses with CHD than normal controls and that with advancing gestation this difference became more marked, implying that CHD results in a slower rate of brain growth.

5.6.2 Structural brain abnormalities

Of the 29 patients in the study there was one patient (3.4%) with a mild structural abnormality (prominent lateral and 4th ventricles measuring within normal range and mild cerebellar vermis rotation). This is not dissimilar to the reported prevalence by Limperopoulos et al., where 6/105 (5.7%) had minor brain abnormalities with similar

exclusion criteria of any patient that had a structural brain abnormality on cerebral ultrasound or those with identified underlying genetic abnormalities. Brossard-Racine reported that a significantly higher proportion (23%) of their study population had structural brain abnormalities - the abnormalities identified are summarised in table 12 [107]. This wide variation in the reported incidence of structural brain abnormalities is likely to represent differences in methodology of patients that are included; for example, in the present study, there may have been increased rates of genetic testing antenatally, resulting in more patients with known genetic abnormalities being excluded. It is not clear if any of the patients were identified postnatally to have genetic abnormalities, which may be important, as these may not always be apparent until after birth or in some cases on longer term follow up.

Despite the differences in reported prevalence, it is clear that there is an association between CHD and structural brain abnormalities[154]. Further studies are required to achieve a greater understanding of the genetic basis of CHD and this may, in time, help to explain the increased association of structural brain abnormality in these patients.

Table 12: Structural brain abnormalities in fetuses with CHD

Structural brain abnormalities recognised in fetuses with CHD[107]
Ventriculomegaly
Increase extra axial spaces
Vermis hypoplasia
Cysts
Increased WM signal intensity
Immature appearance

5.6.3 White matter injury

Another feature of interest is that postnatal preoperative studies have reported that up to 50% of babies exhibit abnormalities on brain MRI[110], most of these as focal white matter lesions[101, 113]. However, in this study, none of the cohort had white matter lesions and this appears to be less prevalent in the fetal population with CHD. One explanation for this may be the difficulty seeing punctate white matter lesions on fetal brain imaging. Postnatally they are best seen on T1 weighted sequences, which have a much more limited use in the fetus due to fetal movement. If it is not simply a case of reduced detection, this then raises the question of how and when these white matter lesions are acquired. Post mortem studies have suggested that these lesions represent hypoxic damage to immature oligodendroglia during the myelineation process, which is a time at which they exhibit particular vulnerability[155]. Added to this, previous studies have suggested that, in addition to the brains being smaller in CHD compared to normal fetuses, the brains may be

immature[156]. This may suggest that these immature brains have a vulnerability that is not present in term infants, which leads to the acquisition of white matter injury during the delivery and early preoperative days in a similar manner to premature infants. Clinically this is supported by more recent evidence of worse outcome after surgery in patients with CHD that are born at early term (37-38 weeks)[157].

5.6.4 Supratentorial brain volumes

The finding in this chapter that age adjusted brain volumes are smaller in fetuses with CHD than normal controls is consistent with the fetal MRI findings of Limperopoulos et al[120].

To fully elucidate the mechanisms involved in this process, it is vital to be able to clearly describe the oxygen delivery and blood flow to the cerebral circulation. Other studies have shown brain volumes to be reduced in specific subgroups of CHD, in particular hypoplastic left heart syndrome and transposition of the great arteries. The present study had a diverse mixture of underlying heart defects but still demonstrated a reduction in brain volumes over the third trimester of pregnancy, suggesting that the effects are widespread in congenital heart defects.

In this study, it was attempted to group patients on the basis of the delivery of oxygen that would be expected to the fetal brain rather than anatomical variables; however, in the majority of defects the main abnormality is the mixing of oxygenated and deoxygenated blood in the heart without the ability to quantify this. It is notable that in the subgrouping of patients for this study, there was not always complete agreement on how the observers thought that the cardiac abnormality would affect

cerebral oxygen delivery and blood flow. Some evidence for reduced oxygen delivery to the developing fetal brain is gained from the findings of Chapter 4, where a reduction in MCA PI suggests evidence of alterations in cerebral vasculature, a recognised response to hypoxia in the fetus. Further insight is likely to come from emerging studies using fetal cardiac MRI, which involve methods to quantify the previously assumed reduction in cerebral oxygen delivery and cerebral blood flow. Evidence from these studies also supports the link between reduced cerebral oxygen delivery and impaired brain growth[21].

5.6.5 Rate of brain growth in fetuses with CHD

In this study, the difference in brain volumes became more pronounced with advancing gestation, implying that CHD results in a slower rate of brain growth. If the underlying cause for the reduced brain volume is reduced oxygen delivery to the fetal brain, then this appears to have a greater impact as the third trimester of pregnancy advances. This may perhaps be explained by the high rate of growth in the third trimester. Alternatively, there may be genetic and epigenetic mechanisms common to cardiac and brain development that may result in the abnormalities observed.

5.6.6 Cerebellar growth in CHD

There have been no previous studies that have looked specifically at cerebellar growth in fetuses with congenital heart disease. In this study population, there was a linear increase in cerebellar growth with gestation that did not differ significantly from

controls. There is evidence of a deleterious effect of CHD on cerebellar growth in newborns. Zeng et al. looked at the growth of intracranial structures in fetuses with CHD on ultrasound and found a reduction of global and regional volumes including cerebellar volumes [34]. Ortinau and colleagues showed that infants with CHD had smaller brain and cerebellar volumes but that there was a trend towards greater growth of the cerebellum over the first 3 months of life compared other brain structures. This contrasts with the cerebellum being reported to be smaller than controls in adolescents with CHD in the setting of globally reduced brain volumes [37]. This may suggest that the rate of cerebellar growth differs from general brain growth.

5.7 Clinical Implications

The slower rate of brain growth in fetuses with CHD seen in this study supports the theory that the abnormalities seen postnatally in children with CHD may have its origins, at least in part, in the in utero environment. This has important clinical implications as a number of studies have shown that smaller brain volumes correlate with reduced cognitive performance[38, 158] and therefore a better understanding of how abnormal brain development in fetuses with CHD may be the first step to improving the long term neurological outcome of these children.

Studies of the fetal brain in CHD remain limited and there remains much to be learned about fetal brain development in CHD.

5.8 Limitations

The main limitation of this chapter is the difficulty of comparison by subgroups due to the low prevalence of individual lesions. This information is needed to further define the relationship between the haemodynamics and the effect on brain development. A limited amount of longitudinal data was obtained. Some patients may find the MRI scan time consuming, noisy or uncomfortable which contributes to the reluctance to undergo a second scan in pregnancy. Fetal MRI studies are all subject the limitation of fetal movement that resulted in the exclusion of one patient due to poor quality images.

Chapter 6: Short and long term outcomes in fetuses with CHD

6.1 Introduction

There are many factors that contribute to the long-term outcome of a child with CHD. This starts with genetic factors and the in utero environment that we have considered in the last 3 chapters. The next logical point at which the baby may be vulnerable to neurological injury is at the time of labour and delivery particularly if, as suggested in some studies, the brain of these fetuses is immature which may potentially increase their vulnerability to insults. In cases with a prenatal diagnosis of CHD there is the opportunity to make an individualised plan for labour and delivery taking into account maternal indications and risk factors and thereby minimise the risk to the fetus. Following delivery, the treatment of the baby depends on the nature of the heart abnormality, but many cases of major congenital heart disease will need major surgery over the first year of life and not uncommonly over the first 2 weeks post delivery, providing further time point at which there may be an accumulation of injury.

This chapter describes the outcome of the cases enrolled in the study from labour and delivery over the first 2 years of life including neurodevelopment assessment when this was performed.

6.2 Methods

Outcome data was sought on all 29 patients in the study. Delivery data was obtained from the clinical notes and birth summary. Details of postnatal care and procedures were obtained from electronic patient records at the tertiary cardiac unit. Outcome data to a minimum of 2 years of age was available for all except one patient who had postnatal care at another cardiac unit, so minimal details only were available.

6.3 Results

6.3.1 Survival

28/29 (96.6%) of fetuses included in the study were live born.

Stillbirth: One fetus died in utero following fetal aortic valvototomy for critical aortic stenosis at 27 weeks gestation.

Neonatal Death: Three infants died within 28 days: one died on day 1 of life with pulmonary , tracheal atresia, tracheoesophageal fistula and imperforate anus; the second died at 16 days of age following a Blalock-Taussig shunt surgery; and the third died at 17 days of age following an arterial switch procedure for TGA.

Infant deaths: Two children died after 30 days and within the first year of life: one died at 30 days of age following coarctation repair and pulmonary artery banding; the other died at 4 moths of age in their local unit. The details were not available.

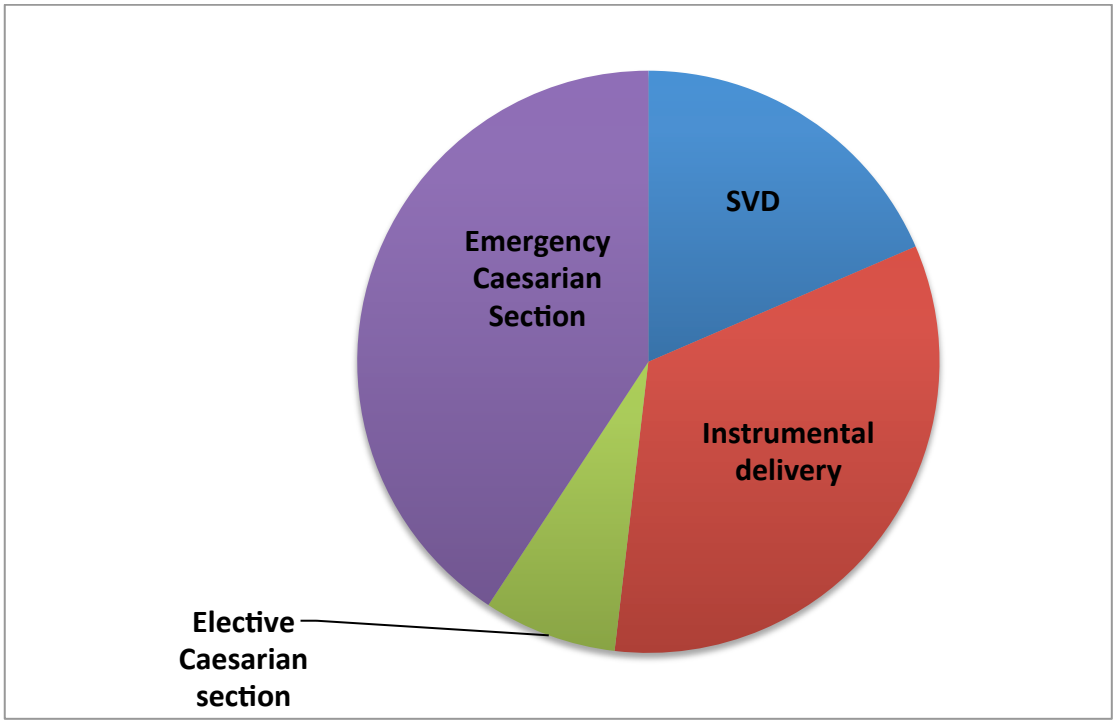
6.3.2 Birth Weight and gestation

The gestation at birth ranged from 32-43 weeks gestation. Three babies delivered preterm, one at 32 weeks, two at 36 weeks. All others were born between 37 and 43 weeks. Of 28 live born infants, 20 were male and 8 were female. The mean birth weight was 3.1kg (excluding one baby born at 32 weeks gestation).

6.3.3 Mode of delivery

The mode of delivery was obtained on 27/28 of the live born infants (see fig 28). 13/28 (46.4%) of patients had a caesarean section with 11/27 (41%) by emergency caesarean and 2/27 (7%) by planned caesarean section; 9/27 (33%) had an instrumental delivery; 5 patients (19%) were born by vaginal delivery.

Figure 28: Pie chart showing break down of mode of delivery of live born fetuses in study



6.3.4 Apgar scores

Apgar scores were recorded on 26/28 of the live born infants. Three infants required initial resuscitation due to poor respiratory effort at birth. One of these, born by elective section, despite needing brief resuscitation, had an Apgar score of 8 by 1 minute and 9 at 5 minutes. The other two (one born by SVD and one by crash caesarean section) had Apgar scores of 8 at 5 minutes. Figure 29 and 30 below show the one and five minute Apgar scores for the study group.

Figure 29: Bar chart of one minute Apgar scores

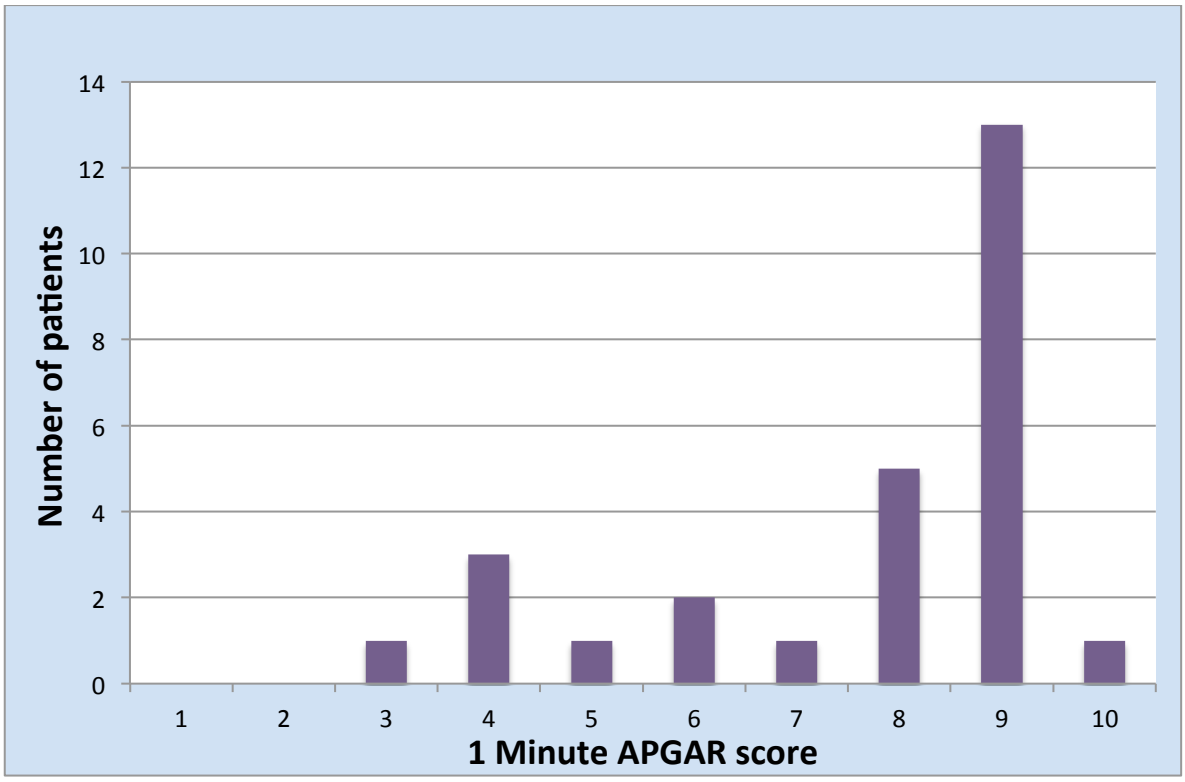
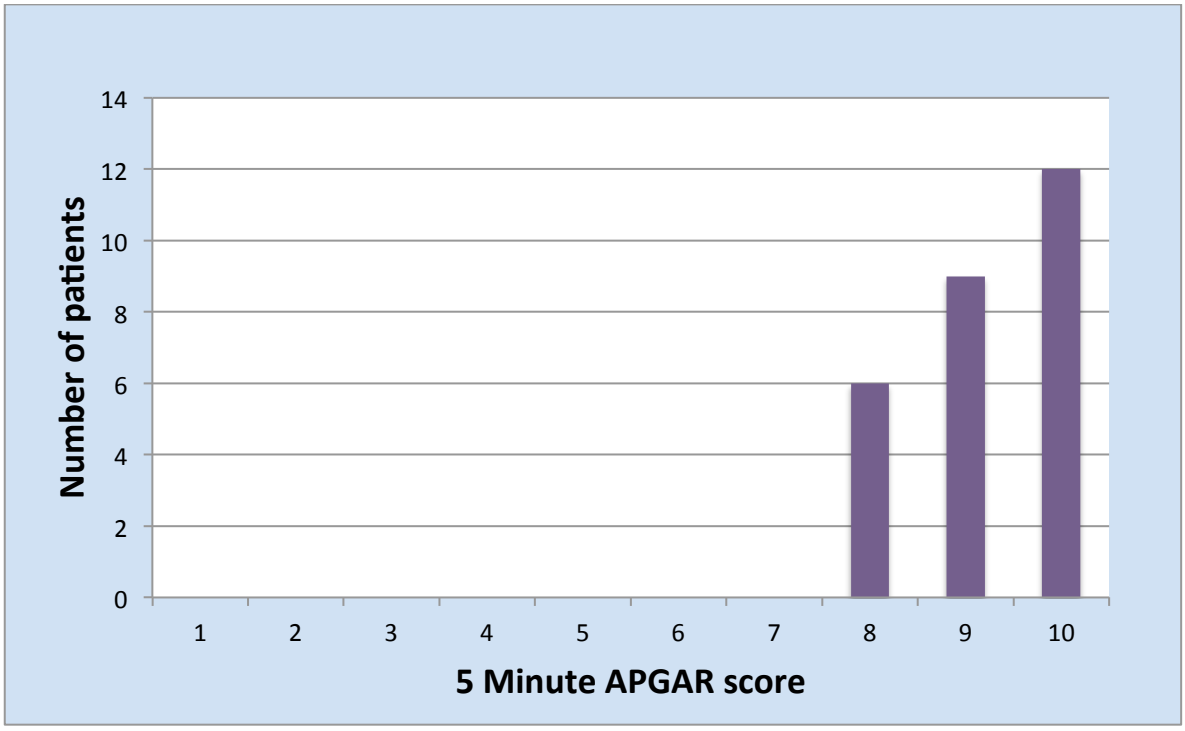


Figure 30 – Bar chart of 5 minute Apgar scores



6.3.5 Additional abnormalities detected postnatally

Three patients had additional extracardiac abnormalities detected postnatally:

Case 13 was found to have a Duodenal web, laryngeal cleft, bronchomalacia

Case 16 was born at term and developed necrotising enterocolitis in the newborn period. The baby was also found to have hemivertebrae.

Case 23 was born prematurely at another centre at 32 weeks. The baby had tracheal atresia with a tracheoesophageal fistula, pulmonary hypoplasia and an imperforate anus and died shortly after birth.

6.3.6 Surgery and intervention in first 2 years of life

Of the 23 children alive at 2 years of age, details of interventional procedures that they had undergone was gained from review of electronic patient records at The Royal Brompton Hospital. This includes surgical and catheter reports, clinic letters and recordings of multidisciplinary meetings. One patient was transferred to another cardiac unit and full follow up details were not available. Procedures were categorised as follows:

- Cardiac surgery involving cardiopulmonary bypass
- Cardiac surgery not involving cardiopulmonary bypass
- Interventional cardiac catheterization
- Other non cardiac procedures

A total of 53 procedures were performed on the 23 surviving children over the first 2 years of life (see figure 31). The median number of procedures was 2, ranging from 1 to 8. 26/53 (46%) of procedures performed involved cardiopulmonary bypass; 18/27 (32%) interventional catheter and 10/53 (18%) cardiac surgery not involving cardiopulmonary bypass. Two (4%) patients had non-cardiac procedures (one a tracheostomy and the other a laparotomy for necrotising enterocolitis; Figure 32).

Figure 31: Bar chart showing number of surgical or interventional catheter procedures undergone by study cohort over first 2 years of life.

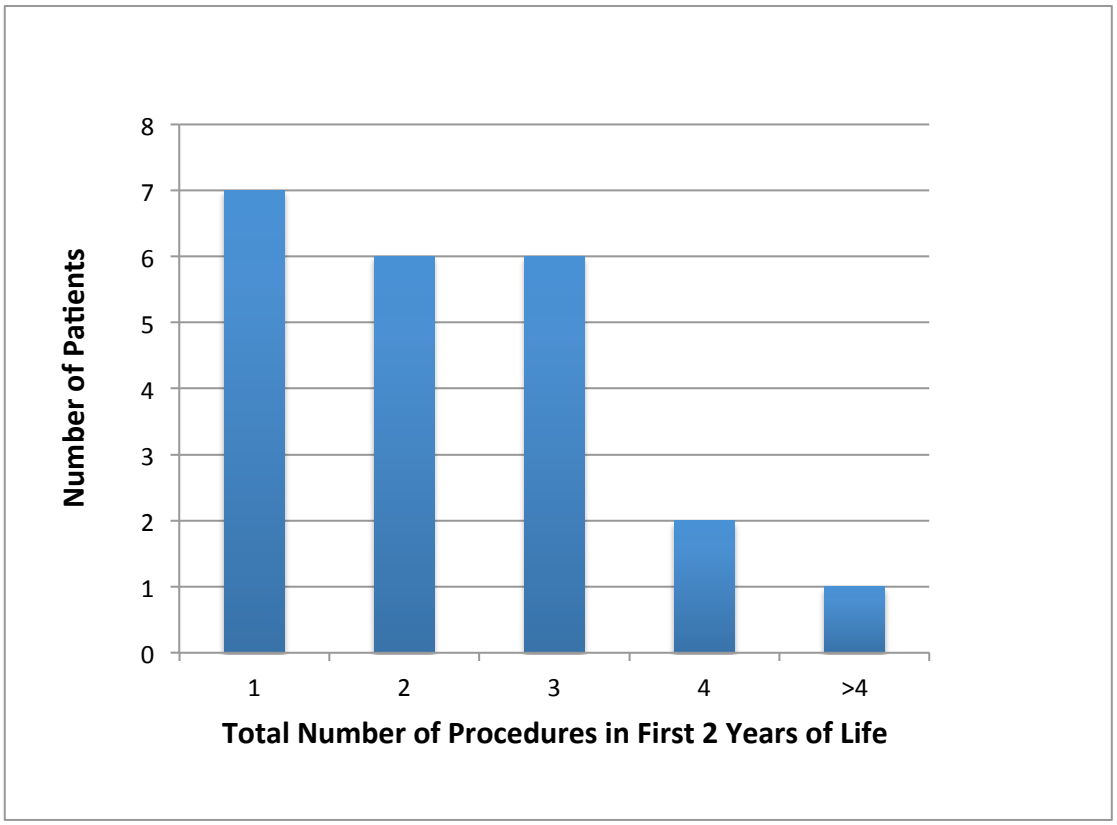
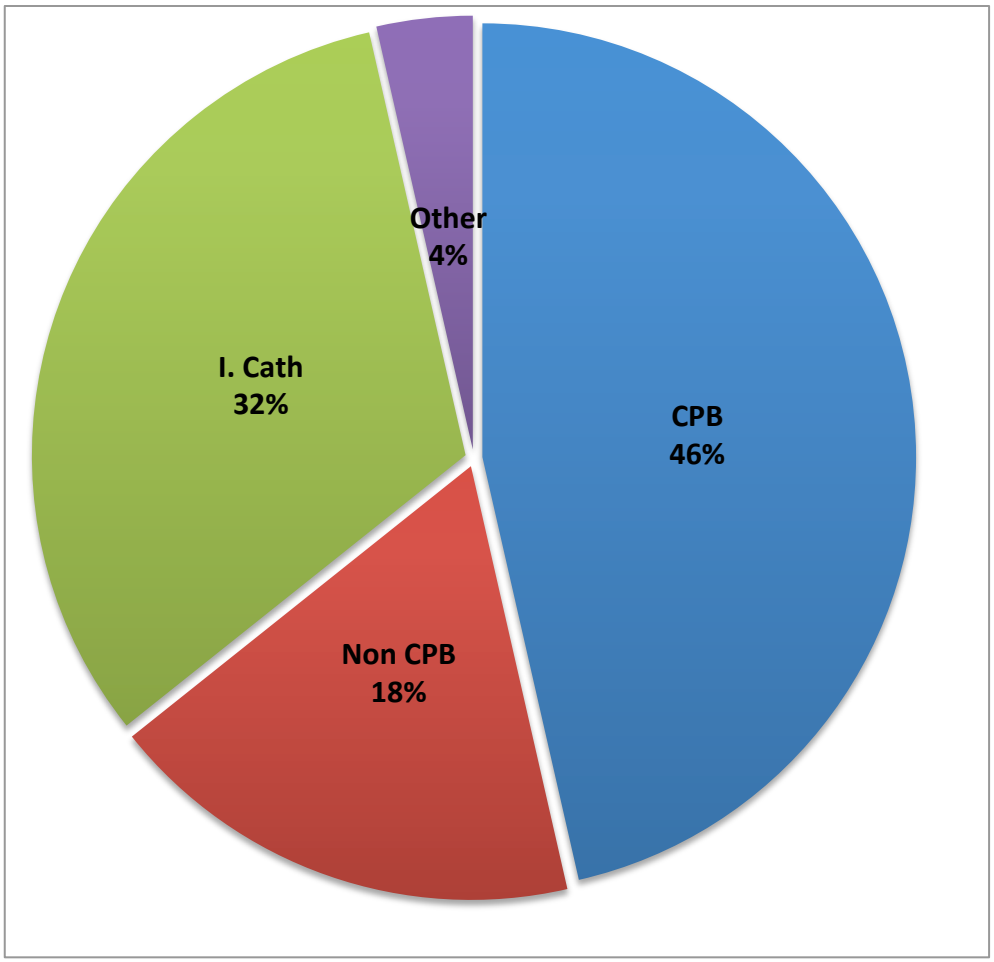


Figure 32: Pie chart showing breakdown of subtype of procedures for all procedures undergone by study cohort



CPB – cardiopulmonary bypass, non CPB cardiac surgery not requiring cardiopulmonary bypass, I. Cath - Interventional cardiac catheter, Other – no cardiac surgery

6.3.7 Neurodevelopmental assessment

At the time of initial MRI, consent was sought from the parents for permission to contact them to ascertain postnatal data and offer neurodevelopmental assessment as part of the on-going study. All patients that could be contacted were offered formal neurodevelopmental assessment at 1 year of age. This was performed using the Griffiths Mental Development Scales (GMDS 0-2). The examinations were performed by either a clinical psychologist (BH) or paediatrician (MM or AC). One child who had previously been recruited was assessed using the Bayley Scales of infant and toddler development III (BSID-III), whereby a score of less than 1SD below the mean is indicative of neurodevelopmental delay.

At the end of the study, patients who had declined attending for neurodevelopmental assessment or had not been possible to contact were contacted again and offered an ages and stages (ASQ) questionnaire. If they agreed, this was then posted to the parent so that they could complete and return to fill in a neurodevelopmental questionnaire.

1-year neurodevelopmental assessment was accepted and performed in 5 of the 23 patients alive at 1 year. An additional 3/23 patients returned the ASQ questionnaire.

Table 13: Reason for not performing neurodevelopmental assessment

Explanation for no neurodevelopmental test	Number of patients	Comments
Died at under 1 year of age	5	
Formal Neurodevelopmental Assessment	7	1 result missing
ASQ questionnaires returned	3	
Agreed to ASQ but didn't return	4	One mother made comment that child being investigated for possible autism, obsessive compulsive disorder and panic attacks.
Unable to contact	3	1 case transferred to another unit with know long term admission over 1 year
Language difficulty	2	
Declined due to illness	2	Child in hospital for surgery Long term hospital admission
Declined other	2	One stated too far away

Of 7 children who underwent neurodevelopmental assessment, the results for 6 were available. 5 children had the test performed at 1 year. The Griffiths mental

development score (GMDS 0-2) was preferentially used at the one-year assessment, as the test is shorter and better tolerated by this age group. One child had a two-year assessment using the Bayley scales of infant and toddler development (BSIDIII). Of the 5 children undergoing the 1-year GMDS assessment, 1 child had a developmental age 1 month behind that expected for chronological age. The other 4 children had development one month ahead of that expected. The head circumference in all 5 children was on the 25th centile or below, although only 2/5 had weight on the 25th centile or below.

The one child who underwent the 2-year assessment had scores in the three subsets of cognition, language and motor of 100, 97 and 107 respectively. The normal range for scores in all scales is 85-115, indicating that at 2 years, this child's development was within the normal range.

3 cases returned ages and stages questionnaires. One was performed at 48 months of age and two performed at 60 months of age. 2 children scored within the normal range for all domains. One child, at 60 months, scored below the normal range in all domains with the exception of problem solving which was in the borderline range. The child is followed up clinically for global developmental delay and autism.

Table 14: Summary of results of neurodevelopmental assessment.

Case No	Birth Gestation (weeks)	Age at assessment (months)	Test used	Developmental age (months)	Head circumference centile	Weight centile
6	41+6	13	Griffiths	12	2	50
14	40+0	12	Griffiths	13	<0.4	0.4-2nd
4	39+0	11.5	Griffiths	12.7	9	46
5	40+1	11	Griffiths	12.4	25	25
9	40+0	13	Griffiths	14	15	50
3	40+4	24	Bayley III	In normal range for age	30	60

6.4 Discussion

This chapter describes the follow up of the study cohort over the first 2 years of life including pregnancy outcome, interventions over the first 2 years of life and neurodevelopment.

28/29 (96.6 %) of fetuses in the study survived until term. There is an increased rate of intrauterine death in fetuses with CHD, however, this is seen most frequently in cases with right atrio-ventricular valve regurgitation such as Ebstein's anomaly of the tricuspid valve or atrioventricular septal defect with severe atrio-ventricular valve regurgitation; in hydrops; or in those fetuses with extracardiac or chromosomal abnormalities. No cases in the cohort had this condition and chromosomal abnormalities were excluded so the low mortality is as expected. The one fetus that

died did so during the course of fetal balloon aortic valvuloplasty, which is known to be a very high risk procedure with a procedural mortality in the region of 25%.

The condition of the baby at birth as judged by the Apgar scores showed that 3 infants required resuscitation at birth. All Apgar scores were above 8 after 5 minutes. The delivery plan for fetuses with CHD is generally determined by maternal indications, with the exception of hypoplastic left heart with a restrictive atrial septum and transposition of the great arteries with potentially restrictive atrial septum which require immediate intervention after birth and therefore a meticulously planned time of delivery. 46% of patients delivered by caesarean section, which contrasts with the published caesarean section rate of 26.5% for 2014-15 (hsic.co.uk). This may be contributed to by 2 factors:

The accepted practice is that these patients are offered an induction of labour at 38-40 weeks gestation, in part due to the practicalities of coordinating the postnatal management of the baby and in patients who live some distance from the tertiary unit to lower the risk of delivery outside the hospital environment. Induction of labour is known to be associated with a higher rate of caesarean section.

Another contributing factor may be that due to the prenatal diagnosis of the heart defect, there may be a greater degree of caution at the time of delivery resulting in a higher caesarean section rate.

Three patients were born prematurely. This is important as prematurity carries its own burden of neurodevelopmental impairment. It is recognised that even for babies born ‘near term’, the outcome is suboptimal and this needs to be considered and balanced against the risk of continuing pregnancy for this group.

There was a relatively high incidence of additional problems detected after the birth in our infants. This is in keeping with published data of a high incidence of underlying genetic and chromosomal abnormalities in CHD[159]. There was also a high mortality rate in the study cohort over the fist year postnatal year. In children with major CHD mortality is highest during the first month from delivery. This is due to a combination of extracardiac abnormalities seen in association with CHD and the mortality associated with neonatal surgery. In this study 2 patients died as a result of surgical complications and one due to an extracardiac abnormality.

Many children with congenital heart disease require multiple procedures over the first few years of life. This is important as these interventions also carry the risk of neurological insult and contribute to subsequent neurological impairment. A major limitation of many imaging studies in children with CHD, including this one, is that they are cross sectional and there is a lack of serial imaging of individuals within the cohort to clearly determine the timing and likely aetiology of any neurological insults. In the original study design we aimed to perform brain MRI both in fetal life and postnatally before cardiac surgery. Ideally this would also be extended to include imaging post surgery to look for any change or progression in findings. This was not achieved due to newborns with CHD being transferred to the tertiary cardiac unit, which was located on a different site, on the first day of life.

There was a disappointingly low rate of take up for neurodevelopmental examinations in this study, which is detailed in the limitations section below. In all 6 patients who had formal assessment (5 at 1 year and 1 at 2 years) the results were within the normal range, but the age at follow up is still too young to detect potential cognitive and behavioural problems. Of interest, the head circumference centile was calculated at the time of neurodevelopmental testing and all 5 of the patients tested

at 1 year had an HC at or below the 25th centile. It is generally considered that the 2-year neurodevelopmental follow up is more informative about the potential for longer-term neurodevelopmental problems.

6.5 Limitations

A major limitation of this study was the low number of children who underwent neurodevelopmental follow up. The reason for this is multifactorial and these are detailed in table 15. Many patients with complex heart lesions have a particularly difficult time over the first year of life, with some children spending many months in hospital and most others attending frequent out patient appointments. When contacted, some parents expressed that they simply could not manage another, non-essential, appointment. This is added to by the regional nature of a fetal and paediatric cardiology service; some patients may live 2-3 hour from the tertiary unit, again increasing the difficulty of attending. When patients were contacted to offer the ASQ questionnaire, even though some parents agreed to fill these in, they were not returned. This included one mother who discussed on the phone that her child was undergoing assessments for possible autism. Children with CHD are now recognised to be at high risk of neurodevelopmental impairment and a structured integrated follow up should be available clinically. Research studies could then take advantage of the information provided without adding to the burden of repeated hospital visits for both the child and their family.

In the original study protocol the aim was to perform postnatal MRI scan on the study cohort. In practise this was extremely difficult to achieve as neonates born with a major congenital heart defect were transferred from the hospital with the maternity

unit to the cardiac centre. This transfer took place over the first few hours of life, often out of hours, and it was not possible for both logistic reasons and clinical reasons to perform a research MRI scan before transfer. This practice was discussed with clinical staff involved but the uncertainty of length of clinical stability in an individual neonate prevented our attempts to scan prior to transfer. In order to overcome this difficulty similar studies would need to be performed at a unit where maternity and cardiac services are co-located or the MRI brain scan would need to be performed after transfer but before cardiac surgery at the cardiac surgical unit.

Chapter 7: Summary of main findings and discussion

7.1 Introduction

Children with congenital heart disease have a high burden of neurodevelopmental abnormality that has important consequences across the spectrum of academic achievement, behaviour and social function and overall quality of life. Considerable improvements have been made in many aspects of care of these children. This includes higher rates of prenatal diagnosis allowing for a detailed delivery and early postnatal plan, advances in surgical management, cardiopulmonary bypass techniques and perioperative care. Despite this these children continue to demonstrate a high rate of neurodevelopmental impairments prompting research to identify and treat any modifiable factors that may contribute to this.

7.2 Summary of main aims and findings

This research study aimed to describe brain growth and blood flow in a cohort of fetuses with congenital heart disease using MRI, ultrasound and Doppler techniques with the hypothesis that alterations in brain growth would be seen in patients with reduced brain oxygen delivery. Evidence to support an underlying aetiology was sought by examining whether the severity of any brain abnormality was predicted by different circulatory patterns as a result of the heart defect.

The findings of this thesis have shown that fetuses with congenital heart disease (CHD) have smaller brain volumes compared to controls when adjusted for advancing gestation. This difference becomes more pronounced with advancing gestation suggesting a slower rate of in utero brain growth in fetuses with CHD. Measurements of growth in the CHD cohort showed that the fetuses were smaller,

for head and abdominal circumference, both at the initial growth scan, and final growth scan with a highly significant difference at the later growth scan.

Cerebral and umbilical artery Doppler data showed evidence of reduced cerebrovascular resistance in fetuses with CHD compared to normal population controls but did not show a difference in the umbilical artery Doppler or reduction in the cerebroplacental ratio that is characteristic of IUGR fetuses.

7.3 Discussion

The importance of the in utero environment for fetal growth is well recognised. Epidemiological studies have shown that children who are growth restricted in fetal life have a higher incidence of cardiovascular disease and noninsulin-dependent diabetes later life[160] showing the potential for far reaching consequences of a fetus developing in an unfavourable environment.

7.3.1 Evidence for reduced oxygen delivery to the fetal brain

In the fetus with a congenital heart defect the in utero environment is very likely to be abnormal. Many congenital heart defects will result in interruptions to the normal patterns of streaming of blood with potential to lower the oxygen saturation of cerebral blood flow. At the most severe end of the spectrum blood may only reach the cerebral circulation in a retrograde manner due to left heart outflow obstruction with potential for reduced volume of flow and therefore an additional limitation on oxygen delivery. In addition to the physiological studies on fetal lambs from Rudolph et al in San Francisco, recent developments in fetal cardiovascular MRI have

produced oximetry and flow data that support this assumption of reduced oxygen delivery by demonstrating in vivo evidence of reduced oxygen saturation in blood delivered to the fetal brain in CHD.

Evidence from the Doppler data in chapter 4 supports a degree of cerebral vasodilation in this study group. This suggests that there is a reduction in cerebral vascular resistance that may represent an adaptive response to attempt to increase blood flow and therefore oxygen delivery to the brain. This finding lends support to the theory of reduced oxygen delivery as an underlying aetiology of the brain volume reduction that was seen in the cases in this study. The finding that the brain volumes are reduced suggests that if this is an adaptive response, it is incomplete and does not fully protect against the abnormality of oxygen delivery. In this study the Doppler measurements were made at a single time point. It has been suggested that evidence of brain sparing at an early stage predicts a problem with substrate supply and demand that is detrimental to development in a way that later changes are not[161], raising the interesting question of whether lower cerebral resistance has different implications depending on when in pregnancy it is observed.

One study by Williams et al. showed that in children with single ventricle anomalies, those fetuses with lower cerebrovascular resistance had higher neurodevelopmental scores[72], suggesting that these fetuses may in fact have adapted better to the hemodynamic disturbance than those that do not show these changes.

The normal umbilical artery Doppler flow in the present study does not show evidence of an elevation of placental vascular resistance. A recent study by Ruiz et al. showed a higher rate of pre-eclampsia in addition to IUGR in pregnancies with CHD, suggesting possible abnormalities of placentation [162]. This conflicts with the

findings in this study but is clearly an area that would be of further research interest due to the vital role of the placenta in fetal growth and development.

7.2.3 Fetal growth restriction in study patients

The finding of a smaller body size of the fetuses in this study is in keeping with other reports of a high rate of fetal growth restriction in CHD. The underlying mechanism may involve a number of factors. Abnormal mixing of blood may result in lower saturation of blood reaching tissue other than the brain. Reduced cardiac output may also be important. One study showed reduced cardiac output in fetuses with CHD[163]. This is also been supported by fetal MRI data showing reduced cardiac output in single ventricle fetuses. This study supports the theory that these fetuses are developing in an environment that is suboptimal for growth. It was not possible in this dataset to draw meaningful conclusions about the subgroups. As mentioned previously this is a major limitation of studies, even those from very large centres, due to the broad spectrum of CHD and low prevalence of individual lesions. A large study of distinct subgroups would be very informative about which aspects of particular lesions have the most significant effect on growth and development.

7.2.4 Reduced brain volumes on fetal MRI

The finding that brain volumes are reduced in CHD is consistent with other published research. The Doppler data described above support a haemodynamic cause being the underlying aetiology for this. In the patient subgrouping, although no significant results were gained due to small numbers, there was a trend towards lower Z-scores

(and therefore the smallest brains) in cases in group 3 when compared to control population Z-scores. This is in keeping with what would be expected for the subgroup that had the most significant haemodynamic disturbance with an abnormality both of anticipated oxygen concentration and flow to the brain. In this study the supratentorial brain volume was measured along with ventricular volumes. It would be of interest to look in detail at other brain structures. One study of adolescents with CHD found reduced volumes of the hippocampi, limbic cortices and mesocortices and corpus callosum in CHD[37], although there may be limitations to the accuracy of measuring small structures that may not be so clearly defined on fetal imaging.

It was not possible to draw any meaningful conclusions from the neurodevelopmental follow up in the study other than the awareness that 2 patients from the cohort have developmental abnormalities that were not formally assessed. Unfortunately one of these is based on the informal report of a parents who declined the formal assessments and the other on the results of an ASQ which highlighted a patient who is receiving developmental follow up for global developmental delay. The finding of all 5 patients being in the lower 25% of normal at the 1 year follow up highlights the need for longer term follow up in these children.

There are likely to be many, as yet undefined, patient specific factors that influence the individual response of the developing brain to the presence of congenital heart disease. One example of this is the Apolipoprotein E (APOE) genotype. Work by Gaynor and colleagues [164] showed that APOE, which is an important regulator of cholesterol metabolism and important in neuronal repair, has a role in determining the neurodevelopmental outcome of infants with CHD. It was found that carriers of the APOE epsilon2 allele had a worse neurodevelopmental outcome. Further

research will need to investigate this further, and identify additional potential modifiers of outcome.

7.4 Clinical implications of the thesis

The main finding of this study, that fetuses with CHD have a high rate of abnormalities of growth and brain development, are in keeping with previous reports. This has important clinical implications. Appropriate surveillance of these children is needed after birth in order to detect any deviations from normal development and intervene if neurodevelopmental abnormalities are present. In the longer term, further research is needed to determine whether there are any factors of the in utero environment that can be modified to improve outcomes. Studies on the impact of perinatal management, in particular in relation to timing and mode of delivery, will be important given the potential for increased susceptibility of the brain to injury in these children.

7.5 Limitations

There were a number of important limitations in this study.

Challenges to recruiting patients into the study: Participation in the study was offered to all women with on-going pregnancies with congenital heart defects over a 2-year period. There are a number of reasons that contributed to difficult recruitment. The first is that a diagnosis of CHD in the fetus is a very distressing for the parents and, as such, involvement in a research study is not something they wish to discuss or consider. Despite reassurance of the safety of the procedure a number of patients

had concerns about having a scan in pregnancy others about the discomfort of the procedure. Despite these difficulties a total of 42 patient agreed to the study 12 of which were excluded due to other abnormalities.

Postnatal MRI was intended following delivery and before transfer to the tertiary cardiac centre. This was not possible as the patients were inevitably transferred before a scan could be performed. This is in accordance with the clinical pathway for major cardiac defects to transfer postnatally to the cardiac centre, which is located on a different site. Due to the relocation of our group to a site with colocation of maternity and cardiac services this will be the subject of further longitudinal studies.

Towards the end of the recruitment period the department of perinatal imaging moved from the hospital Trust at which the research was performed to another Trust. This significantly complicated completion of the project. In particular on-going aspects such as the neurodevelopmental follow up of patients. This was in part overcome by some aspects of follow up being continued on the original site and regular meetings across sites.

Low rate of return for neurodevelopmental assessments: This has been detailed at the end of chapter 5 but in summary it was difficult to contact and get agreement for 1 and 2 year follow up. However, the results obtained suggest an important effect on neurodevelopment, and this will be explored further within our centre.

7.6 Further studies

7.6.1 Longitudinal studies

A major limitation of the majority of postnatal and all fetal studies in brain MRI and neurodevelopment in congenital heart are limited in that they are cross sectional studies. The Boston Circulatory Arrest Trial shows that much can be learnt from following a single large cohort with assessment at serial intervals but this study was started in an era before the importance of the in utero environment was fully appreciated and before the development of fetal MRI techniques. Our understanding of the origins of neurodevelopmental abnormalities would be greatly enhanced by studying a large cohort of fetuses with CHD. Imaging the brains of these children in utero and before and after interventions and along with serial neurodevelopmental assessment and following these children up through childhood.

7.6.2 Neuroimaging findings and neurodevelopmental outcome in CHD

Further studies into the relationship between neuroimaging findings and neurodevelopmental outcome are needed as the current data are limited in CHD. This has been studied in the preterm population where Martinussen [35] has shown regional brain volume reductions correlated with cognitive and perceptual function outcome.

7.6.2 Fetal cardiac MRI

In adult and paediatric cardiology over the past 15 years we have seen cardiovascular MRI evolve into an imaging modality of fundamental importance in decision-making in the treatment of CHD. Until relatively recently its use in the fetus has been significantly limited by the difficulty of synchronising the images to the fetal

heart beat (gating). Methods to overcome this problem are under development and we are starting to see the great potential this method can offer in providing direct measurements of haemodynamic parameters. Further research is needed to validate these techniques and correlate different haemomodynamic patterns with that of brain development. At the moment the use of fetal MRI is limited to late gestation fetuses but with advances in imaging techniques it may become possible to gain useful haemodynamic information earlier in pregnancy. This would open up the possibility of studying factors which may alter the haemodynamics with the hope that this in turn would have an impact on brain development and long term outcome. The evidence from this project and others implicating reduced cerebral oxygen delivery in the aetiology of abnormal brain development leads on to the question of whether administration of maternal oxygen could modify the process and improve outcome. This is potentially a very exciting possibility but further work is needed on validating measures of the oxygen delivery and brain oxygen consumption before this step can be considered.

7.7 Planned publications from this work

Middle Cerebral artery and umbilical artery Doppler measures in fetuses with CHD

Cardiac brain volumes in relation to expected volumes for gestation in a cohort of fetuses with CHD

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Appendix

Appendix 1: Confirmation of ethical approval

Appendix 2: Information sheet for parents

Appendix 3: Consent Form

Appendix 4: Poster/Leaflet to advertise study

Appendix 5: Standard reporting proforma for Fetal MRI

Appendix 6: Guideline for fetal brain MRI

Appendix 7: Ages and stages questionnaire form

Appendix 1: Ethical approval confirmation

NRES Committee London - Central
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09 June 2011

Dr Victoria C Jowett
Clincial Research Fellow
Imperial College, London
Department of Surgery & Cancer,IRDB
Imperial College,Hammersmith Campus
Du Cane Rd. London
W12 0NN

Dear Dr Jowett

Study title: **Cerebral development in a case-control study of fetuses with congenital heart disease.**
REC reference: **11/LO/0394**

Thank you for your letter of 07 June 2011, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC at a meeting held on 9th June 2011 A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement	1	
Covering Letter		10 March 2011
Evidence of insurance or indemnity		29 July 2010
GP/Consultant Information Sheets	1	24 February 2011
Investigator CV		10 November 2011
Other: Patient Information Sheet for control group	1	24 February 2011
Other: CV Mary Rutherford		
Other: CV Helena Gardiner		01 February 2010
Participant Consent Form	2	03 June 2011
Participant Consent Form: control group	2	03 June 2011
Participant Information Sheet	2	03 June 2011
Participant Information Sheet: control group	2	03 June 2011
Protocol	1	24 February 2011
REC application		08 March 2011
Response to Request for Further Information		07 June 2011

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

Notifying substantial amendments
Adding new sites and investigators
Progress and safety reports
Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

11/LO/0394	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr John keen
Chair

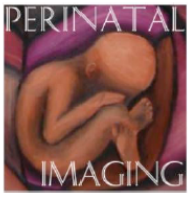
Email: Julie.kidd@nwlh.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

Copy to: *Ms Becky Ward, Imperial College London and Imperial College Healthcare NHS Trust*

Appendix 2: Information sheet for parents

Imperial College Healthcare NHS
NHS Trust



Information Sheet for Patients: Fetal magnetic resonance imaging.
3rd June 2011 V2

Study title: Head Start for Hearts Study

Quantification of brain development and growth in fetuses with congenital heart disease using magnetic resonance imaging

Invitation to take part

We are inviting you to take part in a research study. We are inviting all pregnant women whose baby is affected by congenital heart disease. The study uses a technique called magnetic resonance (MR) to image your baby. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

Magnetic resonance imaging is a method of obtaining pictures of the inside of the body. While ultrasound is excellent for showing the anatomy of the baby (fetus), magnetic resonance gives additional information, particularly of the brain.

There is evidence from other research studies that brain development may be abnormal in a small proportion of fetuses with congenital heart disease. We would like to study this association in detail to see if there are differences in the structure or function of babies' brains that might help to explain this.

The diagnosis of congenital heart disease is made by performing a baby heart scan (fetal echocardiogram). This procedure is normally repeated a number of times in pregnancy as part of your routine clinical care. The information from these scans will be used in combination with the brain MR information.

We would like to image your baby by MR once or twice before delivery (ideally at around 6 and 8 months of pregnancy). We also seek your permission to scan your baby after delivery, this would usually be in the first few weeks of life. We would also like to assess your child's development at age 1 and 2 years. This would involve attending a clinic and a neurodevelopment specialist assessing your child.

MR uses a large magnet and radio-waves. It does not involve the use of either X-rays or radiation. It is not believed to have any hazard associated with it, although care is necessary to keep certain metallic objects away from the magnet and, at the present time, we exclude women who are in the first three months of an uncomplicated pregnancy. This is only a precaution as there are no reports of any side effects, even in the very young fetus. However in the first months of pregnancy the fetus is very small and many structures are not completely formed therefore we do not feel that very early MR imaging would be particularly useful with current MR techniques. MR can also be used to obtain information about the way a tissue is functioning, this is called MR spectroscopy.

Baby heart scan (fetal echocardiography) uses ultrasound and is considered to be a safe procedure in pregnancy.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. If you do not wish to take part we will only take those images that we consider necessary to help with the management of your pregnancy. We still require your permission to do this.

What will happen to me if I take part?

For the MR scan you will be asked to lie feet first inside a magnet. Your head will be level with the opening of the magnet. We will make you as comfortable as possible. You will hear a knocking noise during the procedure. We will give you some headphones and you can listen to some music during the scan if you wish. Your partner or a friend or relative may accompany you into the scanning room once they have had a safety check. You will be given an alarm bell to sound at any time if you are upset or worried during the examination. For your scans we will ask you to lie as still as possible within a magnetic scanner while the machine produces images. If the procedure does not suit you for any reason, it can be stopped at any time.

For the purposes of this research we wish to collect some extra images from your baby's brain. We may also collect information on brain function (MR spectroscopy) from your baby's and images of the blood flow from the heart to the brain. We hope that information on these organs will help to increase our understanding the effect on brain growth and development of congenital heart disease. The total duration of the examination is unlikely to be longer than 45 minutes. If you become uncomfortable we can stop the scan. It is possible to continue scanning after you have had a break or a change in position.

You may at any stage decline any further studies.

What are the side effects of taking part?

The MR examination itself is not believed to have any side effects for you or your baby. The machine is enclosed and people who cannot travel in a lift because of claustrophobia may find it unacceptable. However because you will be going in feet first your head will be level with the opening and not deep inside the scanner. Occasionally we find that the magnet is not wide enough to take women who are large e.g. twin pregnancy or women who are nearing term. The machine is noisy when it is acquiring images and that is why we give you some headphones whilst you are being scanned. We can play music through these and if you want you could bring your own CD.

What are the possible disadvantages and risks of taking part?

The MR examination is not believed to have hazards associated with it when operated within National Radiological Protection Board Guidelines (which we do).

What are the possible benefits of taking part?

A scan may have been requested by your doctor to help him/her with the management of your pregnancy. The further MR images that we take may be of benefit to you in improving the diagnostic information of your baby. Otherwise the benefit is likely to be in improving the diagnostic technique for the benefit of others.
As part of this study we would like to follow your baby's development. You may find information that is produced from these assessments reassuring or it may help plan any treatment or extra therapy that your baby may require.

What if new information becomes available?

MR may just confirm the findings on your antenatal ultrasound or it may show an abnormality that has not been detected previously. This improved detection is why you may have been referred for an MR scan. All the findings on your scan will be discussed with yourself and with your obstetrician. If any new information became available you would immediately be informed. This will allow your obstetrician to advise on the management of your pregnancy with all the available information. If there is any impact on care from the new information you may be asked to sign an updated informed consent form.

What happens when the research study stops?

At the end of the follow up period of the study the participant would go back to their routine care. The present research has been on-going for over 20 years and the results have been published regularly. We intend to continue this.

What if something goes wrong?

Imperial College London holds insurance policies that apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study,

you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform Dr Victoria Jowett. The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.
Insurance Reference number PIMP00510

Will my taking part in this study be kept confidential?

The information obtained from your study is covered by the Data Protection Act. The computerised information is protected by a software and hardware barrier and the records are handled in the same way as hospital records. We also seek permission to include clinical details about your pregnancy in the study, for instance baby's gestation and ultrasound details of your baby. This information would be given to us by your obstetrician or GP and would be handled confidentially.

What will happen to the results of the research study?

The results are usually published in the medical literature. No patients' names will be included.

Who is organising and funding the research?

The research is organised and funded by the Medical Research Council, Imperial College School of Medicine and charities such as Action for Medical Research, the Richard and Jack Wiseman Trust and Tiny Tickers. The research is being conducted as part of the PhD project of Dr Victoria Jowett.

Who has reviewed the study?

The study has been reviewed by the Hammersmith Hospitals Research Ethics Committee.

Thank you for considering to take part in this study.

Contact for further information

Dr Victoria Jowett
Clinical Research Fellow in Fetal Cardiology
Phone 020 8383 3298
Victoria.Jowett10@imperial.ac.
uk

Mary Rutherford
Professor in Perinatal Imaging
MRC Clinical Sciences Centre
Imaging Sciences Department
Hammersmith Hospital
Du Cane Road,
London W12 OHS

Appendix 3: consent form

Consent Form 3rd June 2011 V2

NAME:_____

Head Start for Hearts Study – Quantification of brain development and growth in fetuses with congenital heart disease using MRI

(please tick each statement if it applies to you)

I have read the Information Sheet for this study 3rd June 2011 V2 ☐

I have been given the opportunity to ask questions and discuss this study. I ☐

have received satisfactory answers to all my questions. ☐

I have received enough information about the study. ☐

I am happy for my GP to be informed of my/my child's participation in this study ☐

I agree to my medical records/research data relevant to the study being accessed by Imperial college NHS trust or regulatory agencies in order to ensure that the research is being conducted properly. ☐

I understand that my/my child's data my be stored on Imperial College London computer systems ☐

The study has been explained to me by:
Prof/Dr/Mr/Mrs/Ms_____

I understand that I am free to withdraw myself/my child from the study at any time, without having to give a reason for withdrawing and without affecting my/ my child's future medical care.

I agree to take part in this study.

Signed.....Date.....

(NAME IN BLOCK CAPITALS).....……..

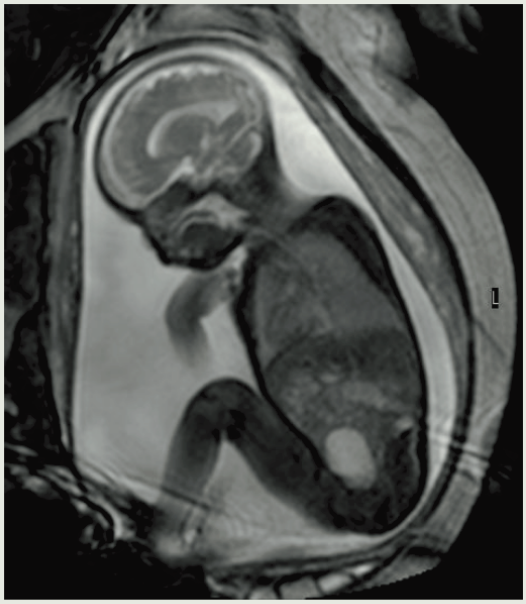
Investigator's signature.....…Date: .....

(NAME IN BLOCK CAPITALS).....……..

Appendix 4: Poster/Leaflet to advertising recrutment for study

HEAD START FOR HEART BABIES

Find out more about your baby's heart and brain development before birth



We are studying brain development and blood flow using magnetic resonance imaging (MRI) and ultrasound, which are safe in pregnancy.

We are asking all parents to participate in this study. You will receive a DVD of your baby's scans and we will be able to tell you more about your baby's development.

Please contact Dr Victoria Jowett to discuss this study in more detail.

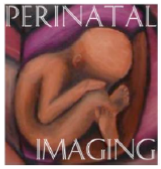
Email: victoria.jowett10@imperial.ac.uk

Tel: 07506 981 425

Appendix 5: Standard reporting proforma for fetal MRI

PERINATAL PAEDIATRIC IMAGING REPORTING SERVICE

Mary A Rutherford, MD FRCR MRCPCH
Perinatal Imaging
Division of Imaging Sciences
Robert Steiner MRI Unit
The Hammersmith Hospital
London W12 OHS
Tel: 0207 188 9156
Fax: 0207 188 9154



FETAL MR BRAIN IMAGING REPORT **DATE:**

NAME OF MOTHER	

Date of Birth		**MRI Scan No**		**Date of Scan**	

Hospital		**HCN**		**Consultant**

Expected Date of Delivery	
Gestation	
Number of Fetuses	
Clinical history	
Ultrasound Findings	
Reason for fetal brain MRI	

Magnet System	**1.5 Tesla** **3 Tesla**			
Sequences	**SSh T2 Three planes**		**Diffusion**	
	SNAPIR		**Dynamic TSE**	loops
	Gradient Echo		**Functional**	

Other sequences:

Fetal MR Imaging Report (Continued) Name:

REGION OF BRAIN	FETUS 1
BPD	= mm (centile)
Cortex	Normal configuration for age
White Matter	Normal appearances
Basal Ganglia & Thalami	Normal appearances
Internal Capsule	Present
Corpus Callosum	Present. Normal shape and size
Cerebellum	Transcerebellar diameter (TCD) = mm (centile) Vermis height in sagittal plane = mm (centile)
Brainstem	AP pons diameter = mm (centile)
Extracerebral Space	Normal width. No collections
Ventricles - lateral	Post horn diameter =
- 3rd	Normal size and shape
- 4th	Normal size and shape
Germinal Matrix	Normal appearances for age No haemorrhage. No subependymal cysts.
Myelin	Appropriate for age
Cavum Septum pellucidum	Present . Septum width =
Eyes	Globe diameter: Lenses present. Optic nerves present
Teeth	Present
Ears	Present

Other comments:

Summary:

Mary Rutherford
Perinatal Imaging
mary.rutherford@kcl.ac.uk

Appendix 6: Guideline for fetal brain MRI

MRI of the fetal brain: guidelines

These guidelines represent only a guide as to an approach to imaging the fetal brain and have been distributed to delegates of the course. March 2011
Mary A Rutherford

WHOM

- Any fetus with an abnormal cranial ultrasound
- In the event of a severe illness or accident in the mother
- Following certain invasive procedures e.g. fetal transfusion
- In congenital infection e.g. parvovirus, CMV, toxoplasmosis
- Following the death or fetocide of a co- twin
- In pregnancies where there is a high risk of a fetal genetic disorder. e.g. previous fetus with a pontocerebellar hypoplasia.

WHY

- Confirm US findings and detect additional abnormalities
- Ensure normal brain appearances for age in high risk fetuses
- Guide clinical management
- Counsel parents

HOW

- 1-1.5 Tesla scanner
- Ensure mother not claustrophobic e.g. can she go in an elevator
- Ensure not too large e.g. high BMI, twin pregnancies, later gestations. Absolute weight a guide only.
- Ensure adequate resuscitation facilities near to scanner. Resuscitation should take place outside of the scanner room.

- Perform full metal safety check. Remove all piercings, jewellery.
- Image in lateral tilt or on side to avoid vena cava compression by pregnant uterus
- Ear protection. Listen to music, relaxing and distracting.
- Avoid overheating, No blankets. Keep fan on in bore of magnet.
- Record temperature before and after scan. (If temperature over 37 degrees C consider whether to postpone scan)

- Use phased array body or cardiac coil. Place coil as near to fetal head as possible. Try and ascertain position prior to scan.
- Use single shot T2 weighted sequences. Obtain at least acquisition in each plane. Pilot of last scan. Use maximum 4mmm slice thickness. Also obtain thinner slice 2-3mm sagittal images.
- T2 sequences the mainstay of fetal MRI but progress being made with T1 and DWI. Consider a dynamic approach (see below) e.g. a modified cardiac sequence or navigator echo if fetal movement repetitive and unidirectional
- Fast T1 weighted imaging with breath hold. Poor anatomical definition but allows detection of short T1 lesions such as haemorrhage and lipomas. Try modified inversion recovery.
- Diffusion weighted imaging: In presence of large infarction may show restricted diffusion.

Ensure interpretation by experienced person. Obtain second opinion of in doubt.

Suggested acquisition parameters: T2 weighted single shot spin echo

TR	TE	Thick/Gap	FOV	NSA	Slices	Matrix	Time
1000	98	4/0.4	430	2	20	218/512	20s

Dynamic scanning. Obtain several loops for maximum brain coverage.

TR	TE	Thick/Gap	FOV	NSA	Slices	Matrix	Time
15000	110	2.5/-1.3	339	1	64	272/288	4m

Appendix 7 : Ages and stages questionnaire

ASQ-3

24 Month Questionnaire

23 months 0 days through 25 months 15 days

On the following pages are questions about activities children may do. Your child may have already done some of the activities described here, and there may be some your child has not begun doing yet. For each item, please fill in the circle that indicates whether your child is doing the activity regularly, sometimes, or not yet.

Important Points to Remember:

- ☑ Try each activity with your child before marking a response.
- ☑ Make completing this questionnaire a game that is fun for you and your child.
- ☑ Make sure your child is rested and fed.
- ☑ Please return this questionnaire by _____.

Notes:

_____

_____

_____

_____

At this age, many toddlers may not be cooperative when asked to do things. You may need to try the following activities with your child more than one time. If possible, try the activities when your child is cooperative. If your child can do the activity but refuses, mark "yes" for the item.

COMMUNICATION

	YES	SOMETIMES	NOT YET	
1. Without your showing him, does your child *point* to the correct picture when you say, "Show me the kitty," or ask, "Where is the dog?" *(She needs to identify only one picture correctly.)*	○	○	○	___
2. Does your child imitate a two-word sentence? For example, when you say a two-word phrase, such as "Mama eat," "Daddy play," "Go home," or "What's this?" does your child say both words back to you? *(Mark "yes" even if her words are difficult to understand.)*	○	○	○	___
3. Without your giving him clues by pointing or using gestures, can your child carry out at least *three* of these kinds of directions?	○	○	○	___
○ a. "Put the toy on the table." ○ d. "Find your coat."				
○ b. "Close the door." ○ e. "Take my hand."				
○ c. "Bring me a towel." ○ f. "Get your book."				
4. If you point to a picture of a ball (kitty, cup, hat, etc.) and ask your child, "What is this?" does your child correctly *name* at least one picture?	○	○	○	___
5. Does your child say two or three words that represent different ideas together, such as "See dog," "Mommy come home," or "Kitty gone"? *(Don't count word combinations that express one idea, such as "bye-bye," "all gone," "all right," and "What's that?")* Please give an example of your child's word combinations:	○	○	○	___

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COMMUNICATION *(continued)*

	YES	SOMETIMES	NOT YET	
6. Does your child correctly use at least two words like "me," "I," "mine," and "you"?	○	○	○	____

COMMUNICATION TOTAL ____

GROSS MOTOR

	YES	SOMETIMES	NOT YET	
1. Does your child walk down stairs if you hold onto one of her hands? She may also hold onto the railing or wall. *(You can look for this at a store, on a playground, or at home.)*	○	○	○	____
2. When you show your child how to kick a large ball, does he try to kick the ball by moving his leg forward or by walking into it? *(If your child already kicks a ball, mark "yes" for this item.)* 	○	○	○	____
3. Does your child walk either up or down at least two steps by herself? She may hold onto the railing or wall. 	○	○	○	____
4. Does your child run fairly well, stopping herself without bumping into things or falling? 	○	○	○	____
5. Does your child jump with both feet leaving the floor at the same time? 	○	○	○	____
6. Without holding onto anything for support, does your child kick a ball by swinging his leg forward? 	○	○	○	____ *

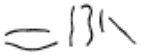
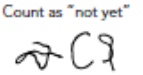
GROSS MOTOR TOTAL ____

**If Gross Motor Item 6 is marked "yes" or "sometimes," mark*

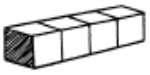
FINE MOTOR

	YES	SOMETIMES	NOT YET	
1. Does your child get a spoon into his mouth right side up so that the food usually doesn't spill?	○	○	○	___
2. Does your child turn the pages of a book by herself? *(She may turn more than one page at a time.)*	○	○	○	___
3. Does your child use a turning motion with his hand while trying to turn doorknobs, wind up toys, twist tops, or screw lids on and off jars?	○	○	○	___
4. Does your child flip switches off and on?	○	○	○	___
5. Does your child stack seven small blocks or toys on top of each other by herself? *(You could also use spools of thread, small boxes, or toys that are about 1 inch in size.)*	○	○	○	___
6. Can your child string small items such as beads, macaroni, or pasta "wagon wheels" onto a string or shoelace? 	○	○	○	___
		FINE MOTOR TOTAL		___

PROBLEM SOLVING

	YES	SOMETIMES	NOT YET	
1. After watching you draw a line from the top of the paper to the bottom with a crayon (or pencil or pen), does your child copy you by drawing a single line on the paper in *any direction*? *(Mark "not yet" if your child scribbles back and forth.)* Count as "yes"  Count as "not yet" 	○	○	○	___
2. After a crumb or Cheerio is dropped into a small, clear bottle, does your child turn the bottle upside down to dump out the crumb or Cheerio? *(Do not show him how.) (You can use a soda-pop bottle or baby bottle.)*	○	○	○	___
3. Does your child pretend objects are something else? For example, does your child hold a cup to her ear, pretending it is a telephone? Does she put a box on her head, pretending it is a hat? Does she use a block or small toy to stir food?	○	○	○	___
4. Does your child put things away where they belong? For example, does he know his toys belong on the toy shelf, his blanket goes on his bed, and dishes go in the kitchen?	○	○	○	___
5. If your child wants something she cannot reach, does she find a chair or box to stand on to reach it (for example, to get a toy on a counter or to "help" you in the kitchen)?	○	○	○	___

PROBLEM SOLVING (continued)

	YES	SOMETIMES	NOT YET	
6. While your child watches, line up four objects like blocks or cars in a row. Does your child copy or imitate you and line up *four* objects in a row? *(You can also use spools of thread, small boxes, or other toys.)* 	○	○	○	____

PROBLEM SOLVING TOTAL ____

PERSONAL-SOCIAL

	YES	SOMETIMES	NOT YET	
1. Does your child drink from a cup or glass, putting it down again with little spilling?	○	○	○	____
2. Does your child copy the activities you do, such as wipe up a spill, sweep, shave, or comb hair?	○	○	○	____
3. Does your child eat with a fork?	○	○	○	____
4. When playing with either a stuffed animal or a doll, does your child pretend to rock it, feed it, change its diapers, put it to bed, and so forth?	○	○	○	____
5. Does your child push a little wagon, stroller, or other toy on wheels, steering it around objects and backing out of corners if he cannot turn?	○	○	○	____
6. Does your child call herself "I" or "me" more often than her own name? For example, "I do it," more often than "Juanita do it."	○	○	○	____

PERSONAL-SOCIAL TOTAL ____

OVERALL

Parents and providers may use the space below for additional comments.

1. Do you think your child hears well? If no, explain: ○ YES ○ NO

2. Do you think your child talks like other toddlers her age? If no, explain: ○ YES ○ NO

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OVERALL (continued)

3. Can you understand most of what your child says? If no, explain: ○ YES ○ NO

4. Do you think your child walks, runs, and climbs like other toddlers his age? If no, explain: ○ YES ○ NO

5. Does either parent have a family history of childhood deafness or hearing impairment? If yes, explain: ○ YES ○ NO

6. Do you have any concerns about your child's vision? If yes, explain: ○ YES ○ NO

7. Has your child had any medical problems in the last several months? If yes, explain: ○ YES ○ NO

OVERALL *(continued)*

8. Do you have any concerns about your child's behavior? If yes, explain: ○ YES ○ NO

9. Does anything about your child worry you? If yes, explain: ○ YES ○ NO

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