The fetal brain in co-twins treated for twin-twin transfusion syndrome

Marisa Christina Taylor-Clarke

Imperial College London
Department of Surgery & Cancer

Submitted for the degree of PhD

Declaration of Originality

I declare that the content of this thesis is my own work and that work by others is appropriately referenced or acknowledged.

Copyright Declaration

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

Abstract

Introduction

Twin-twin transfusion syndrome (TTTS) affects 10% of monochorionic pregnancies and provides a unique opportunity to study the impact of early environmental discordance in genetically identical twins. TTTS can be successfully treated with laser therapy but poor long term neurodevelopmental outcome remains a significant problem. Aberrant antenatal brain development may contribute to some of these deficits. The main aim of this study was to investigate differences in fetal brain development in identical twins treated for twin-twin transfusion syndrome.

Methods

Thirty-nine twin pairs that had been treated by laser for TTTS underwent fetal MRI (1.5T) from 21 to 32 weeks gestational age. Volumetric segmentation of the fetal body was performed on 2D images whilst volumetric analysis of the supratentorial brain was performed on 3D reconstructed datasets. Development of the cortical surface was evaluated using an objective cortical scoring system. The metabolic profile of the fetal brain was assessed using magnetic resonance spectroscopy. Neurodevelopmental outcome was assessed at 1 and 2 years of age in surviving children.

Results

Ex-donor fetuses had significantly smaller brains than their ex-recipient co-twins and showed evidence of global brain sparing. Extracerebral fluid volumes were significantly smaller in ex-donors relative to ex-recipient fetuses. Corrected cerebellar and cortical grey matter volumes were similar within twin pairs whereas maturation of the parieto-occipital fissure, central sulcus and Sylvian fissure was delayed in ex-donors compared to ex-recipients. Lactate was detected in 43% of fetal brains. By two years of age 20.8% of children followed up had significant neurodevelopmental impairment. This correlated with cortical maturation discordance within twin pairs.

Conclusions

Significant differences in brain development exist between co-twins following laser treatment for TTTS. Discordance between twin fetuses correlates with later neurodevelopmental delay. Despite treatment and the absence of overt brain injury, in utero blood flow and endocrine influences may create divergent developmental paths in identical twins.

Dedication

To my son William

Contents

List of Figures **9**

List of Tables **11**

Abbreviations **12**

1. Introduction **15**

1.1 Twin-twin transfusion syndrome *15*
1.1.1 Epidemiology 16
1.1.2 Diagnosis 16
1.1.3 Staging 17
1.1.4 Natural History 19
1.1.5 Pathophysiology 20
1.1.6 Management 24
1.1.7 Outcomes following fetoscopic laser therapy 28

1.2 The fetal brain in TTTS *32*
1.2.1 In utero brain injury 32
1.2.2 In utero brain development 37

1.3 Fetal magnetic resonance imaging *40*
1.3.1 Fundamental Principles of MRI 40
1.3.2 Safety 43
1.3.3 MRI in fetal CNS assessment 47
1.3.4 Fetal intracranial volumetry 49
1.3.5 Fetal MR Spectroscopy 54

1.4 Aims and Hypothesis *59*
1.4.1 Specific aims 59

2. Materials and Methods **60**

2.1 Ethics *60*

2.2 Study Design *60*
2.2.1 Inclusion Criteria 60
2.2.2 Exclusion Criteria 60
2.2.3 Cohort 61

2.3 Fetal Imaging *65*
2.3.1 Procedure 65
2.3.2 Data acquisition 66
2.3.3 Identification of fetuses and study blinding 70
2.3.4 Post-Processing 71
2.3.5 Data Analysis Cohort Summary 91

2.4 Perinatal Outcome *94*

2.5 Neurodevelopmental Assessment *94*
2.5.1 Griffiths Mental Development Scales 95
2.5.2 Bayley Scales of Infant and Toddler Development-III 97
2.5.3 Ages and Stages Questionnaires 98

2.6 Statistical Analysis *99*
2.6.1 Power Calculation 99

3. Results 101

3.1 Fetal brain imaging clinical examination findings 101
3.1.1 Background 101
3.1.2 Aims 101
3.1.3 Methods 101
3.1.4 Results 102
3.1.5 Discussion 106
3.1.6 Conclusion 110

3.2 Co-twin differences in cranial biometry 111
3.2.1 Background 111
3.2.2 Aims 111
3.2.3 Methods 111
3.2.4 Results 111
3.2.5 Discussion 117
3.2.6 Conclusion 118

3.3 Measurement of intracranial volumes 119
3.3.1 Background 119
3.3.2 Aims 119
3.3.3 Methods 119
3.3.4 Results 120
3.3.5 Discussion 139
3.3.6 Conclusion 147

3.4 Assessment of cortical development 148
3.4.1 Background 148
3.4.2 Aims 149
3.4.3 Methods 149
3.4.4 Results 151
3.4.5 Discussion 164
3.4.6 Conclusion 167

3.5 ^{1}HMRS assessment of cerebral metabolic ratios 168
3.5.1 Background 168
3.5.2 Aims 168
3.5.3 Methods 168
3.5.4 Results 170
3.5.5 Discussion 177
3.5.6 Conclusion 180

3.6 Neurodevelopmental Assessment 181
3.6.1 Background 181
3.6.2 Aims 181
3.6.3 Methods 181
3.6.4 Results 182
3.6.5 Discussion 189
3.6.6 Conclusion 192

4. Main Discussion 193

5. Future Work 198

5.1 Volumetric segmentation of white matter, basal ganglia and thalami 198

5.2 Placental influence 198

5.3 Lateralisation 198

5.4 Neurodevelopmental follow up *199*

Appendix 1: Fetoscopic laser therapy **200**

Appendix 2: Information Sheet for Patients **201**

Appendix 3: Information Sheet for Patients **205**

Appendix 4: Consent Form 1st December 2011 V4 11/LO/1744 ..... **210**

Appendix 5: Parental Questionnaire **211**

Appendix 6: Ages and Stages Questionnaire **215**

Appendix 7: Singleton Survivor Data **221**

7.1 Aims *221*

7.2 Methods *222*
7.2.1 Statistics 222

7.3 Results *222*
7.3.1 Cohort 222
7.3.2 Singleton survivors of iatrogenic co-twin death 223
7.3.3 Singleton survivors of co-twin death subgroups 226
7.3.4 Singleton survivor outcomes 231

7.4 Discussion and prospective work *232*

References **233**

List of Figures

Figure 1. 'The Swaddled Children'. 15
Figure 2. Ultrasound findings of recipient polyhydramnios and donor oligohydramnios. 17
Figure 3. Ultrasound appearance of stage II TTTS. 18
Figure 4. Ultrasound appearances of stage III TTTS. 19
Figure 5. Uncomplicated MCDA placenta. 20
Figure 6. MCDA placenta complicated by TTTS. 21
Figure 7. Magnetic properties of the hydrogen nucleus. 40
Figure 8. Effect of application of an external magnetic field on the hydrogen nucleus. 40
Figure 9. Evolution of MR image quality. 47
Figure 10. ^{1}HMRS Normal fetal spectrum. 55
Figure 11. Flowchart of cases that did not meet inclusion criteria. 61
Figure 12. Flowchart of cases meeting inclusion criteria. 62
Figure 13. T2-weighted Single Shot Turbo Spin Echo. 67
Figure 14. T2-weighted dynamic Single Shot Turbo Spin Echo. 67
Figure 15. T1-weighted Single Shot Inversion-Recovery. 68
Figure 16. T2*-weighted Gradient Echo. 68
Figure 17. Voxel placement in fetal ^{1}HMRS. 69
Figure 18. Identification of fetuses. 70
Figure 19. Normal clinical examination findings in an ex-recipient fetus at 28 weeks GA. 71
Figure 20. Post processing of MR images: Volume reconstruction and optimisation. 72
Figure 21. Example of 2D measurements. 74
Figure 22. Segmentation of supratentorial brain tissue. 76
Figure 23. Segmentation of the lateral ventricles. 76
Figure 24. Segmentation of the extracerebral fluid. 77
Figure 25. Segmentation of the cerebellum. 77
Figure 26. Segmentation of cavum septum pellucidum and cavum vergae. 78
Figure 27. Segmentation of the fourth ventricle. 78
Figure 28. Segmentation of cortical grey matter. 79
Figure 29. Segmentation of the fetal body. 81
Figure 30. Segmentation of the fetal body: identification of body parts. 82
Figure 31. 3D reconstruction of the fetal body segmentations. 82
Figure 32. Cortical development scoring system. 84
Figure 33. Assessment of the frontal-parietal border. 85
Figure 34. Assessment of the parietal lobe. 85
Figure 35. Assessment of the frontal lobe. 86
Figure 36. Assessment of the temporal lobe. 86
Figure 37. Assessment of the occipital lobe. 87
Figure 38. Assessment of the frontal-parietal-temporal border. 87
Figure 39. Example of a motion corrupted spectrum at echo time 270ms. 88
Figure 40. Example of a lipid contaminated spectrum at echo time 136ms. 89
Figure 41. Antenatal brain injury in Case 1. 103
Figure 42. Antenatal brain injury in Case 2. 104
Figure 43. Antenatal brain injury in Case 3. 104
Figure 44. Antenatal brain injury in Case 4. 105
Figure 45. Antenatal brain injury in Case 5. 106
Figure 46. Flowchart of cases suitable for biometry analysis. 112
Figure 47. The relationship between linear brain measurements and GA in all twins treated for TTTS. 113
Figure 48. The relationship between linear brain measurements and GA in ex-recipients and ex-donors. 115
Figure 49. Differences in BPD-corrected and HC-corrected TCD between ex-recipients and ex-donors. 116
Figure 50. Histogram of GA distribution in singleton controls and twin pairs. 121
Figure 51. Flowchart of cases excluded from volumetric segmentation analysis. 122
Figure 52. Bland-Altman plots for inter-rater reliability. 122
Figure 53. Supratentorial brain tissue volume in ex-recipient / ex-donor co-twins and singleton controls. 124
Figure 54. Relationship between biparietal diameter and total supratentorial brain tissue volume. 125
Figure 55. Relationship between head circumference and total supratentorial brain tissue volume. 125
Figure 56. Cerebellar volume and GA in ex-recipient and ex-donor co-twins and singleton controls. 127
Figure 57. Cerebellar volume corrected by supratentorial brain tissue volume. 127
Figure 58. Relationship between transcerebellar diameter and cerebellar volume. 128
Figure 59. Lateral ventricle volume in ex-recipients, ex-donors and singleton control fetuses. 129
Figure 60. Cortical grey matter volume in ex-recipients, ex-donors and singleton controls. 131
Figure 61. Cortical grey matter volume corrected by supratentorial brain tissue volume. 131
Figure 62. Total intracerebral fluid volume in ex-recipients, ex-donors and singleton control fetuses. 133
Figure 63. Total extracerebral fluid in ex-recipients, ex-donors and singleton control fetuses. 134

Figure 64. Relationship between total fetal volumetry and GA in ex-recipient and ex-donor fetuses. 136
Figure 65. Relationship between intracranial volume and total fetal volume in ex-donors and ex-recipients. 137
Figure 66. Relationship between supratentorial brain tissue and intracranial volume in ex-recipients and ex-donors. 138
Figure 67. Relationship between extracerebral fluid volume and intracranial volume in ex-recipients and ex-donors. 138
Figure 68. Gestational age of early sulcation. 152
Figure 69. Gestational age of early sulcation in ex-recipient and ex-donor fetuses. 154
Figure 70. Cortical development scores for the inferior frontal gyrus in ex-recipient and ex-donor co-twins. 156
Figure 71. Cortical development scores for the central sulcus in ex-recipient and ex-donor co-twins. 157
Figure 72. Cortical development scores for the parieto-occipital fissure in ex-recipient and ex-donor co-twins. 158
Figure 73. Sylvian fissure insular-opercular ratio in ex-recipient and ex-donor co-twins. 159
Figure 74. Sylvian fissure insular distance in ex-recipient and ex-donor co-twins. 160
Figure 75. Sylvian fissure opercular distance in ex-recipient and ex-donor co-twins. 161
Figure 76. Cortical development scores for the superior temporal gyrus in ex-recipient and ex-donor co-twins. 162
Figure 77. Cortical development scores for the calcarine sulcus in ex-recipient and ex-donor co-twins. 163
Figure 78. Example of a typical spectrum acquired at echo time 136ms. 170
Figure 79. NAA:Cr and NAA:Cho at echo time 136ms for ex-recipient and ex-donor fetuses. 171
Figure 80. Relationship between GA and Cho:Cr at echo time 136ms for ex-recipient and ex-donor fetuses. 172
Figure 81. Example of lactate present in a spectrum acquired at echo time 136ms. 173
Figure 82. Example of a typical spectrum acquired at echo time 42ms. 174
Figure 83. Metabolite ratios at echo time 42ms in ex-recipient and ex-donor fetuses. 175
Figure 84. Example of a typical spectrum at echo time 270ms. 176
Figure 85. Participation in Neurodevelopmental Assessment at 1 year. 183
Figure 86. Participation in Neurodevelopmental Assessment at 2 years. 184
Figure 87. Flowchart of cases included and grouping according to mechanism of fetal death 223
Figure 88. The relationship between GA and supratentorial brain tissue volume in singleton survivors of iatrogenic co-twin death, singleton controls and dual survivors of laser treated TTTS. 224
Figure 89. The relationship between GA and lateral ventricle volume in singleton survivors of iatrogenic co-twin death, singleton controls and dual survivors of laser treated TTTS. 225
Figure 89. The relationship between GA and supratentorial brain tissue volume in singleton survivor subgroups. 230
Figure 90. The relationship between GA and supratentorial brain tissue volume in TTTS singleton survivors, singleton controls and surviving TTTS twin pairs. 231

List of Tables

Table 1. Diagnostic criteria in TTTS. 16
Table 2. Quintero severity staging system in TTTS. 18
Table 3. Summary of recent studies reporting perinatal survival following laser treatment in TTTS. 28
Table 4. Summary of recent studies reporting brain injury acquired in utero post laser therapy. 34
Table 5. Summary of studies of normal fetal intracranial volumetry. 51
Table 6. Summary of studies of intracranial volumetry in abnormal fetuses. 53
Table 7. Summary of studies of key metabolite ratios in normal singleton fetuses. 58
Table 8. Pregnancy outcome and maternal characteristics for the study and non-study cohort. 62
Table 9. Case characteristics at study entry and outcome. 64
Table 10. Fetal imaging parameters. 66
Table 11. Gestational age of first appearance of cortical landmarks in healthy singleton fetuses. 83
Table 12. Metabolite ratios quantified at each echo time. 90
Table 13. Summary of data acquired for each case. 92
Table 14. Griffiths Mental Development Scales. 96
Table 15. Bayley Scales of Infant and Toddler Development-III. 97
Table 16. Scoring of Bayley Scales of Infant and Toddler Development-III. 98
Table 17. Summary of cases with abnormal MR findings. 102
Table 18. Correlation between linear measurements and GA for ex-recipients and ex-donors. 114
Table 19. Correlation coefficients between linear measures of BPD, HC and TCD. 116
Table 20. Referral reasons in cases subsequently included as healthy controls. 120
Table 21. Correlation between GA and intracranial volumes in singleton controls, ex-recipients and ex-donors. 135
Table 22. Cortical landmarks chosen for assessment and corresponding brain region. 150
Table 23. GA of early sulcation in ex-recipient twins, ex-donor twins and singleton controls. 153
Table 24. Strength of association between cortical development score above 1 and GA in twin fetuses. 155
Table 25. Metabolite ratios quantified at each echo time. 169
Table 26. Summary characteristics and outcomes for fetuses with cerebral lactate detected. 173
Table 27. Assessment at 1 year of age (corrected for prematurity) cohort characteristics. 182
Table 28. Assessment at 2 years of age (corrected for prematurity) cohort characteristics. 184
Table 29. Comparison between developmental outcomes in three children that were assessed at both 1 and 2 years. 187
Table 30. Relationship between MR measurements and developmental outcome scores. 188
Table 31. Main study aims and findings. 194
Table 32. Demographic data for singleton survivors, double TTTS survivors and singleton controls. 223
Table 33. Correlation coefficients, relative growth rates and estimated volumes in singleton survivors and controls. 226
Table 34. Pregnancy characteristics and outcome (singleton death post laser). 227
Table 35. Pregnancy characteristics and outcome (selective feticide for TTTS). 227
Table 36. Pregnancy characteristics and outcome (selective feticide for non-TTTS pathology). 228
Table 37. Pregnancy characteristics and outcome (spontaneous single fetal death). 229
Table 38. GA at which fetal death occurred and GA at MRI in singleton survivor subgroups. 229
Table 39. Perinatal outcomes in singleton survivors. 232

Abbreviations

^{1}HMRS	^{1}H Magnetic Resonance Spectroscopy
AAA	Arterio-arterial anastomoses
ACE	Angiotensin converting enzyme
ADC	Apparent Diffusion Coefficient
ADHD	Attention deficit hyperactivity disorder
AFI	Amniotic fluid index
AGA	Appropriately grown for gestational age
ANP	Atrial natriuretic peptide
ASQ	Ages and Stages Questionnaires
AT	Angiotensin
B_0	External Magnetic Field
BNP	Brain natriuretic peptide
BPD	Biparietal diameter
BSID	Bayley Scales of Infant Development
CHD	Congenital heart disease
Cho	Choline
CNS	Central Nervous System
CP	Cerebral palsy
Cr	Creatine
CSF	Cerebro-Spinal Fluid
CSP	Cavum Septum Pellucidum
DCDA	Dichorionic diamniotic
DICOM	Digital Imaging and Communications in Medicine
DQ	Developmental Quotient
DTI	Diffusion Tensor Imaging
DV	Ductus venosus
DVP	Deepest vertical pool
EDF	End diastolic flow
EFW	Estimated fetal weight
FGR	Fetal growth restriction
FID	Free Induction Decay
FOV	Field of View
GA	Gestational Age
GMDS	Griffiths Mental Development Scales

HC	Head circumference
ICV	Intracranial volume
IFG	Inferior frontal gyrus
IVH	Interventricular haemorrhage
M_0	Net Magnetization Vector
MCA	Middle cerebral artery
MCDA	Monochorionic diamniotic
MI	Myo-inositol
MRI	Magnetic Resonance Imaging
MVP	Maximum vertical pocket
NAA	N-acetylaspartate
NDA	Neurodevelopmental assessment
NIfTI	Neuroimaging Informatics Technology Initiative
ppm	Parts per million
POF	Parieto-occipital fissure
PRESS	Point Resolved Spectroscopy
PVL	Periventricular leukomalacia
RAS	Renin-angiotensin system
REC	Research Ethics Committee
RFA	Radiofrequency ablation
ROI	Region of Interest
SAR	Specific Absorption Rate
SD	Standard Deviation
sFGR	Selective fetal growth restriction
SGA	Small for gestational age
SQ	Sub-Quotient
ssTSE	Single Shot Turbo Spin Echo
STB	Supratentorial brain
STG	Superior temporal gyrus
SVR	Snapshot MRI with Volume Reconstruction
TCD	Transcerebellar diameter
TE	Echo Time
TOP	Termination of pregnancy
TR	Repetition Time
TTTS	Twin-twin transfusion syndrome
UA	Umbilical artery

US	Ultrasonography
VM	Ventriculomegaly

1. Introduction

1.1 Twin-twin transfusion syndrome

Twin-twin transfusion syndrome (TTTS) is characterized by unbalanced blood flow across the shared circulation in monochorionic diamniotic (MCDA) twins. One twin (the 'donor') is hypovolaemic leading to oliguric oligohydramnios. Increased distal impedance and hypervolemia in the other 'recipient' twin results in polyuria and polyhydramnios.



The first reference to a case of TTTS comes from the Book of Genesis (Genesis 25:19–25). The twins of Isaac and Rebekkah "struggled together within her" and the first born, Esau, "came out red," and is speculated to have been the recipient twin. A 17th century painting by an unknown artist called '*De Wikkellkinderen'* or 'The Swaddled Children' has been credited as the first depiction of TTTS (figure 1). The twins appear to be identical, but one is pale, thought to be anaemic, whilst the other is red, attributed to polycythaemia. Historical analysis suggests that the twins died soon after birth (Berger et al. 2000).

Figure 1. 'The Swaddled Children'.
This 17th century painting is credited as the first depiction of TTTS.

A German Obstetrician, Friedrich Schatz, was the first to delineate TTTS in 1882 (Schatz 1882). He injected the placental circulation of twins with dye and demonstrated intertwin anastomoses. He described this as the 'third circulation' because the anastomoses couldn't be seen from inspection of the surface of the placenta. This concept was explored more fully in a later description of a case of twins with classical twin to twin transfusion syndrome. Grossly discordant twins are described; one oedematous twin died in 12 hours, had urinated constantly and had a hugely distended bladder at autopsy. The other growth restricted twin survived for 53 hours, never passed urine and had an empty bladder. Schatz inferred that the polyhydramnios was due to excessive intrauterine urination of the "recipient" resulting from transfusion from the "donor" twin. The atrophied donor was described as the "stuck twin", because there was no fluid in which to move before birth.

1.1.1 Epidemiology

The prevalence of TTTS is around 1-3 in 10,000 live births (Blickstein 2006). Only monochorionic twin gestations are at risk of TTTS. Estimates of the proportion of monochorionic gestations that are affected vary from between 5% to 17.5% (Lutfi et al. 2004; Quintero 2003; Sebire et al. 2000). Although higher incidences of up to 20% have been reported (Hack et al. 2008), this most likely reflects selection bias in the cohort studied, and the most commonly reported figures are around 8% to 10% (Acosta-Rojas et al. 2007; Lewi et al. 2008; Nakayama et al. 2012; Ortibus et al. 2009; Simpson 2013).

1.1.2 Diagnosis

The diagnosis of TTTS is made according to strict ultrasound based criteria and is shown in table 1.

Diagnostic criteria for TTTS
Confirmed monochorionic pregnancy
Polyhydramnios in recipient
Oligohydramnios in donor
Discordant fetal bladders (markedly enlarged in recipient and reduced (very small or non-visible) in donor)

Table 1. Diagnostic criteria in TTTS.

All criteria must be met. Polyhydramnios is defined on US as a deepest vertical pool (DVP) of >8cm. In Europe the definition of polyhydramnios is DVP >8cm at <20 weeks GA or >10cm at >20 weeks GA. Oligohydramnios is defined as DVP <2cm. Adapted from WAPM Consensus group on TTTS (Baschat et al. 2011).

Figure 2 illustrates amniotic fluid findings on ultrasound examination that are necessary to make a diagnosis of TTTS.

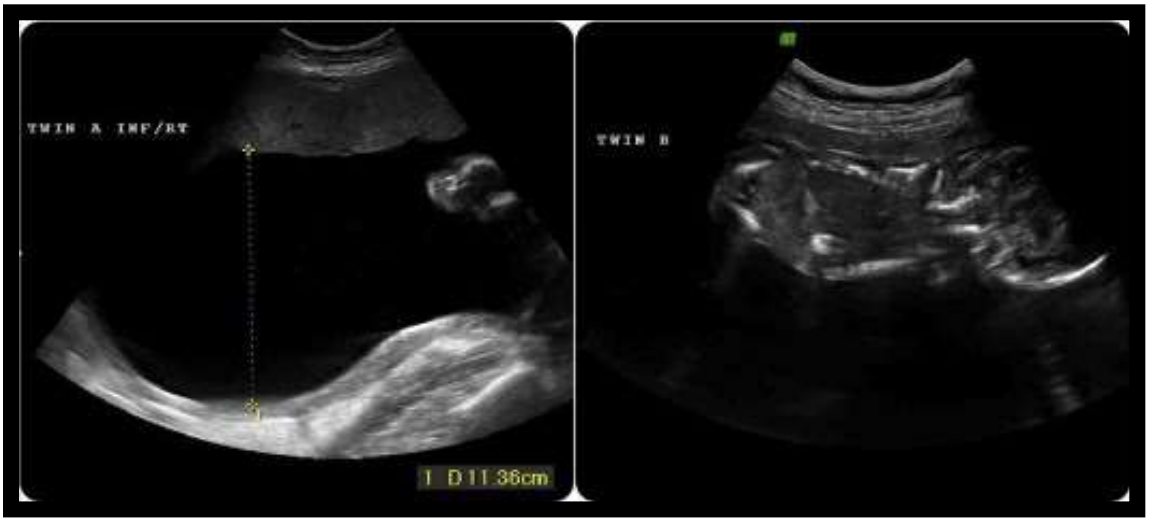


Figure 2. Ultrasound findings of recipient polyhydramnios and donor oligohydramnios.

The left panel shows ultrasound appearances of polyhydramnios with a DVP of >11cm in the recipient (twin A) amniotic sac as indicated by the dashed yellow line. The right panel shows oligohydramnios in the donor amniotic sac causing the donor (twin B) to be stuck to the anterior wall of the uterus. Reproduced from SMFM clinical guideline. Twin-twin transfusion syndrome. Simpson, LL (2013).

The above criteria should reduce misdiagnosis of selective fetal growth restriction (sFGR) or amniotic fluid discordance. A discordance in estimated fetal weight (EFW) is not a necessary feature in TTTS, although placental insufficiency may co-exist in the donor twin in up to 20% of cases (Habli et al. 2008). Selective fetal growth restriction may be hard to distinguish from TTTS as the growth restricted twin may present similarly to a donor twin with oligohydramnios, a small bladder and abnormal umbilical artery flow. Importantly, normal amniotic fluid volume in the co-twin amniotic cavity should suggest sFGR rather than TTTS (Sueters & Oepkes 2014).

1.1.3 Staging

The Quintero staging system of severity (Quintero et al. 1999) was originally developed as a prognostic tool to aid the comparison of outcome data from different treatment modalities. The system is based on ultrasound and Doppler findings (see table 2) and has become widely used. Although it is based on observations of the pattern of disease progression, the disease may not progress in a predictable manner (Quintero 2003).

Quintero Stage	Ultrasound Finding
I	Donor bladder is still visible
II	Donor bladder is no longer visible
III	Abnormal umbilical artery, umbilical vein or ductus venosus Doppler waveforms in either twin
IV	Fetal hydrops

Table 2. Quintero severity staging system in TTTS.

The Quintero staging system is widely used although the correlation with outcome is debated. Adapted from WAPM Consensus group on TTTS (Baschat et al. 2011).

Figures 3 and 4 illustrate characteristic ultrasound findings at stage II and stage III disease. The correlation with outcome is still debated (Chmait et al. 2011; Gray et al. 2011; Murakoshi et al. 2008; Ortibus et al. 2009; Rossi & Addario 2009; Taylor et al. 2002). Alternative measures for inclusion in a staging system have been proposed (Harkness & Crombleholme 2005; Rossi & D'Addario 2009b; Rychik et al. 2007; Stirnemann et al. 2010) largely based on cardiovascular parameters. Such models are yet to be prospectively validated and a recent expert review panel recommended retaining the existing system (Stamilio et al. 2010).

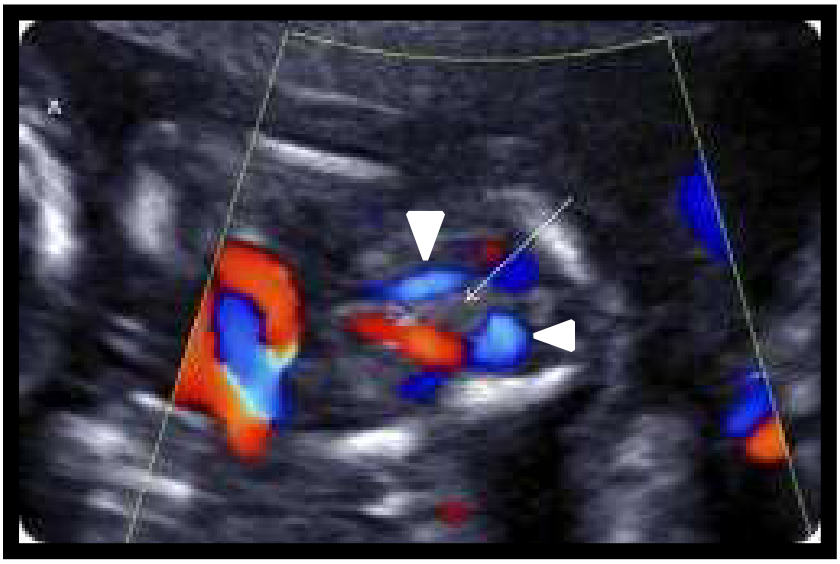


Figure 3. Ultrasound appearance of stage II TTTS.

The fetal bladder (arrow) cannot be visualised between the umbilical arteries (white arrowheads) of the donor twin. Reproduced from SMFM clinical guideline on Twin-twin transfusion syndrome. (Simpson 2013).

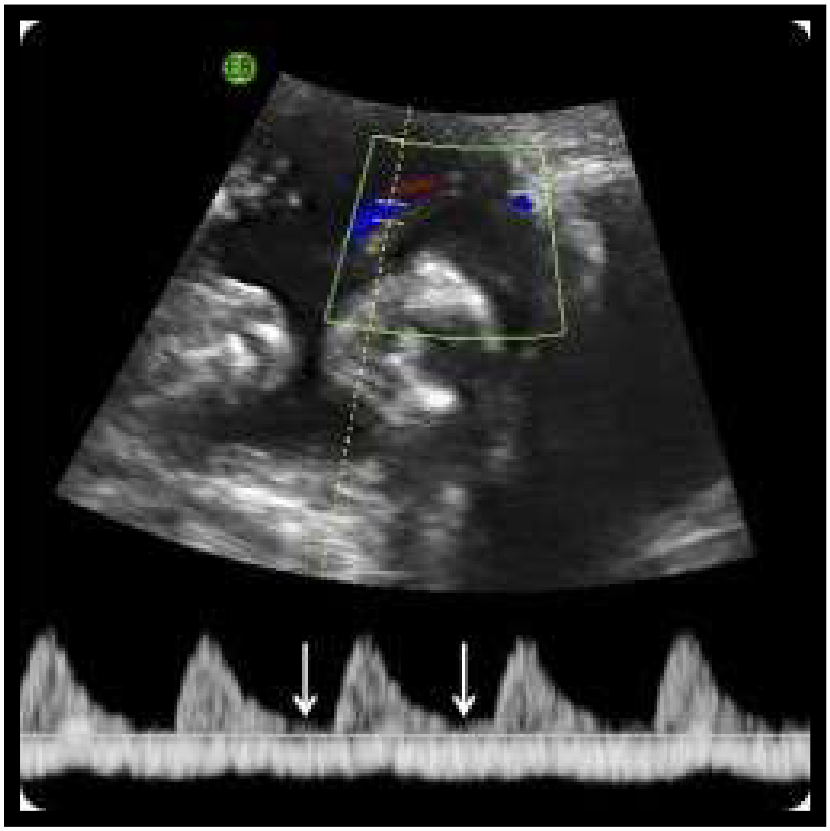


Figure 4. Ultrasound appearances of stage III TTTS.

Measurement of flow in the umbilical artery of the donor twin. Absent UA EDF is shown in the waveform (white arrows). Reproduced from SMFM clinical guideline: Twin-twin transfusion syndrome. (Simpson 2013).

1.1.4 Natural History

Twin-twin transfusion syndrome usually presents in the second trimester (15-28 weeks) but may develop at any time in gestation (Carver et al. 2011; Sueters et al. 2006). The disease may not progress in a predictable manner. There may be no progression from one stage to the next, sequential progression or non-sequential progression at varying rates (Quintero 2003). Natural history data by stage are limited, largely because by the time the staging system was developed in the 1990s, intervention was being performed to treat or limit progression.

Reports of perinatal mortality independent from stage vary from 50% to 100% (Berghella & Kaufmann 2001; Steinberg et al. 1990; Weir et al. 1979) for untreated TTTS. Prognosis is worse when the disease presents earlier in gestation (Berghella & Kaufmann 2001; Chescheir & Seeds 1988; Gul et al. 2003). Stabilization or regression occurs in over two thirds of those presenting with stage 1 disease (Simpson 2013), however disease advancement is rapid in those that do progress (O'Donoghue et al. 2007).

Perinatal mortality occurs from miscarriage or very preterm delivery due to uterine distension from polyhydramnios or fetal demise due to cardiovascular disturbance.

1.1.5 Pathophysiology

The placenta is the anatomical substrate for disease development in TTTS. Blood flow across the placenta is unbalanced and the donor twin pumps blood to the recipient twin as originally proposed by Schatz (Schatz 1882). Several excellent review papers elaborate on the complex nature of the placental aberrant anatomy and the dynamic relationship with the endocrine system (De Lia et al. 2000; Fisk et al. 2009; Lopriore et al. 1995).

1.1.5.1 Placental Features: the normal MC placenta

Dichorionic twins have completely separate placental circulations however vascular anastomoses are characteristic of the normal monochorionic placenta (Denbow et al. 2000; De Paepe et al. 2010; Robertson & Neer 1983). Several large placental injection studies have commented on the frequency of different types of placental anastomoses. Arterio-venous (AV) anastomoses connect the arterial supply from one twin to the venous supply of the co-twin. These are uni-directional and occur in 90-95% of MC placentae (De Paepe et al. 2010; Hack et al. 2008; Nikkels et al. 2008; Umur et al. 2003). In most monochorionic placentae multiple AV anastomoses exist in both directions (Hack et al. 2008) allowing for balanced intertwin exchange of blood. Arterio-arterial (AA) and veno-venous (VV) anastomoses bypass the capillary bed and are theoretically bidirectional depending on baseline haemostatic pressures. AA anastomoses occur in about 85-90% of monochorionic placentae whereas VV anastomoses are the least frequently seen in only 20-25% (Denbow et al. 2000; Nikkels et al. 2008; Umur et al. 2003). The number and diameter of vascular connections are variable in monochorionic pregnancies but critically in uncomplicated pregnancies balanced intertwin exchange should occur (see figure 5).

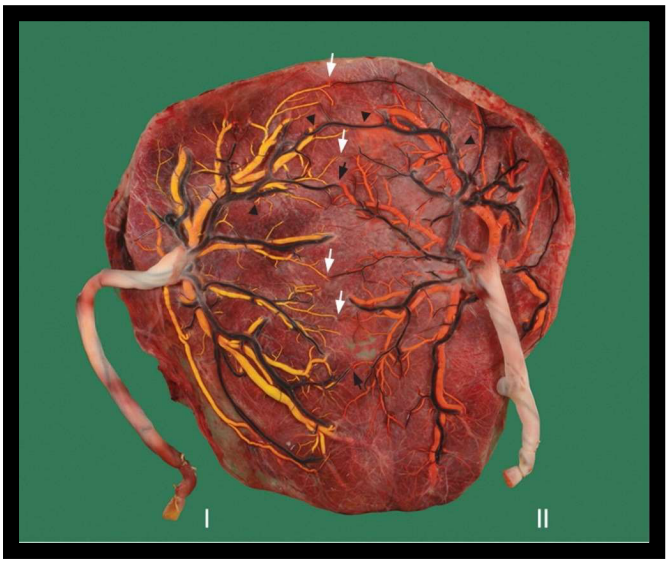


Figure 5. Uncomplicated MCDA placenta.
A normal placenta delivered at 35 weeks gestational age in an uncomplicated MCDA pregnancy is shown. The placental territories for each twin are approximately equal. Both umbilical cords show a paracentral insertion. Arterio-arterial anastomoses are shown by the black arrowheads. Arterio-venous anastomoses in opposite directions are shown by the black and white arrows. Reproduced from Nikkels et al. 2008.

1.1.5.2 Placental Features: the abnormal MC placenta in TTTS

Both placental injection studies and fetoscopic observations confirm that the presence of at least one unidirectional AV anastomoses (see figure 6) is a prerequisite for the development of TTTS (Bermúdez et al. 2002; Denbow et al. 2000; Diehl et al. 2001; Hack et al. 2008).

AA anastomoses are less frequently seen in placentae from pregnancies complicated by TTTS (Denbow et al. 2000; de Villiers et al. 2012; Lanna et al. 2015) compared to uncomplicated MC placentae. It has been suggested that the presence of AA anastomoses protects against the development of TTTS (Denbow et al. 1998; Denbow et al. 2000; De Paepe et al. 2010; de Villiers et al. 2012; Hack et al. 2008; Zhao et al. 2013). This could be because resistance in AA anastomoses, which bypass the capillary bed, may be lower than in AV anastomoses which do not. This would facilitate more efficient compensation for flow imbalance and allow equilibration of intertwin blood volumes (Umur et al. 2002).

In contrast, VV anastomoses are more frequently seen in placentas complicated by TTTS compared to those without (De Paepe et al. 2010; Zhao et al. 2014; Zhao et al. 2015). The clinical significance is unclear but their presence appears to be an independent risk factor for the development of TTTS (de Villiers et al. 2015).

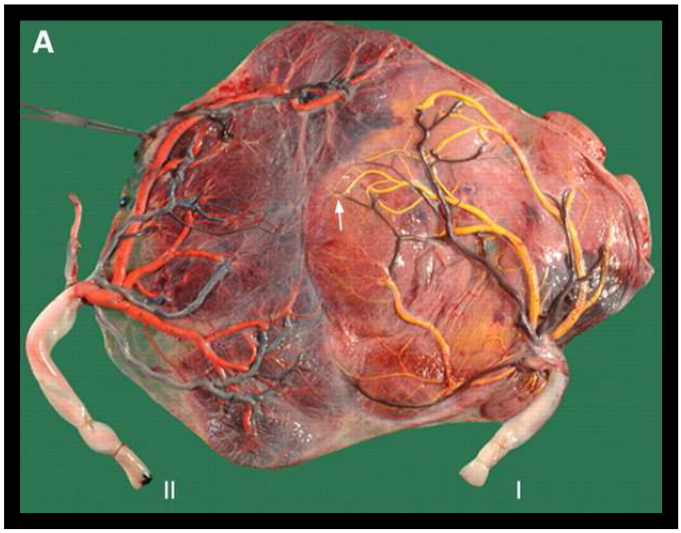


Figure 6. MCDA placenta complicated by TTTS.
This pregnancy delivered at 31 weeks gestational age. Twin II (left side of placenta in image) was the recipient twin with a velamentous cord insertion and smaller placental share. Twin I (right side of placenta in image) had a marginal cord insertion and larger placental share. A single arterio-venous anastomosis was present (white arrow) from twin I to twin II. Reproduced from Nikkels et al 2008 (Nikkels et al. 2008).

Uneven placental sharing between twins and velamentous cord insertion are more frequently found in MCDA pregnancies that develop TTTS compared to those that don't (De Paepe et al. 2010; Denbow et al. 2000) although are not independently critical to its development (Hack et al. 2008; Lopriore et al. 2007). There does not appear to be a consistent difference in the number of anastomoses between twins with TTTS and those without (Bermúdez et al. 2002; De Paepe et al.

2010) and no consistent anastomotic configuration has been identified. However an overall paucity of vascular communications as well as imbalanced flow in those that are present is certainly necessary for the development of the disease. Factors such as net cross sectional area of anastomoses may be important (De Paepe et al. 2010) in combination with placental territory.

1.1.5.3 Endocrine consequences

A net imbalance in placental flow creates hydrostatic differences between twins. Recipient kidneys exhibit down-regulation of the renin-angiotensin system (Kilby et al. 2001), as one would expect as an adaptive response to hypervolaemia. Hypervolemia in the recipient twin leads to release of atrial natriuretic peptide as a result of atrial distension. This increases renal glomerular filtration rate and decreases tubular reabsorption resulting in polyuria with consequent polyhydramnios. Fetal atrial natriuretic peptide and brain natriuretic peptide, both diuretics and potent vasodilators, promote polyuria and have been shown to be higher in the recipient twin (Nageotte et al. 1989; Wieacker et al. 1992). Vasopressin is inhibited by hypervolaemia and both plasma and amniotic fluid levels are significantly lower in recipient fetuses compared to donors and non-TTTS twin fetuses (Bajoria et al. 2004).

Hypovolaemia in the donor twin produces up-regulation of the renin-angiotensin system (RAS) in donor kidneys. Renin gene expression is increased, with forty times more renin secreting cells in donor kidneys than recipient kidneys (Kilby et al. 2001; Mahieu-Caputo et al. 2000) as well as increased angiotensin 2 receptors (Guilherme et al. 2009). Up-regulation of the RAS in donors produces vasoconstriction, oliguria and therefore oligohydramnios. Renal hypoperfusion results in renal tubular hypoplasia and atrophy (Oberg et al. 1999).

However donor RAS up-regulation and recipient RAS down-regulation fail to restore haemodynamic balance in TTTS and the disease is usually progressive. Cord blood levels of renin, aldosterone and angiotensin are similarly high in both donors and recipients compared to controls (Galea et al. 2008; Mahieu-Caputo et al. 2005). This suggests that interfetal transfusion results in recipient exposure to the donor co-twin's high circulating levels of renin and angiotensin. The recipient is therefore also affected by extrarenal paradoxic RAS activation. Recipient polyhydramnios increases intra-uterine pressure and further reduces placental blood flow promoting RAS up-regulation. Renin gene expression and protein has been found to be much higher in the placental territory of recipients compared to donors (Galea et al. 2008; Guilherme et al. 2009). The fetuses are unable to maintain

separate compensatory systems for placental flow imbalance and are negatively affected by the adaptive mechanisms of the other.

1.1.5.4 Cardiac effects

Fetal cardiac function in donors appears relatively unaffected in TTTS (Michelfelder et al. 2007; Moon-Grady 2014;) but at least one fifth of recipients demonstrate TTTS specific cardiomyopathy (Bendon 1995) which lessens following successful laser treatment (Barrea et al. 2006; Habli et al. 2008). Some recipient cardiac findings, such as increased pulmonary and aortic velocities, ventricular hypertrophy and atrioventricular valve regurgitation are consistent with volume overload alone. High levels of vasodilators such as fetal ANP and BNP correlate with cardiac dysfunction (Bajoria et al. 2001; Bajoria et al. 2002; Bajoria et al. 2003; Habli et al. 2010). However myocardial hypertrophy and left sided diastolic dysfunction suggest the influence of afterload effects which can be attributed to paradoxic extrarenal RAS activation in the recipient. Systemic hypertension exacerbates dysfunctional LV filling in the recipient and leads to impaired right to left shunting at the atrial level. This in turn results in right heart loading and many of the common cardiac sequelae recognized in recipient fetuses (Gardiner 2001; Raboisson et al. 2004; Taylor-Clarke et al. 2013). In addition, other vasoactive mediators, such as ET-1, have been found to be increased in TTTS (Bajoria et al. 1999; Galea et al. 2008). These will also contribute to the manifestation of cardiac pathology and long term vascular remodelling demonstrated in childhood in TTTS survivors (Gardiner et al. 2014).

1.1.6 Management

1.1.6.1 Conservative

Watchful waiting is an option for TTTS that presents at stage 1. Up to two thirds will stabilise or spontaneously regress (Emery et al. 2016; O'Donoghue et al. 2007; Taylor et al. 2002). However in cases that do progress, development of advanced disease usually occurs within two weeks (O'Donoghue et al. 2007), and therefore at least weekly ultrasound monitoring is recommended (Simpson 2013). A recent meta-analysis found no significant difference in perinatal outcome for conservative management versus laser therapy in stage 1 TTTS (Rossi & D'Addario 2013). However the largest series reporting outcomes in stage 1 TTTS has suggested that neurodevelopmental outcomes are improved at two years of age with early treatment (Wagner et al. 2009). This is independent of gestational age at birth and further long term studies are necessary.

1.1.6.2 Amnioreduction

Amnioreduction involves removal of excess amniotic fluid from the recipient sac usually when the MVP is >8cm and until the 1990s was the standard treatment for TTTS. This approach provides symptomatic relief from abdominal distension for the mother, particularly due to respiratory distress in later gestation. Removal of excess fluid may also reduce uterine contractions and preterm birth associated with polyhydramnios. It is also theorised that reduction of the intra-amniotic and placental vasculature pressures may improve placental blood flow.

The procedure may only be necessary once in stage 1 or 2 TTTS. This could reflect disease resolution due to improved placental blood flow following reduction of intra-amniotic and placental vasculature pressures. However it may alternatively reflect the high proportion of early stage disease that spontaneously regresses or stabilises, as previously discussed. Poorer perinatal outcomes have been reported following amnioreduction in stage 1 TTTS compared to laser therapy or conservative management (Rossi & D'Addario 2013). Serial procedures are usually necessary, particularly for advanced stage TTTS. Repeated procedures increase the likelihood of complications such as preterm prelabour rupture of the membranes, preterm labour, placental abruption and infection as well as fetal death (Roberts et al. 2008).

1.1.6.3 Fetoscopic laser

Fetoscopic laser ablation of placental vessels aims to ablate placental vascular anastomoses to remove the underlying haemodynamic imbalance between recipient and donor twins. Ultrasound is

used to map the vasculature to determine the placental angioarchitecture. The procedure is performed by passing an endoscope through the maternal abdominal wall into the recipient amniotic sac under ultrasound guidance. De Lia et al (De Lia et al. 1990) were the first to report its use in 1990. This original approach aimed for complete ablation of all visible anastomoses and involved a 10cm laparotomy under general or regional anaesthetic (De Lia et al. 1990).

The technique itself has evolved considerably since then and outcomes have been unsurprisingly been shown to improve with operator experience (Hecher et al. 2000; Morris et al. 2010; Rustico et al. 2012). In addition technological advances in both surgical and ultrasound equipment improve visualisation and access thereby increasing speed and accuracy. Following the original approach, the method was subsequently refined to a less invasive percutaneous technique that could be performed under local anaesthetic (Ville et al. 1992). The dividing membrane was later used as a landmark and a non-selective approach, in which all vessels which crossed the membrane were ablated, was proposed (Ville et al. 1995). A more selective approach was later advocated 'selective laser photocoagulation of communicating vessels' or S-LPCV (Quintero et al. 2000) which requires identification of only vessels involved in blood exchange between fetuses. More recently, sequential coagulation of the donor artery to recipient vein, followed by recipient artery to donor vein, has been proposed as it may theoretically allow some return of flow from the recipient to the donor prior to severing other connections (Quintero et al. 2007). It appears to offer a perinatal survival benefit compared to the non-sequential approach (Akkermans et al. 2014; Chmait et al. 2011; Chmait et al. 2014).

However, recurrence or persistence of disease occurs in up to 16% (Morris et al. 2010; Robyr et al. 2006; Rustico et al. 2012; Walsh & McAuliffe 2012) and may be due to incomplete initial ablation or recanalization of anastomoses (Lewi et al. 2006). Twin anaemia-polycythaemia sequence (TAPS) has been reported in up to 13% (Habli et al. 2009; Robyr et al. 2006; Rustico et al. 2012). Further significant complications include premature rupture of membranes in 7-18% of cases (Habli et al. 2009; Morris et al. 2010; Rustico et al. 2012; Yamamoto et al. 2005), placental abruption in 3-8% (Habli et al. 2009, Yamamoto et al. 2005), amniotic band syndrome (Habli et al. 2009). There is also the possibility of iatrogenic monoamniocity with the associated consequent risks of cord entanglement as well as potential maternal haemorrhage and laparotomy, infection and amniotic fluid embolism. Coagulation of the surface of the chorionic plate between anastomoses or 'equatorial dichorionisation' (ED-LASER or Solomon technique) has been shown to reduce complications of TTTS recurrence and TAPS without impacting perinatal mortality (Baschat et al. 2013; Ruano et al. 2013). Longer term outcome results of this approach are awaited.

1.1.6.4 Selective feticide

Selective feticide involves interrupting the blood flow of the umbilical cord in one twin to cause intentional death of the twin. It is usually performed with radiofrequency ablation or cord coagulation. Clearly the maximum perinatal survival rate cannot exceed 50%. It is therefore an option when alternative treatments are not possible or prognosis of a co-twin is particularly poor as outcomes for the survivor are better than following spontaneous death of the co-twin (O'Donoghue et al. 2009; Paramasivam et al. 2010; Robyr et al. 2005).

1.1.6.5 Outcomes for amnioreduction versus laser treatment

There have been two Cochrane Reviews comparing amnioreduction and laser treatments for TTTS. The earliest (Roberts et al. 2008) concluded that laser treatment should be considered to improve perinatal outcome in TTTS. Laser treatment was found to result in less death overall, less perinatal death, as well as less neonatal death, compared to amnioreduction. This earlier review included only one trial, the Eurofetus trial (Senat et al. 2004). The study randomised 142 women to laser or amnioreduction. The vast majority had stage 2 or 3 disease, with a small number treated at stage 1. The trial was stopped early as interim analyses showed a significantly higher chance of survival at both 28 days and 6 months in the laser treatment arm compared to amnioreduction. In addition cerebral injury and neurological complications at 6 months were significantly higher following amnioreduction compared to laser.

The more recent Cochrane Review (Roberts et al. 2014) concluded that laser treatment should continue to be considered in the treatment of TTTS to improve neurodevelopmental outcome. In contrast to the earlier review, they found no significant difference in overall death, death of at least one infant or death of both infants between laser and amnioreduction treatments. There was no difference between numbers of children alive at six years with major neurological abnormality, however significantly more children were alive without neurological abnormality at the age of six years following laser treatment compared to amnioreduction. Current evidence from level 1 meta-analysis data finds no significant overall survival benefit with laser treatment relative to amnioreduction.

The later Cochrane Review included the American NICHD trial (Crombleholme et al. 2007) in addition to the Eurofetus trial. The NICHD trial was also terminated early, due to poor recruitment; only 42 women (less than one third of the planned sample size) were recruited over five years.

Although no significant differences in perinatal outcomes were found between the treatment groups, a trend was observed towards lower survival rates of at least one twin and higher perinatal mortality following laser treatment. There was also a significantly increased recipient twin mortality in the laser group compared to the amnioreduction group. The authors acknowledge that the outcome of the trial did not conclusively answer the question of which treatment is superior.

In effect the two trials included in the most recent Cochrane Review appear to have found opposite directions of effect on the main outcome of overall death, resulting in a shift in conclusion. However, several important considerations must be made. Quintero stage at treatment in this cohort was more advanced than in the Eurofetus trial as participants with stage 1 disease were excluded and there were more cases of stage 4 disease. The upper gestational age limit for inclusion was also lower in the NICHD trial; 24 weeks compared to 26 weeks in Eurofetus. The authors of the NICHD trial comment that referring centres were unwilling to refer to a centre where laser treatment was only available within a trial setting and that both arms of the study will have excluded many eligible participants. Other criticisms, including the lack of power and operator experience with the laser technique have been raised, leading some to suggest that the results of the trial should not be considered regarding the management of TTTS (Battin et al. 2008; Johnson & Moise 2008; Quintero & Chmait 2009).

A meta-analysis in 2008 (Rossi & D'Addario 2008) included the Eurofetus randomised trial data as well as data from three additional non-randomised series comparing outcomes between amnioreduction and laser treatment. They report an overall survival benefit following laser compared to amnioreduction with an odds ratio of 2.04 (95% CI 1.52-2.76). Results from a further six published studies were also commented upon; two small amnioreduction outcome series (n<20) and four laser outcome series (n<100). Analysis of this data from over 600 cases found overall survival rate following laser treatment of 57%-77% with perinatal survival rate of at least one twin as 76-87% compared to 38%-81% overall survival and 51%-90% for survival of at least one twin following amnioreduction. Cerebral anomalies ranged from 2%-33% following laser treatment and 18%-83% following amnioreduction. A more recent meta-analysis reviewed cerebral injury after amnioreduction versus laser surgery (Van Klink et al 2013) and included data from over 600 children from non-randomised comparison studies. They found a significantly higher risk of severe cerebral injury in children following treatment with amnioreduction compared to laser (OR 7.69; 95% CI 2.78-20.0). This increased risk remained after excluding infants that died in the neonatal period.

Given the limitations of the available evidence, fetoscopic laser therapy is considered to be broadly preferable to amnioreduction for the treatment of TTTS stage 2 and above at less than 26 weeks

(Moise et al. 2013; Quintero et al. 2013; Rossi & D'Addario 2008; Salomon et al. 2010; Simpson 2013). The participants in the current study were exclusively treated by fetoscopic laser therapy. The following section reviews the available data from recent non-randomised series of outcomes following laser treatment.

1.1.7 Outcomes following fetoscopic laser therapy

1.1.7.1 Perinatal outcome

In a recent summary of six non-randomised series including over 1000 cases of TTTS treated with laser, overall perinatal survival was 65% (Simpson 2013). Survival of at least one twin was 81%. Table 3 below summarises more recent large outcome series (>100 participants) that were not included in the report by Simpson (2013). Perinatal survival of at least 1 twin ranges from 81-91% and overall survival rates are 75-79% Outcomes from smaller series must be interpreted cautiously as results may be skewed due to operator inexperience or inclusion bias (Nakata et al. 2009; Morris et al. 2010; Crombleholme et al. 2007).

Study	Country	N	≥ 1 survivor %	Overall survival %
(Cincotta et al. 2009)	Australia	100	86	75
(Habli et al. 2009)	US (NIH)	152	88	77%
(Sago et al. 2010)	Japan	181	91	78
(Chmait et al. 2011)	USFetus	682	91	79
(Rustico et al. 2012)	Italy	150	74	57

Table 3. Summary of recent studies reporting perinatal survival following laser treatment in TTTS.
Survival refers to survival in the perinatal period. The figure for overall survival is not explicitly reported in the USFetus publication (Chmait et al. 2011) but has been calculated from the data published.

Rustico and colleagues (Rustico et al. 2012) report a relatively low 74% perinatal survival of at least one twin over the study period. However as a new centre performing fetoscopic laser therapy they have demonstrated improvement in outcomes of 1% with each 10 procedures performed, and

perinatal survival of at least one twin has risen to over 80% with increasing operator experience. Each of these studies has used the selective laser technique. The largest study (Chmait et al. 2011) used the sequential selective technique (Quintero et al. 2007) in over 50% of cases, and also reports the best outcomes. One study excluded cases of stage 1 TTTS (Cincotta et al. 2009). In the remaining studies the majority of cases (74-80%) were for stage 2 and 3 TTTS, but included cases from all stages. Treatment was undertaken between 16 weeks and 26 weeks in all studies except one, which had an upper limit of 28 weeks (Cincotta et al. 2009), and another study which included three exceptional cases (Chmait et al. 2011). The exact time frame of survival was not always clear but when defined was within a range from 28 days to 6 weeks after delivery.

Robyr et al (2006) report on the prevalence and management of fetal complications following successful laser. In their cohort of 151 cases, single fetal death occurred in 30%. However the perinatal survival rates cannot be ascertained as the study outcomes reported are only for those 101 pregnancies in which both twins were alive seven days following the laser procedure. This study included participants from two centres and it is likely that outcomes from at least one of the centres have been reported as part of larger series elsewhere.

Four other outcome studies have reported lower overall survival rates of 40-60% and survival of at least one twin as 58-79% (Sepulveda et al. 2007; Ruano et al. 2013; Hernandez-Andrade et al. 2011; Has et al. 2014). However all of these studies are from developing countries and have consequent limitations as the authors point out. Importantly the non-selective technique, which is known to have poorer outcomes (Quintero et al. 2000), was used in a number of cases and follow up was often at non-specialist and distant centres. Access to obstetric and neonatal services in these circumstances is not comparable to those in developed countries.

Although some studies report equal perinatal survival and fetal death rates for ex-recipients and ex-donors (Senat et al. 2004; Huber et al. 2006; Cavicchioni et al. 2006), results from meta-analysis (Rossi & D'Addario 2009a) found that overall perinatal survival rates were higher for ex-recipients than for ex-donors. More recent series also confirm significantly higher perinatal loss rates for ex-donors compared to ex-recipients (Skupski et al. 2010; Has et al. 2014). This is probably mostly attributable to a higher rate of fetal, rather than neonatal, loss for ex-donors following laser treatment. Multiple studies have observed an increased risk of fetal death for ex-donors relative to their co-twin (Cincotta et al. 2009; Chmait et al. 2011; Sago et al. 2010; Morris et al. 2010), whereas outcomes in the neonatal period are more equivocal. Morris et al (Morris et al. 2010) did not observe any difference in neonatal mortality in their cohort. However in a much larger series, the risk of neonatal death was significantly higher for ex-donors compared to ex-recipients, particularly in those

treated for stage 3 disease (Chmait et al. 2011). This may be related to increased prevalence of donor sFGR in this subgroup.

1.1.7.2 Neurodevelopmental outcome

As the use of fetoscopic laser becomes more widespread, an increasing number of studies have examined neurodevelopmental outcomes in TTTS. Various criteria are used to define impairment but usually include cerebral palsy (CP), performance on cognitive / developmental tests, blindness or deafness. Rates of impaired function have reduced over time as techniques have improved (Maschke et al. 2011), particularly in groups treated with fetoscopic laser surgery.

Outcome studies of laser treatment have assessed children up to 6 years of age and report an incidence of approximately 10-20% impairment (Salomon et al. 2010; Lenclen et al. 2009; Ortibus et al. 2009; Sutcliffe et al. 2001; Gray et al. 2011; Graeve et al. 2012). A recent meta-analysis found neurological impairment of 13.3% of which 7.1% was defined as cerebral palsy (van Klink et al. 2011). Rustico et al (Rustico et al. 2012) have assessed neurological morbidity at a mean age of 30 months (range 15 months to 6 years) and report an overall rate of 7.6% impairment. This constitutes 3.5% severe impairment (classified as CP or bilateral deafness), 1.7% moderate (unilateral deafness or ADHD) and 2.3% mild impairment (learning disability or attention deficit).

However a recent small study (n=38) reporting neurological morbidity at a median follow up of four years found no difference compared to gestational age matched twin controls and report that most deficits were prematurity related (Kowitt et al. 2012). Several fetoscopic laser series which have reported perinatal outcomes are yet to report long term neurodevelopmental follow up (Sago et al. 2010; Chmait et al. 2011; Morris et al. 2010; Habli et al. 2009; Quintero et al. 2007).

Unsurprisingly lower gestational age at birth and birth weight are associated with increased risk of neurological impairment (Graef et al. 2006; Ortibus et al. 2009; Lenclen et al. 2009; Maschke et al. 2011). Some studies have found that higher Quintero stage is an independent risk factor for neurological impairment (Gray et al. 2011; Lopriore et al. 2007; Salomon et al. 2010). Multiple follow up studies have failed to find a significant association between recipient or donor survivors and risk of neurodevelopmental impairment (Senat et al. 2004; van Klink et al. 2011; Maschke et al. 2011). However the increased mortality in ex-donor twins may have confounded this observed lack of association. Higher incidence of severe cerebral injury in ex-donors (Quarello et al. 2007) results in increased likelihood of fetal death for donors compared to recipients (Rossi & D'Addario 2009a;

Cincotta et al. 2009; Chmait et al. 2011). Furthermore the higher incidence of severe cerebral injury (Quarello et al. 2007) combined with lower birth weights (Maschke et al. 2010) increases neonatal death rate in ex-donors (Rossi & D'Addario 2009a; Chmait et al. 2011). If these babies survived it is unlikely that NDI would be equally seen in ex-recipients and ex-donors.

The impact of single fetal death on neurodevelopmental impairment in the surviving co-twin is unclear. Maschke and colleagues (2011) found no difference in neurodevelopmental outcome between infants born as twins or singletons. Some studies suggest that severe neurodevelopmental impairment is less common in singleton relative to double survivors (Sutcliffe et al. 2001; Gray et al. 2011) whereas others have found a higher incidence of 11-16% (Cavicchioni et al. 2006; Graef et al. 2006).

The aetiology of long-term neurological deficit is complex. A significant proportion will relate to the effects of premature birth (Blickstein 2002). The co-existence of congenital anomalies is higher in MC pregnancies complicated by TTTS (Patel et al. 2012; Springer et al. 2014) and may lead to higher rates of neurological impairment secondary to the impact of long term hospital treatment. Other mechanisms specific to TTTS have been suggested. Brain injury following death of a co-twin (Glinianaia et al. 2002) may or may not lead to increased rates of neurodevelopmental impairment. Haemodynamic instability or fluctuations in blood pressure and volume through direct (AA, VV) anastomoses (Grafe 1993), as well as donor anaemia and hypotension and recipient polycythaemia and hyperviscosity may contribute. Nutritional insufficiency causing qualitative alterations in brain myelination (Ulfig et al. 1998) has also been proposed.

1.2 The fetal brain in TTTS

1.2.1 In utero brain injury

1.2.1.1 Pathophysiology of brain injury

Haemodynamic imbalance causing intertwin blood shifts is the most likely mechanism by which intrauterine brain injury occurs in untreated TTTS. Although the net flow of blood is from donor to recipient, it is likely that fluctuations can occur in either direction (Degani et al. 2006). Periods of low flow during hypoperfusion lead to hypoxic injury and high flow during hyperperfusion carries a risk of haemorrhage. High flow and low flow lesions may occur in both donors and recipients.

Both neurological morbidity and cerebral injury rates are higher following amnioreduction compared to laser (Rossi & D'Addario 2008). This supports the hypothesis that placental anastomoses are central to the development of brain lesions. Although laser therapy reduces the risk of brain injury (Senat et al. 2004), acute disturbance in blood flow between twins at the time of laser therapy, or ongoing chronic injury due to incomplete ablation or recanalization, may occur.

Fetal anaemia may negatively influence cerebral oxygenation and cause hypoxic – ischaemic injury. Polycythaemia is also a theoretical mechanism by which intravascular sludging may result in ischaemic injury (Carr et al. 2004). Whilst neither have been found to be independently associated with overall cerebral injury (Lopriore et al. 2006), anaemia does appear to associate with severe brain injury (Lenclen et al. 2007). Anaemia may be compatible with persistence or early recurrence of TTTS despite surgery (Lewi et al. 2006).

1.2.1.2 Classification of brain injury

Multiple types of brain injury have been described in TTTS. The appearance of brain lesions will vary depending on the duration since the injury as well as the maturity of the fetal brain at the time of the insult. Patterns of injury most commonly seen include intraventricular haemorrhage (IVH) and periventricular leukomalacia (PVL) although isolated ventriculomegaly is also frequently described (Lopriore et al. 2006; Quarello et al. 2007; Merhar et al. 2013; Tarui et al. 2012).

Intraventricular haemorrhage may be graded according to extent and severity as follows Papile (1978) (Papile et al. 1978):

I : Germinal matrix (GM) haemorrhage
II : GM haemorrhage with extension into lateral ventricles without ventriculomegaly
III : GM haemorrhage with extension and ventriculomegaly
IV : Intraparenchymal haemorrhage

The germinal matrix is a metabolically active region supplied by a fragile capillary network and sensitive to ischaemia. During fetal life neuronal proliferation occurs at the subependymal germinal matrix as neuroblasts divide and migrate into the cerebral parenchyma towards the cerebral cortex. Neuronal proliferation is complete by about 20 weeks GA although glial cell proliferation continues until around 32 weeks. Alterations in cerebral blood flow and pressure may cause rupture of the fragile capillary network supplying the GM resulting in grade I-III IVH. Grade IV IVH most likely occurs through haemorrhage of the draining veins of the capillary network into the parenchyma. Eventually necrotic areas form cysts that may become continuous with the ventricles (porencephalic cysts).

Periventricular leukomalacia is a white matter lesion that results from ischaemia induced injury to oligodendrocytes in the periventricular area of the fetal brain. It is graded in severity from grade I to IV (de Vries et al. 1992). The deep penetrating arteries of the MCA that supply the watershed zone of the periventricular white matter have few vascular anastomoses. Consequently perfusion is difficult to maintain during periods of hypotension. The region is therefore particularly susceptible to cerebral blood flow disturbances causing ischaemia.

1.2.1.3 Incidence of brain injury

The reported incidence of antenatally acquired cerebral injury following laser treatment varies from 1.4% to 10% (Lopriore et al. 2006; Lenclen et al. 2007; Rustico et al. 2012; Cincotta et al. 2009; Spruijt et al. 2012; Quarello et al. 2007). The table below (table 4) presents the major studies published over the last decade that have commented on post laser cerebral injury acquired in utero.

Study	Sample (n)	Mode	Timing	Incidence (%)	Recipient/donor differences
Lopriore et al (2006)	84	US	day 1	10%	No difference at d/c
Lenclen et al (2007) [a]	101	US / MRI	week 1 (US) +/- antenatal (MRI)	9%	No difference at d/c
Rustico et al (2012) [b]	300	US +/- MRI	antenatal	2%	All donors
Cincotta et al (2009) [c]	167	US	week 1	1.4%	Not reported
Sprujit et al (2012)	267	US	day 1	4.5%	No difference at d/c
Quarello et al (2007)	222	US +/- MRI	antenatal	5.4%	Donors > Recipients

Table 4. Summary of recent studies reporting brain injury acquired in utero post laser therapy.

Sample size refers to number of infants/fetuses. With the exception of the study by Rustico et al (2012) all injury rates are for postnatal imaging in survivors. The definition of survivors varies from alive at 1 week to alive at 6 months depending on the study. All studies included single survivors of co-twin death with the percentage included varying from 12-18%. [a] The cohort was limited to preterm delivery <34 weeks GA. [b] MRI was performed in utero in all survivors of single fetal death and in other cases if abnormality was suspected on ultrasound. [c] Study included triplets; 8% of neonates died before imaging could be performed. (d/c : discharge).

The variation in estimates reflects a number of methodological inconsistencies. The definition of severe cerebral abnormalities usually includes the presence of severe intraventricular haemorrhage (grade III or IV) or cystic periventricular leukomalacia (grade III or IV). Some studies have also included hydrocephalus, lenticulostriate vasculopathy, subependymal pseudocysts, enlargement of the extra-axial space and ventriculomegaly (Lopriore et al. 2006). As one would expect studies with broader definitions also report higher rates of neurological injury.

Most studies have ascribed abnormalities diagnosed on cranial ultrasound within the first week of life to antenatal events (Lopriore et al. 2006; Lenclen et al. 2007; Cincotta et al. 2009; Spruijt et al. 2012). There are a number of issues with this approach. Firstly ultrasound is less sensitive at detecting non-haemorrhagic brain injury compared to MRI (Mirmiran et al. 2004; Merhar et al. 2013) and therefore may underestimate injury. Secondly those fetuses with brain injury that do not survive

to birth, or who die before cranial ultrasound can be performed, are inherently excluded. This will obviously lower the estimate of antenatally acquired injury. On the other hand prematurity is common in TTTS with an average GA at delivery of 32 weeks in the largest reported series of TTTS (Chmait et al. 2011). Premature infants are at increased risk of brain injury (particularly PVL) during labour and delivery and in the early neonatal period (Volpe 2009). Such estimates of the incidence of antenatal injury may therefore be confounded by the inclusion of perinatal insult. Reports of the incidence of antenatally acquired injury that are based on postnatal ultrasound must therefore be interpreted with caution.

A further consideration is that most studies included single survivors of co-twin death following laser in their analysis (Lenclen et al. 2007; Rustico et al. 2012; Cincotta et al. 2009; Lopriore et al. 2006; Spruijt et al. 2012). Whether or not co-twin death following laser treatment has an impact on risk of brain injury compared to cases in which both fetuses survive is unclear. Quarello et al 2007 (Quarello et al. 2007) report that cerebral lesions were twice as common in single survivors compared to double survivors after laser treatment. One other study has analysed these outcomes separately and found no difference in incidence (Vanderbilt et al. 2012). However this study assessed cerebral injury in neonatal survivors at 30 days and therefore excludes cases that may not have survived due to severe injury.

At least one study has included triplet pregnancies with a monochorionic pair treated for TTTS in their cohort (Cincotta et al. 2009). The significance of this is unclear as no studies have compared brain injury in triplet pregnancies treated for TTTS with similarly affected twin pregnancies. However overall perinatal morbidity and mortality rates are higher in triplet pregnancies (Diemert et al. 2010) treated for TTTS. Increased brain injury is likely to account for some of this.

In a meta-analysis of trials reporting outcomes in those treated with laser therapy, the rate of severe injury was found to be 13% (Rossi & D'Addario 2008). This estimate is based on four studies published between 1999 and 2007 but does not refer directly to injury acquired in utero following laser treatment. The figures included in the meta-analysis include injury diagnosed neonatally in survivors, usually at discharge, and attributed retrospectively to the antenatal period. Two more recent outcome studies have reported cerebral injury incidence in survivors of 9.9% at 28 days (Morris et al. 2010) and 5.4% at six months (Sago et al. 2010). A number of large studies reporting pregnancy and perinatal outcome following laser therapy have not commented explicitly on rates of in utero cerebral injury (Ville et al. 1998; Quintero et al. 2007; Huber et al. 2006; Middeldorp et al. 2007; Chmait et al. 2011; Hecher et al. 2000; Yamamoto et al. 2005).

The study by Quarello et al (Quarello et al. 2007) probably represents the closest estimate of the incidence of antenatally acquired injury following laser treatment. In this large study of 222 fetuses, reported incidence was 5.4%. Cerebral injuries were diagnosed by ultrasound or MRI in utero and all abnormalities were included. Imaging was also performed within the three days of delivery in case of missed injury acquired in the interval between antenatal imaging and delivery. No new lesions were identified. Fetuses in which pregnancy was terminated due to severe cerebral abnormalities, as well as single survivors of co-twin death, were included. However fetuses that did not survive to 7 days of life were excluded. Injury was significantly more common in single survivors of co-twin death (11%) compared to double survivors (4%).

Antenatally acquired cerebral injury is more common in ex-donors compared to ex-recipients (5.6% and 0.9% respectively) (Quarello et al. 2007). Rustico et al (2012) (Rustico et al. 2012) performed in utero brain MRI following all cases of single fetal death and in cases where injury was suspected on antenatal ultrasound. Cerebral lesions were seen exclusively in ex-donor fetuses. There were two cases of grade 3 haemorrhagic lesions following death of the recipient co-twin, and an ischaemic lesion in the ex-donor fetus in a case where the EFW discordance was >40%.

Several other studies have reported no difference in brain injury rates for ex-recipients and ex-donors. However it is important to note that these studies report incidence either in neonates at 30 days (Vanderbilt et al. 2012) or in neonates at discharge (Lopriore et al. 2006; Lenclen et al. 2007; Spruijt et al. 2012). Ex-donors are at increased risk of fetal and neonatal death (Rossi & D'Addario 2009a), often with coexistent brain injury. Incidence of brain injury in neonatal survivors therefore excludes lethal cerebral injury acquired both in utero and in the neonatal period.

One meta-analysis has separated ex-donor and ex-recipient findings of cerebral injury and reported rates of 10% and 9% respectively (Rossi & D'Addario 2009a). Five studies published between 2003 and 2007 were included in this meta-analysis. Although one included study (Quarello et al. 2007) described in utero acquired injury, the remainder report different outcomes. Two studies actually report neurodevelopmental impairment in survivors over one year of age rather than radiological cerebral injury (Banek et al. 2003; Graef et al. 2006). Whilst this is an important outcome, it is clearly not the same as in utero acquired injury following laser treatment for TTTS. The meta-analysis also included the data from the Eurofetus trial (Senat et al. 2004) (included in the later study by Lenclen et al (Lenclen et al. 2007)) as well as the study by Lopriore et al 2006 (Lopriore et al. 2006) which reported injury in survivors at discharge. With the exception of Quarello et al 2007 (Quarello et al. 2007), all of the studies are likely to have excluded a significant proportion of injury in ex-donor

fetuses that do not survive to follow up. This may account for the lack of difference found in that meta-analysis between ex-recipients and ex-donors.

1.2.2 In utero brain development

1.2.2.1 Development in twins

Early anatomic studies report a two week lag in cortical maturation in twins, as described by the appearance of sulci and fissures, compared to singletons (Chi & Dooling 1977). The limited imaging studies of brain development in twins have mostly been restricted to ultrasound biometry comparisons with singletons. Ultrasound based Biparietal diameter (BPD) studies in twins have produced no consensus regarding findings. Consistently smaller BPDs have been shown in twins relative to singletons throughout pregnancy (Leveno et al. 1979) whilst other studies have shown similar BPDs and head circumference (HC) throughout (Reece et al. 1991). Investigators have found that BPD growth in twins mirrors singletons until the third trimester and then slows (Persson & Grennert 1979) whereas alternative studies report significantly different BPDs, HCs and ventricular widths in the second trimester (Gilmore et al. 1996). Ong et al 2002 (Ong et al. 2002) report consistently larger BPDs from mid to early third trimester in twins relative to singletons followed by a significant reduction in growth into the third trimester. Furthermore these studies have rarely accounted for chorionicity or growth discordance. Some authors have criticised the use of the BPD as a reliable measure in twins due to the increased incidence of dolichocephaly resulting from non-vertex presentation and uterine crowding (Goldstein et al. 2002).

The development of the cerebellum in utero has been more successfully studied. It has been of particular interest because of its position within the posterior fossa providing relative protection from compressive effects more common in multiple gestations. Transverse cerebellar diameter (TCD) measurements by antenatal ultrasound show comparable growth patterns in both singleton and twin gestations (Dias et al. 2011; Goldstein et al. 2002; Shimizu et al. 1992). TCDs appear concordant within twin pairs irrespective of chorionicity and growth discordance (Shimizu et al. 1992; Goldstein & Reece 1995) although small TCD differences have been observed in dizygous (DZ) growth discordant twins (Charlemaine et al. 2000).

A preferential protective mechanism in brain development may underlie the consistency of TCD measures observed between twins and compared to singletons and reflect its more established evolutionary status. Similar cerebellar circuits are seen in all classes of vertebrates (Bell et al. 2008)

and whilst it is certainly important in motor control, its full role is yet to be fully understood. It demonstrates unusual plasticity and whilst the neocortex has reduced in size over recent human evolution, the cerebellum has increased (Weaver 2005). Furthermore, in growth restricted fetuses regional blood flow redistribution appears to favour the cerebellum over other cortical areas (Hernandez-Andrade et al. 2008) and relative reduction in cerebellar growth in utero may correlate to poorer neurodevelopmental outcomes (Padilla et al. 2011). One caveat in interpreting TCDs is that biometry only correlates directly to volume in normal brains although normal measures may exist in spite of structural cerebellar abnormality.

Animal models suggest that the cerebellum is the last to be affected by a decrease in blood flow (Behrman et al. 1970). These authors found that the blood flow to the cerebellum was significantly higher relative to cerebral cortex in the control group. However, in asphyxiated primate fetuses, although cortical blood flow decreased, blood flow to the cerebellum remained unchanged. Furthermore the cerebellum is rarely injured in term neonates with hypoxic ischaemic encephalopathy (Chao et al. 2006). This may make its investigation of particular interest in TTTS where fluctuation in blood flow is a central issue.

Parameters of brain growth and maturation have been studied by postnatal magnetic resonance (MR) imaging in preterm twins. Dubois and colleagues (2008) (Dubois et al. 2008) compared cortical volume, surface and sulcation index in 12 twin pairs and 22 singletons at comparable gestational age. Twins had smaller cortical volumes and surface area at a given gestation but showed similar patterns of folding for equivalent surface areas suggesting delayed but harmonious maturation compared to singletons. However this study did not examine the influence of placentation or growth concordance in the small twin group.

One study has compared neonatal intracranial volumetry from MRI within the first month of life in singletons and uncomplicated monozygous (MZ) and dizygous (DZ) twins (Knickmeyer et al. 2011). Chorionicity however was not commented upon in the MZ group. Whilst lateral ventricular volumes were similar between singletons, monozygous and dizygous twins, total cerebral CSF volumes were largest in MZ twins. Neonatal growth trajectories of the cortical grey matter were accelerated in MZ twins and suggest that in utero growth is delayed in this group relative to singletons and DZ twins. Total intracranial volume, cortical brain volume and cerebellar volumes were otherwise similar between MZ twins and singletons.

1.2.2.2 Brain growth and development in TTTS

Whilst the increased incidence of perinatal brain injury in twins affected by TTTS is well documented, only three studies have commented on in utero brain development in the absence of overt brain injury in these complex twin pregnancies.

Jelin and colleagues (Jelin et al. 2008) reported fetal brain MR findings in a cohort of survivors of spontaneous co-twin demise, which included pregnancies with a confirmed or probable diagnosis of TTTS. Delayed sulcation, most prominent at the Sylvian fissure, was noted in two TTTS cases although one of these fetuses also had evidence of parietal infarct.

The second study described fetal MR findings in a cohort of 25 consecutive pregnancies with TTTS (Kline-Fath et al. 2007). Gestational age at imaging ranged from 18 to 23 weeks although it is not clear whether any pregnancies had undergone treatment for TTTS. Cerebral venous sinus enlargement was observed in 50% of donor fetuses relative to their co-twins.

More recently biometric MRI measures have been compared in 17 cases of TTTS with structurally normal brains (Tarui et al. 2012). Smaller bitemporal diameter (BTD) was seen in donors compared to both recipients and singleton controls. TCD was also smaller in donors even when corrected by BTD and the authors report that growth trajectories with gestational age are different between recipients and donors. However this study has a number of limitations. It was a relatively small sample with no follow up reported. The median gestational age at imaging was 21 weeks and the authors do not report when or whether the cases received treatment for TTTS nor the stage of disease. Furthermore non-motion corrected MR images were used for measurement and therefore more likely to be distorted by motion artefact.

1.3 Fetal magnetic resonance imaging

MRI is a non-invasive imaging technique based on the principles of electromagnetism. It exploits the inherent atomic magnetic properties of the hydrogen nucleus, which is abundantly found in biological tissues. As it is not therefore reliant on ionizing radiation, it has become an increasingly popular tool in fetal medicine.

1.3.1 Fundamental Principles of MRI

Nuclear spin **:** The hydrogen nucleus comprises a single proton that has magnetic properties (nuclear spin). It behaves like a tiny rotating magnet, spinning around its axis. The axis of spin is normally randomly oriented (see figure 7), and therefore the sum of all the spins gives a 'null net magnetisation'.

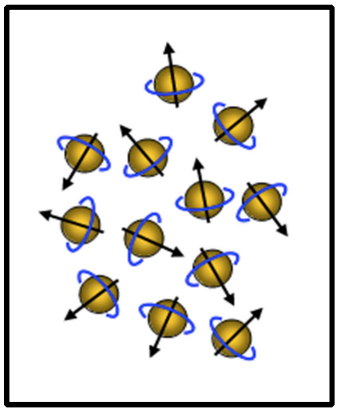


Figure 7. Magnetic properties of the hydrogen nucleus.
Under normal conditions the axis of spin of the hydrogen nucleus is randomly oriented.

Precession **:** When an external magnetic field is applied (B_0), hydrogen nuclei either align with (parallel) or against (antiparallel) the external field (see figure 8). In fetal MR a static external magnetic field (B_0) is usually generated by superconducting electromagnets. The maximum strength routinely used in fetal imaging is 1.5T. The nuclei 'precess' or move similarly to the wobbling of a spinning top when spun.

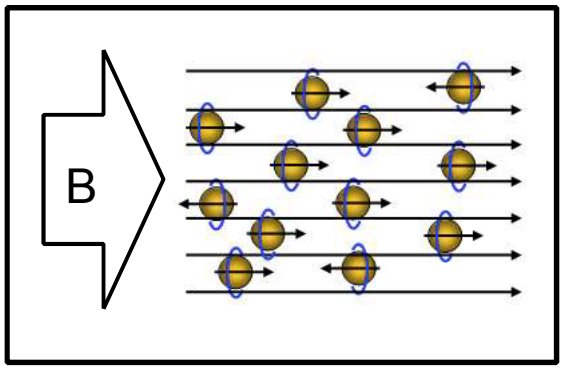


Figure 8. Effect of application of an external magnetic field on the hydrogen nucleus.
When an external magnetic field (B_0) is applied, the hydrogen nuclei align parallel or anti-parallel to B_0 and precess.

The number of times the protons precess per second (Larmor frequency) is proportional to the strength of the magnetic field :

$$\omega_0 = \gamma B_0$$

Where ω_0 = Precessional or Larmor frequency (MHz)

γ = Gyro magnetic Ratio (MHz/T)

B_0 = Magnetic field strength (T)

Net magnetization : Parallel and anti-parallel alignment states represent lower and higher energy states respectively. The nuclei are constantly oscillating between states although at any one time slightly more favour the low energy parallel state. This results in a sum magnetic field or the net magnetization vector (M_0). M_0 has a longitudinal component (parallel to B_0) and a transverse component. In the undisturbed state net magnetization is parallel to B_0 (longitudinal magnetization) and its magnitude is proportional to the strength of B_0.

Excitation : Transmission of a radiofrequency pulse disturbs protons by transfer of energy so that they move to a higher energy state and fall out of alignment with B_0 (excitation). The frequency of the RF pulse must be the same as the precessional frequency of the protons in order to achieve this, a phenomenon called ‘resonance’. Excitation results in reduction of longitudinal magnetization as more protons move to an antiparallel alignment. It also results in the appearance of ‘transverse magnetisation’ in the xy plane as protons move ‘in phase’ with each other rather than random directions. The degree to which transverse magnetization occurs is referred to as the ‘flip angle’ and its amplitude will depend on the strength and duration of the RF pulse.

Relaxation : As soon as an RF pulse is turned off protons fall out of phase and return to a lower energy state. The time for the transverse magnetisation to decay is called T2 relaxation and the time for longitudinal magnetisation to recover is called T1 relaxation. Both follow an exponential curve and therefore T1 and T2 refer to the time taken to reach approximately 63% of the final value. T1 is related to magnetic field strength. T2 is always shorter than T1. Both are tissue specific. Free water and bound hydrogen have a long T1, whereas fat typically has a short T1 value.

Signal detection and image generation: Oscillations in the magnetic field can be detected by a receiver coil as electrical current and this forms the basis of production of the MR signal. This electrical signal is known as free induction decay (FID). Pulsed RF fields are used to generate the signal. The magnitude of the signal generated is proportional to the number of protons within the tissue imaged. Gradient coils apply time-varying EMFs to encode this signal with spatial information in an intermediate form, the k-space. K-space data are then transformed into the final image using Fourier transformation.

Signal weighting and tissue contrast: Timing and application of the RF and gradient EMFs are programmed into sequences. The time between each RF pulse is termed the repetition time (TR). Echo time (TE) is the time between the RF pulse and sampling of the MR signal. Contrast is due to differences in MR signal. In general the higher the signal, the brighter it will appear on the MR image. Images with different contrasts are produced by altering the TE and TR and exploit the tissue specific differences in T1 and T2. Sequences with short TR and short TE are usually described as T1-weighted. The short TR allows differences in longitudinal magnetisation to develop before the next RF pulse whilst the short TE limits the T2 effects. Sequences with long TR and long TE are usually described as T2-weighted. The long TR allows recovery of longitudinal magnetisation (so limiting T1 effects) whilst the T2 effects become pronounced are the long TE. Therefore a tissue with a long T1 and T2 (like water) will be dark in the T1-weighted image and bright in the T2-weighted image. A tissue with a short T1 and a long T2 (like fat) will appear bright in the T1-weighted image and grey in the T2-weighted image.

1.3.2 Safety

MR imaging of the fetus is generally considered to be safe when performed within appropriate limits. However establishing risk is complicated by understanding what effects reach the fetus and the impact at different gestational ages. The main potential sources of concern specific to fetal MR are the bio-effects of both static and gradient magnetic fields, the heating effects of RF pulses and the impact of acoustic noise generated by time-varying gradient fields.

1.3.2.1 Bio-effects of static magnetic fields

A review of the effects of high static magnetic fields on human health has found no firmly established evidence of any adverse effects (Schenck 2000). Cell studies in a variety of species, including humans, of exposure to static magnetic fields ranging from 0.49T to 10T have found no evidence of detrimental effects on cell growth, differentiation, function or survival (Santini et al. 1994; Wiskirchen et al. 1999; Nakahara et al. 2002; Sato et al. 1992). However studies in non-human embryos have found abnormal development and reduced survival following exposure to static magnetic fields ranging from 10mT to 7T (Mevissen et al. 1994; Pan 1996; Espinar et al. 1997; Levin & Ernst 1997; Denegre et al. 1998). Duration of exposure appears to be a significant factor (Narra et al. 1996). Importantly a large human study of over 1900 female MR workers did not find any association with adverse effects on fertility, pregnancy outcome, birthweight or gender (Kanal et al. 1993).

1.3.2.2 Bio-effects of gradient magnetic fields

There has been some suggestion from epidemiological studies of an association between miscarriage and exposure to the low frequency time-varying electromagnetic fields used in MRI (Li et al. 2002; Lee et al. 2002; Savitz 2002). However the NRPB 2004 have concluded that these studies provide insufficient evidence due to selection bias. A review of intrauterine effects of EMFs by Robert (Robert 1999) found the evidence to be inconclusive. Subsequent in-vitro studies have not found any evidence of adverse effects (Rodegerdts et al. 2000; Guisasola et al. 2002).

1.3.2.3 Potential adverse effects of RF energy

The concern regarding RF pulses relates to tissue heating in the fetus through the dissipation of energy. Manufactures design MRI systems in accordance to an international standard (IEC_60601-2-33, 2002). Any pulse sequence must not cause a body temperature rise above 0. 5 ºC. SAR limits are set and must be established for each pulse sequence. The ICNIRP guidelines (2004) state: 'It seems reasonable to assume that adverse developmental effects will be avoided with a margin of safety if the body temperature of pregnant women does not rise by more than 0.5 °C and the temperature of the fetus is less than 38 °C'.

It has been difficult to ascertain the degree of heating to the fetus during an MRI scan. The fetus has a temperature of +0.5 °C above the maternal temperature (Schroder & Power 1997). It has no homeostatic mechanisms to control its own temperature and is therefore dependent on regulation by the maternal temperature and placental blood flow. Tissue heating appears greatest at the maternal body surface, and approaches negligible levels near the body centre (Kanal et al. 1990). This would make thermal damage to the fetus unlikely. Limited animal studies have not detected any significant MRI induced temperature rises in utero (Levine et al. 2001; Kawabata et al. 2003). Mathematical modelling studies used to estimate regional heat absorption during fetal scanning have shown that maximum heat absorption is in the mother (Hand et al. 2006) and levels in the fetus appear safe in both 1.5T and 3T when performed in compliance with IEC conditions (Hand et al. 2010).

In a review of the effect of heat on embryos and fetus, Edwards et al. 2006 conclude that an elevation in fetal temperature is potentially hazardous with specific windows of vulnerability. Raised temperature in utero may cause miscarriage, growth restriction and developmental abnormalities. The risks are dependent on the elevation as well as duration of increased temperature. Organogenesis is a particularly sensitive period and the central nervous system appears to be especially susceptible to development of abnormalities (Ziskin & Morrissey 2011).

1.3.2.4 Effect of acoustic noise

The potential impact of acoustic noise has been most extensively investigated. Lorentz forces, resulting from rapidly switched magnetic gradients interacting with the main magnetic field, cause minute expansions and contractions of the coil itself and produce the characteristic knocking sound.

Sound intensity can reach 120 dB (Price et al. 2001), equivalent to a jet engine at take-off. The mother is protected by headphones or earplugs but there have been concerns over the potential for an effect of acoustic noise on the fetus.

How sound transition is influenced by passing through the maternal abdomen and amniotic fluid is unclear. The fluid filled gravid uterus appears to attenuate transmitted sound by more than 30dB (Glover et al. 1995). This is sufficient to reduce sound intensity to a more acceptable level of 90dB, equivalent to an underground train or large orchestra. However other models have demonstrated a more complex relationship where attenuation is variable depending on sound frequency (Lecanuet et al. 1998; Richards et al. 1992).

An early report for the American Academy of Pediatrics (Etzel et al. 1997) concluded that 'excessive' exposure to high intensity noise during pregnancy may associate with high frequency hearing loss and decreased birth weight. However this report was based largely on research related to occupational noise exposure and the authors acknowledge that noise could be a marker for other risk factors. Furthermore it is difficult to extrapolate this possible association to the effects of MR imaging as occupational noise represents chronic, as opposed to time limited, exposure. Studies specific to the potential effect of exposure to MRI noise have shown no evidence of a negative effect on hearing. A three year follow up study of 20 children exposed in utero to echoplanar imaging (EPI) showed no demonstrable impact on hearing (Baker et al. 1994). Reeves et al. (Reeves et al. 2010) assessed the cochlear function of 96 neonates that had undergone fetal MRI at 1.5T during the second and third trimesters. They did not find an excess risk of neonatal hearing impairment compared to a large unexposed reference sample. In a study by Kok and colleagues (Kok et al. 2004) 41 children that had undergone fetal MRI (1.5T) during the third trimester were not found to have any increased hearing impairment that could be associated with MRI by follow up at three years of age.

1.3.2.5 Animal studies

A number of animal studies have reported adverse effects of exposure to MRI at cell, embryo and fetal stages, whereas others have found no evidence of harm. These studies have not distinguished between particular hazard sources but combine a variety of static, gradient and radiofrequency magnetic field exposures. Reduced CRL has been reported in pregnant mice exposed at mid gestation to 0.35T (Heinrichs et al. 1988) and following prolonged exposure (eight hours) to 4.7T (Carnes & Magin 1996). Magin and colleagues (Magin et al. 2000) found no effect on fetal growth in a similar population following prolonged exposure at 4.7T. Eye malformations in genetically predisposed mice embryos appear to be increased by in utero MRI at 1.5T following exposure for

36 minutes (Tyndall & Sulik 1991) and developmental abnormalities have been reported in chick embryos exposed for four to six hours (Yip et al. 1994). These authors also reported higher rates of embryo mortality whilst other studies have found no evidence of adverse effects on mortality, vitality of chickens, cell growth and migration or blastocyst formation following exposure of up to six hours (Yip et al. 1995; Chew et al. 2001).

1.3.2.6 Human studies

A limited number of human studies have not found any evidence of adverse effects. Baker et al (Baker et al. 1994) found no evidence of increased disease or disability that could be related to in utero echo-planar imaging in a three-year follow up of 20 children. A study of 74 pregnant healthy volunteers exposed to 0.5T EPI compared to unexposed matched controls reported no significant reduction in fetal growth (Myers et al. 1998). In a prospective observational cohort study of 20 infants exposed to in utero MRI, a small decrease in length was noted as well as an increase in gross motor function, compared to matched controls. Paediatric assessment at 9 months was otherwise similar in both groups (Clements et al. 2000). Several studies have performed fetal heart rate monitoring during MRI in the third trimester and observed no effects (Poutamo et al. 1998; Vadeyar et al. 2000; Michel et al. 2003).

1.3.2.7 Guidelines

The ICNIRP 2004, HPA Radiation Protection Division 2008 (guidelines based on ICNIRP) and MHRA 2007 have all produced substantial reviews of the safety issues surrounding MR imaging and guidelines on its use in pregnancy. Whilst there is currently no clear evidence for adverse outcomes related to exposure to static or gradient magnetic fields, some uncertainty remains regarding RF dosimetry in pregnancy and the transmission of sound to the fetus. An additional concern in the first trimester is the underlying relatively high rate of miscarriage in this period. An MRI study could be coincidentally followed by a spontaneous pregnancy loss and give rise to parental concerns regarding causal effect. ICNIRP conclude that ‘insufficient evidence exists to establish unequivocal guidance’ and therefore MRI is always performed in pregnant patients with written informed consent after critical risk/benefit analysis.

1.3.3 MRI in fetal CNS assessment

MRI was first used for fetal imaging in 1983 (Smith 1983). Motion from both fetus and mother represents a significant limitation as it can result in incomplete brain coverage, artefact creation and poor image quality. Strategies including fetal paralysis (Girard et al. 2001), breath holding, maternal sedation and fasting have been used in attempts to overcome this. The development of the single-shot rapid acquisition sequence in the 1990s, in which each slice is acquired in under a second, was a major advancement producing good quality images despite fetal motion. Technological advances in the acquisition and post-processing of images have continued since then (see figure 9) and its clinical value is now well established in fetal medicine.

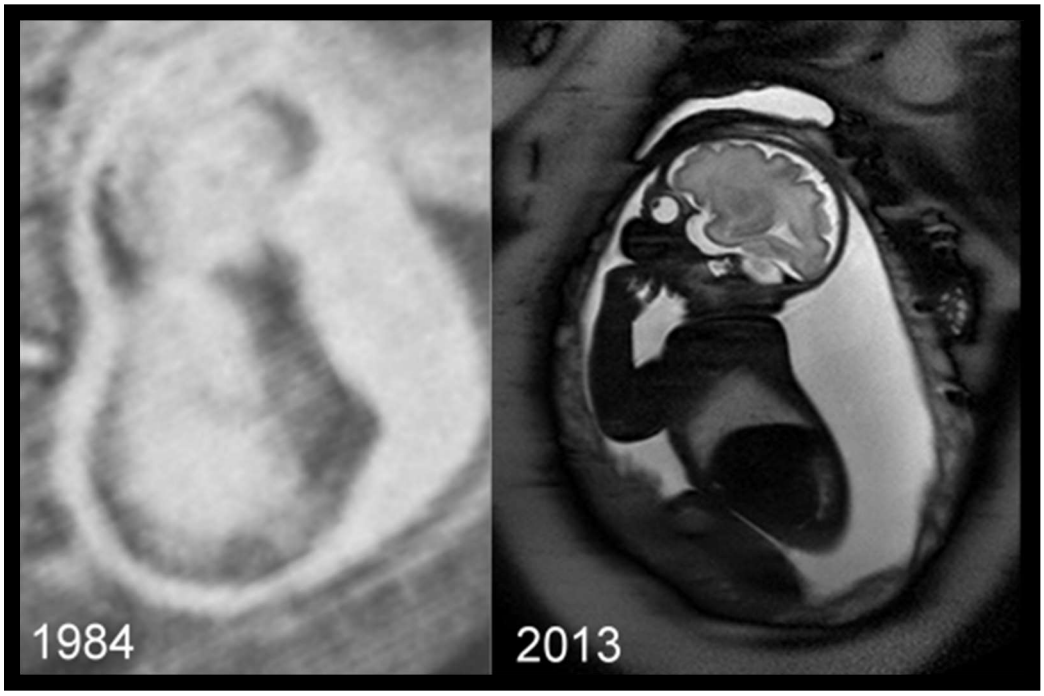


Figure 9. Evolution of MR image quality.
Comparison between an early image reproduced from Smith et al 1984 (Smith & MacLennan 1984) and modern capabilities (reproduced from Kyriakopoulou 2014).

Fast T2-weighted images are routinely used in fetal brain imaging to assess brain anatomy as they provide excellent contrast between fluid and tissue. These single shot fast spin echo (ssFSE) sequences are obtained in less than one second in transverse, coronal and sagittal planes. Superb soft tissue contrast allows different intracranial structures to be visualised as well as identification of early myelination and developing white matter (Rutherford et al. 2008; Rutherford 2009; Glenn 2010; Girard & Chaumoitre 2012).

In contrast to ultrasound, MRI has a large field of view allowing visualisation of the whole uterus at all gestations. It provides good visualisation of the posterior fossa which can be difficult to assess on ultrasound later in gestation when the fetal head is low in the pelvis. Image quality is not limited by maternal or fetal factors such as oligohydramnios, fetal position or, increasingly commonly, the presence of excessive adipose tissue. However imaging of patients with a particularly high BMI may be limited by the diameter of the bore of the scanner. Some patients find claustrophobia and restlessness during the scan unacceptable. MRI is more expensive than ultrasound and contraindicated in patients that have ferrous metal inside their body.

Given this, MRI is now well established as an important complimentary imaging modality to ultrasound (Frick et al. 2015; Hagmann et al. 2008; Trompoukis et al. 2012). MRI is used when confirmation or additional information is requested for a diagnosed or suspected abnormality or in pathological cases where brain changes may not be detectable by ultrasound (Whitby et al. 2004; Rutherford 2009).

1.3.4 Fetal intracranial volumetry

Magnetic resonance imaging as a tool for volumetric measurement of intracranial structures and brain parenchyma is a rapidly evolving approach that began almost 20 years ago. The earliest study performed echoplanar imaging using a 0.5T scanner (Baker et al. 1995). These authors demonstrated a linear increase in brain volume with gestational age in 32 growth restricted singleton pregnancies between 23 and 37 weeks. Fetal brain volumetry using gradient echo MR imaging on a 1.5T scanner was first reported in four healthy fetuses (Garden & Roberts 1996) and later in a larger cohort of both healthy and abnormal fetuses from the same centre (Gong et al. 1998).

A major problem with these early attempts was the use of 10mm thick slices and even larger slice gaps. Considering the average BPD in a 28 week fetus is around 70mm, these parameters were too large to produce reliable measurements. Studies published over the subsequent decade used thinner slices of 5mm or less and attempted to establish growth trajectories for brain parenchyma and other intracranial structures in both normal and abnormal fetuses (see table 5a and 5b). Despite the use of thinner slices, accurate measurement may still be impaired by inter-slice fetal or maternal motion induced error as well as partial volume effects due to the slice thickness.

Recently more sophisticated techniques of motion correction have been applied in selected centres (Malamateniou et al. 2013; Keraudren et al. 2014). In the current project we have overcome this issue using the Snapshot MRI with Volume Reconstruction (SVR) technique (Jiang et al. 2007) which will be described later in more detail in the Methods chapter. This technique produces high resolution datasets with complete brain coverage necessary for accurate volumetric measurement. This is particularly important when attempting to measure smaller intracranial structures or to detect subtle differences between groups.

Normative growth trajectories for brain parenchymal volume as well as intracranial structures have been published using motion corrected datasets (see table 5). However most of these are small samples of less than 50 and there is great variation between studies in defining anatomical regions of interest. Volumetric analysis has been applied to motion corrected data in selected abnormal fetal populations (see table 6). Brain tissue growth in the third trimester has been shown to be reduced in fetuses with Congenital Heart Disease (CHD) relative to healthy controls (Limperopoulos et al. 2011) and brain to body volume ratios are reduced in fetal growth restriction (FGR) relative to healthy singletons (Damodaram et al. 2012). In a small comparison of fetuses with ventriculomegaly compared to healthy controls, cerebral mantle and cortical plate volumes appear to be similar (Scott et al. 2013). In contrast a more recent study (Kyriakopoulou et al. 2014) observed larger cerebral

parenchymal volumes in fetuses with ventriculomegaly relative to healthy controls, and that this increase was largely attributable to overgrowth of the cortical grey matter.

Whilst most of these studies have been carried out using 1.5T, 3T scanners are beginning to be used (Sanz-Cortes et al. 2014). Lack of consistency in coil use, slice and gap width and delineation of brain sub-structures remain. The majority of volumetric studies have measured volumes of interest by manual segmentation techniques. This involves manually tracing around the region of interest on each image slice and then multiplying this area by voxel thickness. Although reliable, this method is time consuming and potentially sensitive to inter-rater error. Promising automatic segmentation techniques are developing but often remain limited by issues caused by motion.

Automatic techniques involve an individual or atlas-based approach. Both are intensity based techniques that rely on differences in signal intensity between brain structures to delineate boundaries. This method is sensitive to image degradation resulting from fetal motion and potentially low contrast between structures of interest. High contrast between fluid and cortical grey matter allows reliable delineation between the two however other boundaries such as between grey and white matter may not be as easy to distinguish resulting in incorrect tissue classifications. Individual segmentation approaches analyse each image de novo. The atlas-based approach involves construction of a 3D statistical spatiotemporal atlas of tissue distribution generated from manual delineations at a range of gestational ages (Habas et al. 2010). Any new subject images can be registered to the space of the atlas to generate volumetric segmentations. The current project uses an individual supervised semi-automatic intensity-based technique and will be described in further detail later.

Study Author/Year	Country	Sample (n)	Slice width (mm)	Motion correction	Segmentation	GA (weeks)	Intracranial volume assessed
(Hatab et al. 2008)	Texas, USA	93	4-5	No	manual	16-40	Cerebellum
(Hu et al. 2009)	China	43	4-5	No	semi-automatic	21-37	Cerebrum
(Habas et al. 2010)	California, USA	20	3	SIMC	manual and atlas based	20-24	Cortical grey matter, white matter, germinal matrix, ventricles
(Jacob et al. 2011)	California, USA	20	3	SIMC	manual	21-32	Hippocampus
(Clouchoux et al. 2011)	USA & Canada	62	2	Atlas constrained registration	manual and atlas based	24-37	ICC, cerebrum, cerebellum, brainstem, CSF, ventricles
(Gholipour et al. 2011)	Boston, USA	25	3-4	ML-SVR	semi-automatic	19-38	Parenchyma (includes cerebellum, brainstem); brain (includes ICF)
(Scott et al. 2011)	California, USA	39	3	SIMC	atlas based	20-32	Supratentorium (excludes brainstem and cerebellum)
(Scott et al. 2012)	California, USA	46	3	SIMC	manual	20-31	Cerebellum

Table 5. Summary of studies of normal fetal intracranial volumetry.

SIMC: Slice intersection motion correction (Kim et al. 2010); ML-SVR: Maximum Likelihood Super-resolution Volume Reconstruction (Gholipour et al. 2010); ICC: Intracranial cavity; CSF: Cerebrospinal fluid; ICF: Intracranial fluid

Study Author/Year	Country	Sample (n)	Slice width (mm)	Motion correction	Segmentation	GA (weeks)	Intracranial volume assessed
(Schierlitz et al. 2001)	Boston, USA	1 normal 3 abnormal	4	No	manual	28-37	Lateral ventricles, haemorrhage
(Grossman et al. 2006)	Israel	27 normal 25 abnormal	3	No	manual	25-41	Cerebrum, lateral ventricles, cerebellum
(Kazan-Tannus et al. 2007)	Boston, USA	8 normal 42 VM	4-5	No	manual	17-36	Supratentorial parenchyma, lateral ventricles
(Hoffmann et al. 2010)	Israel	52 normal 27 CMV	3-4	No	manual	23-39	Temporal lobe
(Limperopoulos et al. 2011)	Boston, USA	55 normal CHD 55	2	non-linear registration	manual	25-37	Intracranial volume, total brain, cerebral fluid
(Haratz et al. 2011)	Brazil	30 VM	4	No	manual	20-36	Lateral ventricles
(Pier et al. 2011)	Boston, USA	32 normal 275 VM	3-5	No	manual	16-40	Lateral ventricles, supratentorial brain tissue
(Damodaram et al. 2012)	UK	19 normal 20 FGR	2.5	SVR	manual	20-36	Total brain volume

(Scott et al. 2013)	California, USA	16 normal 16 VM	3	SIMC	atlas based	22-26	Cortical plate, cerebral mantle, deep grey matter, lateral ventricles
(Sanz-Cortes et al. 2014)	Spain	47 normal 51 SGA	3.5	No	semi-automatic	37	Ratio cerebellum : ICC
(Kyriakopoulou et al. 2014)	UK	60 normal 65 VM	2.5	SVR	semi-automatic	22-38	Supratentorial brain, lateral ventricles, extracerebral fluid, cerebellum

Table 6. Summary of studies of intracranial volumetry in abnormal fetuses.

VM: Ventriculomegaly; CMV: Cytomegalovirus; CHD: Congenital heart disease; FGR: Fetal growth restriction; SGA: Small for gestational age; SVR: Snapshot MRI with Volume Reconstruction (Jiang et al. 2007) SIMC: Slice intersection motion correction (Kim et al. 2010); ICC: Intracranial cavity;

1.3.5 Fetal MR Spectroscopy

Magnetic Resonance Spectroscopy (MRS) allows non-invasive and in vivo exploration of the molecular composition of tissue. This can provide insight into the function of the developing brain. The feasibility of the technique in fetal brain imaging was first demonstrated in 2001 (Fenton et al. 2001). As previously discussed, the MR signal involved in creating anatomical images is generated by the hydrogen protons in water. In 1HMRS, the signal is generated by the protons attached to other molecules.

1.3.5.1 Basic Principles

The resonance frequency of ^{1}H protons varies depending on its molecular environment. This is because the presence of an electron cloud creates an electronic shield that slightly lowers the B_0 magnetic field to which the nucleus would normally be subjected. Variation in the resonance frequency of the proton, resulting from the electron distribution, is called the chemical shift. The value of the chemical shift therefore provides information about the molecular group carrying the hydrogen nuclei. It is usually expressed in ppm and calculated relative to a reference standard:

δ = difference between resonance frequency and reference standard / operating frequency of spectrometer

The signal generated in $^{1}HMRS$ is visualised as a spectrum of peaks at different radiofrequencies representing protons in different molecular environments. Particular molecules corresponding to small molecular weight amino acids, carbohydrates, fatty acids and lipids can therefore be identified by position. Molecules may be mobile or incorporated into cell membranes and myelin in the developing brain. The area under each peak is essentially proportional to the number of protons producing the signal, or the metabolite concentration. Mobile molecules tend to generate narrow peaks whilst molecules attached to other structures produce short and broad resonances are therefore more difficult to reliably identify.

1.3.5.2 Technical considerations in fetal imaging

Fetal studies use a monovoxel acquisition technique such as PRESS or STEAM. A region of interest is identified on a structural scan and then data acquired from within a particular voxel. The size of the nominal volume of interest (VOI) is typically 20x20x20 mm^3 although smaller volumes have been reported (Girard et al. 2006). The voxel size must be large enough to obtain spectra with sufficient

signal to noise ratio (SNR). Increasing voxel size leads to increased tissue heterogeneity of the brain region studied and for most studies, particularly at lower gestations, it is not possible to distinguish between grey and white matter.

Multiple acquisitions are necessary to obtain enough signal. These are then averaged and contamination due to water, lipid or motion are edited from individual spectra. Consequently acquisition time is long compared to neonatal studies at around three to four minutes. Given the issue of fetal motion, this remains a major limitation in its clinical application.

1.3.5.3 Application in fetal imaging

Studies in normal fetuses from 22 weeks gestational age (Kok et al. 2001; Kok et al. 2002; Girard et al. 2006) have successfully identified a number of main peaks : myo-inositol (MI), choline (Cho), creatine (Cr), N-acetylaspartate (NAA), lactate (Lac) (figure 10). By 34 weeks the fetal spectrum is comparable to that of a term born neonate with dominant resonances of Cho, Cr and NAA at a long echo time and MI, Cho, Cr and NAA dominant resonances at a short echo time (Girard et al. 2006).

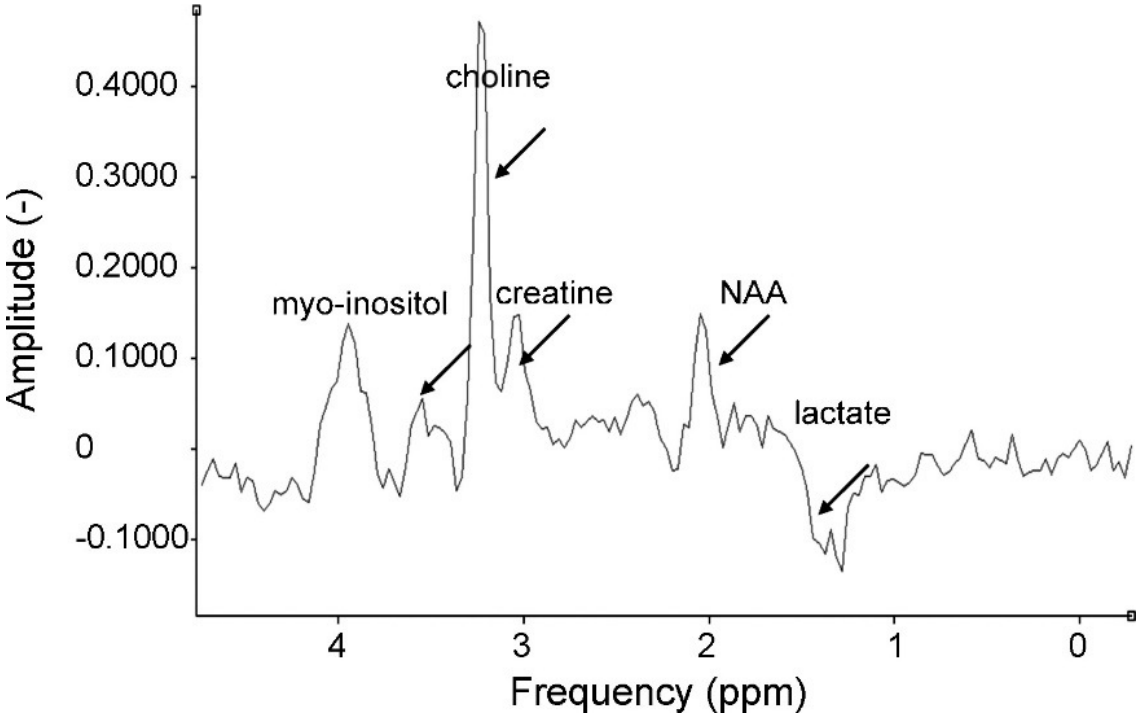


Figure 10. ^{1}HMRS Normal fetal spectrum.

Spectrum at echo time 136ms from a 24 week normal singleton fetus. The black arrows from left to right indicate myo-inositol (3.5 ppm), choline (3.2 ppm), creatine (3 ppm), NAA (2 ppm) and the bifid inverted peak of lactate (1.3 ppm). (NAA: N-acetylaspartate)

N-acetylaspartate (NAA)

The NAA signal is detected at 2.02ppm and reflects both NAA and NAAG. It is the second most abundant molecule in the CNS and is synthesised in neuronal mitochondria from acetyl-CoA and N-acetyltransferase (Bhakoo et al. 2006). It is involved in multiple metabolic and synthetic pathways including myelination, protein synthesis and mitochondrial energy production. It is classically thought to be a neuronal marker, although it has also been shown to reflect oligodendrocyte differentiation and proliferation. Absolute levels are thought to increase with gestational age (Kok et al. 2002; Girard et al. 2006a) and may reflect increased myelination and activity of neuronal mitochondria (Story et al. 2011).

Creatine

Creatine is detected at 3.04ppm and is a composite peak reflecting both creatine and phosphocreatine. These molecules are essential for cellular energy metabolism and the production of adenosine triphosphate (Brighina et al. 2009). Creatine is much more concentrated in glial cells than in neurons (Urenjak et al. 1993). Large regional variations in creatine distribution have been detected within the brain with highest concentrations in grey compared to white matter and in the cerebellum relative to supratentorial regions (Jacobs et al. 2001). Creatine levels are considered to be relatively stable in utero (Kok et al. 2002; Girard et al. 2006a) although an increase with gestational age has been detected at short echo times (Girard et al. 2006b).

Choline

Choline is detected at 3.20ppm. It is a composite peak of several choline containing molecules as well as a small amount of free choline. Choline is important in the synthesis of membrane phospholipids, myelination and in the formation of acetylcholine and is taken up by both glial and neuronal cells (Zeisel 2000). Choline levels appear to decrease with gestational age in utero (Girard et al. 2006a), likely as a result of acceleration in myelination (Girard et al. 2006b).

Myo-inositol

Myo-inositol is detected at 3.56ppm. It is a pentose sugar synthesised from glucose-6-phosphate. It has functions as an osmolyte, in lipid synthesis and in intracellular second-messenger systems. It is predominantly found in astroglial cells and has a role in neuronal plasticity and regulation of cell growth (Fisher et al. 2002). Decreasing MI levels from 22 weeks gestational age have been recorded at short echo times (Girard et al. 2006a), although appear stable in a study from 31 weeks gestational age (Kok et al. 2002).

Lactate

A lactate peak may be seen at 1.33ppm. It is best detected at long echo times due to the potential for lipid contamination in that signal region at short echo times (Berger-Kulemann et al. 2013). It is traditionally considered to be a product of anaerobic glycolysis and a marker of cerebral injury or underperfusion reflecting impaired mitochondrial function.

Lactate has been identified by ^{1}H MRS in the fetus in a small number of cases; It has been detected in back muscle tissue in a fetus with FGR (Robinson et al. 2004), cerebral lactate has been detected in a fetus with gastroschisis (Wolfberg et al. 2007), and in association with FGR (Azpurua et al. 2008; Cetin et al. 2011; Story et al. 2011). The presence of cerebral lactate has also been demonstrated in 3 apparently normal fetuses without adverse perinatal outcomes, although longer term outcome has not been reported (Story et al. 2011). Most recently in a study of 75 spectra from both normal and abnormal fetuses, no association was found between lactate and presence of fetal pathology (Berger-Kulemann et al. 2013).

The MR signal is influenced by factors such as pulse sequence and acquisition method and therefore absolute concentrations cannot be established without controlling all of these. In addition assumptions must be made regarding spin relaxation times and brain water content. Relative concentrations are therefore more robust than absolute quantification. The current trend in the literature is to express the concentration of specific metabolites as ratios to a metabolite peak with a known concentration or to a stable metabolite within the spectrum.

Table 7 summarises spectroscopy studies quantifying metabolite ratios in normal control fetuses. NAA:Cho ratios increase (Kok et al. 2002; Story et al. 2011) and Cho:Cr ratios appear to decrease with increasing gestational age (Kok et al. 2002; Story et al. 2011; Story et al. 2013; Berger-Kulemann et al. 2013). NAA:Cr has been shown to increase with gestational age (Kok et al. 2002; Berger-Kulemann et al. 2013), whereas Story et al found no alteration at long TE (Story et al. 2011). MI:Cho and MI:Cr ratios have been shown to decrease with gestational age (Story et al. 2013; Berger-Kulemann et al. 2013).

Study Author	Year	N	GA (weeks)	Findings
Berger-Kulemann et al	2013	50	19-38	↑NAA/Cr ↓Cho/Cr (short / longTE) ↓MI/Cr short TE
Story et al	2013	47	22-39	↓Cho/Cr MI/Cho MI/Cr (short TE)
Story et al	2011	41	22-39	↑NAA/Cho ↓Cho/Cr →NAA/Cr (long TE)
Kok et al	2002	36	30-41	↑NAA/Cho NAA/Cr ↓Cho/Cr

Table 7. Summary of studies of key metabolite ratios in normal singleton fetuses.
Arrows indicate increase or decrease in metabolite ratios with respect to gestational age.

MRS has been used in a limited number of cases to illustrate impaired brain metabolism in abnormal fetuses (Girard et al. 2006b; Story et al. 2011). Significantly lower NAA:Cho (Sanz-Cortes et al. 2015; Story et al. 2011) and NAA:Cr (Story et al. 2011) ratios have been observed at long echo times in FGR singletons compared to appropriately grown controls and could be due to decreased levels of NAA. This may reflect reduced production of NAA as a result of impaired neuronal mitochondrial function. Consequent reduction in myelination and energy metabolism may predispose to brain injury associated with prematurity and hypoxia in FGR. Decreasing Cho:Cr, MI:Cho or MI:Cr ratios with gestational age observed at short echo times appear to be similar between AGA and growth restricted fetuses. Reduction in NAA:Cho in the third trimester has been observed in CHD fetuses compared to controls (Limperopoulos et al. 2011) and may reflect causal mechanisms similar to those in FGR. To date no studies have used MRS to investigate brain metabolism in utero in twin gestations, particularly those affected by complications of blood flow such as in TTTS.

1.4 Aims and Hypothesis

The main aim of this thesis is to establish volumetric and maturational differences between the fetal brains of ex-recipients and ex-donors in twin pairs treated for TTTS. It is hypothesised that the ex-donor fetal brain will be smaller as well as relatively immature compared to the ex-recipient brain.

1.4.1 Specific aims

1. To evaluate the incidence and nature of abnormalities diagnosed on brain MRI in the post laser treatment cohort.

2. Establish whether differences exist within twin pairs treated for TTTS in brain size and fluid volumes and whether any differences are proportionate to overall growth.

3. Assess whether ex-donor fetuses have delayed cortical maturation relative to their ex-recipient co-twin.

4. Evaluate feasibility of ^{1}HMRS in twins treated for TTTS and determine differences in brain metabolite ratios between ex-recipients and ex-donors.

5. Assess the neurodevelopmental outcome of laser treated TTTS survivors at 1 and 2 years of age and correlate developmental scores with intertwin discordance in fetal brain MR measurements.

2. Materials and Methods

2.1 Ethics

All participants provided written informed consent to partake in the study. Ethical approval for the study entitled “Quantification of fetal brain growth and development using magnetic resonance imaging” was granted by Hammersmith Hospital Ethics Committee (ethics No. 07/H0707/105). Ethical approval for the study entitled “Fetal brain growth and development in twins: an MR study” was granted by Riverside Research Ethics Committee (ethics No. 11/LO/1744). The majority of participants (75%) were recruited under the earlier ethical approval.

2.2 Study Design

This was a prospective study. Women treated by fetoscopic laser for TTTS at Centre for Fetal Care Queen Charlotte’s and Chelsea Hospital were referred to our department for fetal brain MRI and invited to participate. MRI was not scheduled within 2 weeks of laser treatment or at GA less than 20 weeks to optimise the detection of injury incurred at treatment. Women were scanned within 4 weeks of treatment where possible. Clinical notes were reviewed at each contact. Delivery and neonatal unit summaries were obtained where relevant. Clinical neurodevelopmental assessment at 1 year using Griffith’s Developmental Scale or at 2 years using Bayley’s Scale was offered. Where children were unable to attend for developmental assessment in person, parents were asked to complete an appropriate Ages & Stages questionnaire.

2.2.1 Inclusion Criteria

MCDA pregnancy determined at first trimester ultrasound scan.
Fetoscopic laser treatment at less than 26 weeks GA (see Appendix 1).
Ongoing twin pregnancy within 2 weeks of laser and at GA > 20 weeks.
Normal karyotype at amniocentesis.
Able to provide valid consent.

2.2.2 Exclusion Criteria

Contraindication to MRI (including presence of MR incompatible metal within the body, BMI above limit for scanner, claustrophobia).

2.2.3 Cohort

A total of 126 women underwent laser treatment for TTTS at our centre between 2007 and 2012. Forty women did not meet inclusion criteria as shown in figure 11. Complete pregnancy loss occurred in 12 cases. Termination of pregnancy was undertaken in one case at less than 20 weeks GA. In 24 cases fetal death of one twin occurred within two weeks of treatment or at 20 weeks GA or less. Treatment was performed after 26 weeks in two cases. One patient delivered at 25 weeks and 5 days, 1 week after treatment.

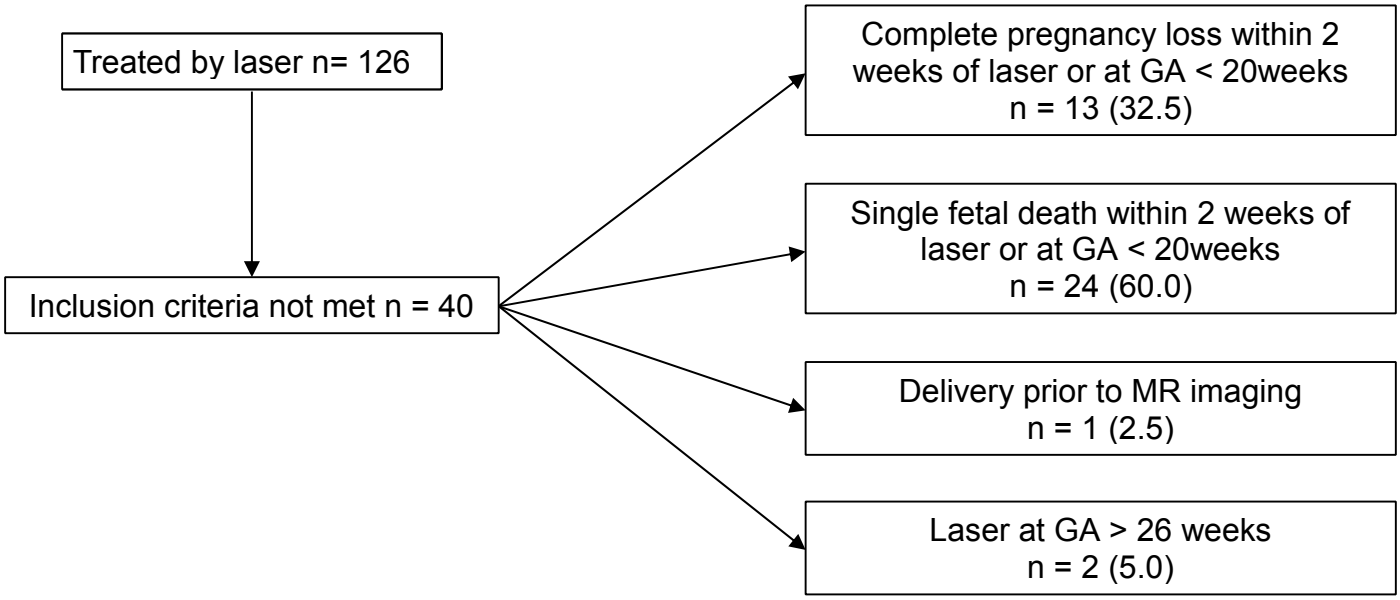


Figure 11. Flowchart of cases that did not meet inclusion criteria.

Numbers in brackets are percentages of the total number (40) excluded.

Eighty six cases therefore met inclusion criteria (see figure 12). In seven cases MR imaging was scheduled but single fetal death occurred before the patient could attend. Thirty women were originally referred from outside of the region and were followed up locally. Ten women declined fetal MRI. Thirty nine women attended for post laser fetal MRI of an ongoing twin pregnancy.

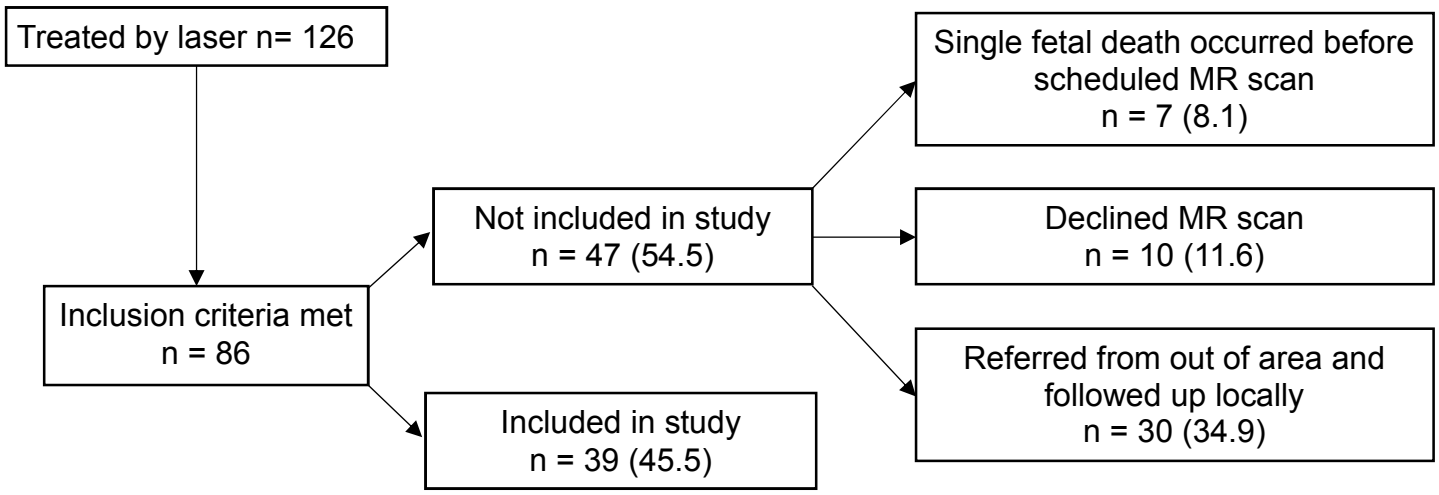


Figure 12. Flowchart of cases meeting inclusion criteria.

Numbers in brackets are percentages of the total number (40) included.

Maternal age and GA at treatment for included in the study and those that were not are shown in table 8. There was no significant difference between the 39 cases meeting inclusion criteria that were included in the study and the 47 cases that were not in maternal age (p = 0.5) or GA at laser (p = 0.28). Pregnancy outcome data were available for 70 out of 86 women (81.4%) meeting inclusion criteria. Outcome data are presented for comparison between the study and non-study groups in table 8. There was no significant difference in pregnancy outcome (Fisher's exact for at least 1 survivor p = 0.6; for 2 survivors p = 0.9) or GA at delivery (p = 0.8) between groups. Table 9 shows the study entry characteristics and pregnancy outcome for each of the 39 cases included in the study.

Cohort (n)	Maternal age (years)	GA laser (weeks)	At least 1 survivor (%)	2 survivors (%)	GA delivery (weeks)
Study (39)	28.8 (25.5 - 34.4)	20.9 (17.6 - 22.6)	95	73	32.4 (30.3 to 34)
Non-study (47)	31.4 (25.6 - 34.6)	19 (18.3 - 20.7)	94	72	32.6 (30 to 34.1)

Table 8. Pregnancy outcome and maternal characteristics for the study and non-study cohort.

Medians and IQR are presented. Percentage survivors refers to survival to discharge.

Case no	Maternal age (years)	GA at laser (weeks)	Stage at laser (Quintero)	GA at MRI (weeks)	Pregnancy outcome ex-recipient	ex-donor
1	28.3	17	3	24	LB	TOP
2	25.5	21 1/7	3	23 6/7	LB	NND
3	34.6	23 5/7	3	26 1/7	LB	NND
4	28.5	24 1/7	3	27	TOP	TOP
5	30.8	22	3	26 2/7	LB	LB
6	28.1	21	3	25	LB	IUD
7	31.2	17 5/7	3	23	IUD	LB
8	38.4	24 1/7	3	26 4/7	LB	LB
9	38.6	16 6/7	3	27 1/7	LB	LB
10	34.1	21 1/7	3	24 2/7	LB	IUD
11	20.3	21 3/7	3	25 2/7	LB	LB
12	27.6	22 4/7	3	30 6/7	LB	IUD
13	31.3	18 1/7	3	27 1/7	LB	LB
14	32.5	16 6/7	3	24 1/7	LB	LB
15	22.8	18 3/7	3	28 5/7	LB	LB
16	29.7	25	3	29	LB	LB
17	23.3	17 4/7	3	23 6/7	LB	LB
18	21.6	17 2/7	3	22 2/7	LB	LB
19	20.9	19 4/7	3	24	LB	NND
20	24.5	17 5/7	3	22 4/7	LB	LB
21	25.4	21 1/7	4	25 3/7	LB	LB
22	24.3	21 6/7	3	26 5/7	LB	LB
23	27.7	19 6/7	3	26 6/7	LB	LB
24	33.0	20 4/7	2	25 5/7	LB	LB
25	35.0	20 6/7	3	26	LB	LB
26	34.0	23 2/7	3	28 4/7	LB	LB
27	41.5	16	2	24 3/7	LB	LB
28	34.9	24	2	27 1/7	LB	LB
29	28.6	24 3/7	3	32	LB	LB
30	37.0	20 4/7	3	27 5/7	LB	LB
31	38.9	24 4/7	3	27 5/7	unknown	unknown
32	28.2	21	4	25 3/7	unknown	unknown
33	26.6	21 3/7	2	25 2/7	LB	LB
34	25.0	17 5/7	3	22 6/7	LB	LB

35	36.4	17 2/7	3	20 5/7	LB	LB
36	28.5	22 1/7	3	25 1/7	NND	IUD
37	25.3	23 6/7	3	26 5/7	LB	LB
38	29.7	23	3	30	LB	LB
39	32.6	17 4/7	3	23 6/7	LB	LB

Table 9. Case characteristics at study entry and outcome.

Case numbers refer to pregnancies that met inclusion criteria and were subsequently included in the study. These cases are referred to in later chapters. (TOP Termination of pregnancy, NND Neonatal death, IUD In utero death, LB Live birth)

2.3 Fetal Imaging

2.3.1 Procedure

Participants were provided with a study information sheet prior to attending for MR scan (appendix 2 for those recruited under ethics No. 07/H0707/105 and appendix 3 for those recruited under ethics No. 11/LO/1744). From September 2012 an information video of the fetal MRI procedure was made available online to parents via http://vimeo.com/37368763. Written informed consent was obtained on the day of examination (appendix 4) and a standard MRI metal safety check questionnaire completed.

Room temperature was set to 18ºC. Air conditioning within the bore of the MR scanner provided extra cooling unless declined. Women were provided with loose clothing to wear during the scan to ensure that clothing was metal free and standardize temperature responses. Maternal temperature was measured with an electronic ear thermometer before entering the magnet and upon leaving each time. If maternal temperature prior to scanning was above 37.5ºC then scanning was rescheduled for another day (in accordance with IRCNIP guidelines 2004). Heat absorption by the fetus was reduced further by interchanging low and high SAR sequences in addition to having short breaks for geometry setting between sequences.

Women were positioned with wedges and pillows for comfort in a lateral tilt position to avoid complications arising from compression of the inferior vena cava. Hands were placed across the chest or by the sides. A 32-channel coil was wrapped around the maternal pelvis and abdomen. Headphones were worn to protect maternal hearing, provide music and allow communication during the scan. Women were advised that scanning both twins may take up to 90 minutes and were given a break between scanning each fetus.

All women were invited to complete a questionnaire regarding their experience following their scan (appendix 5).

2.3.2 Data acquisition

The complete clinical sequence protocol for assessment of the fetal brain is outlined in table 10 below.

Parameters	T2ssTSE	T2dyn	SNAPIR	T2*	PRESS 42	PRESS 136	PRESS 270
TE (ms)	127	160	9.5	80	45	136	270
TR (ms)	1011	15000	23000	3600	1500	1500	1500
Flip angle (deg)	90	90	180	90	-	-	-
Acquisition voxel size (mm)	1.08x1.98x4	1.25x1.25x1.25	1.00x1.01x4	1.2x1.2x2	-	-	-
Slice gap (mm)	0.4	-1.25	0	-1.2	-	-	-
Reconstructed voxel size (mm)	0.84x0.84x4	1.18x1.18x2.5	0.96x0.97x4	0.63x0.63x2	-	-	-
FOV (mm)	430x352x114	320x340x95	320x340x88	400x400x65	-	-	-
SAR (W/Kg)	2	1.7	1	0.4	0.4	0.4	0.4

Table 10. Fetal imaging parameters.
Structural scans included T2ss, T2dyn, T2* and SNAPIR. Spectroscopy sequences at echo times 42ms, 136ms and 270ms included PRESS 42, PRESS 136 and PRESS 270. (T2ss: T2-weighted Single Shot Turbo Spin Echo; T2dyn: T2-weighted dynamic Single Shot Turbo Spin Echo; SNAPIR: T1-weighted Snapshot Inversion-Recovery; T2*:T2*-weighted Gradient Echo; PRESS: Point Resolved Spectroscopy.)

2.3.2.1 T2-weighted Single Shot Turbo Spin Echo (ssTSE)

With this single shot technique single slice acquisition takes less than one second. Detailed acquisition parameters are shown in table 8. Image slices of 4mm thickness with no gap were obtained in transverse, coronal and sagittal planes for structural assessment of the brain. Water dominant fluid such as CSF has a high signal intensity whereas brain parenchyma is isointense (see figure 13).

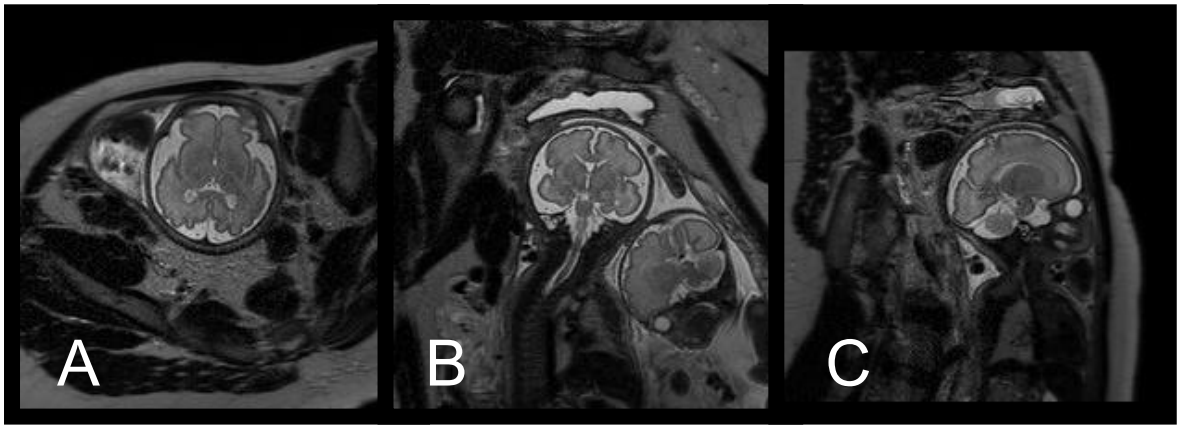


Figure 13. T2-weighted Single Shot Turbo Spin Echo.

T2-weighted Single Shot Turbo Spin Echo sequences of an ex-recipient fetus at 30 weeks and 2 days GA. (A) transverse (B) coronal and (C) sagittal planes.

2.3.2.2 T2-weighted dynamic Single Shot Turbo Spin Echo (ssTSE)

This optimised sequence was designed in our department (Jiang et al 2007) and produces complete datasets suitable for volumetric measurements (figure 14). It is also a single shot technique and images are acquired within the parameters in table 10. Importantly slice thickness is only 2.5mm which improves image quality by reducing partial volume effects. The negative slice gap means that overlapping image slices are acquired. Eight acquisition loops are performed, four in the transverse plane, two coronal and two sagittal. This 'oversampling' with thinner, overlapping slices ensures complete high quality coverage of the brain. Subsequent image registration and reconstruction into 3D datasets suitable for volumetric measurement occurs offline at the post-processing SVR stage described later.

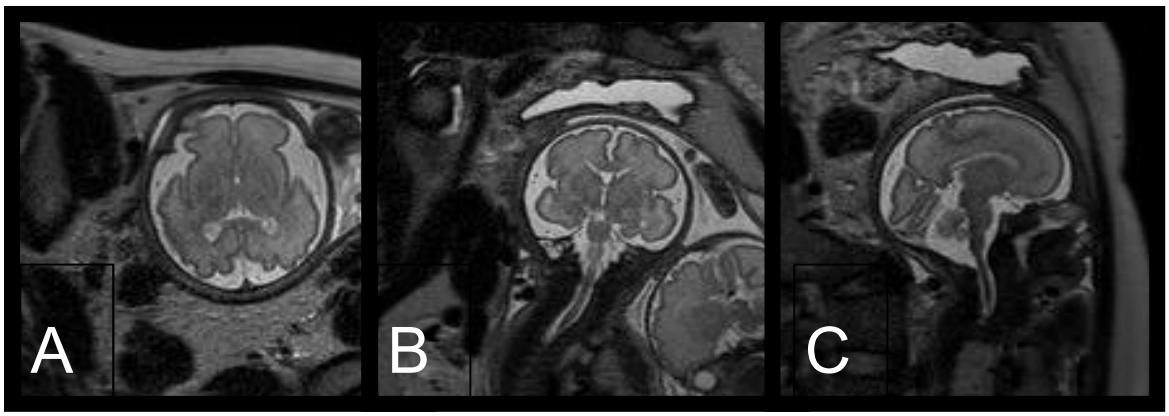


Figure 14. T2-weighted dynamic Single Shot Turbo Spin Echo.

T2-weighted dynamic Single Shot Turbo Spin Echo sequences of an ex-recipient fetus at 30 weeks and 2 days GA. (A) transverse (B) coronal and (C) sagittal planes.

2.3.2.3 T1-weighted Snapshot Inversion-Recovery (SNAPIR)

This fast T1 weighted sequence, using a single shot technique, was designed in our department (Malamateniou et al 2011), and is acquired with the parameters detailed in table 10. It is less susceptible to fetal motion artefact than conventional T1-weighted image sequences and is clinically useful in confirming structural findings from T2 weighted images, particularly in identifying haemorrhage, fat and myelination near term (figure 15).



Figure 15. T1-weighted Single Shot Inversion-Recovery.

T1-weighted Inversion-Recovery sequence a normal control 30^{+5} weeks old fetus (sagittal plane). Note the low signal intensity of CSF and high signal intensity of the cortex.

2.3.2.4 T2*-weighted Gradient Echo

This sequence detects hemosiderin present on iron molecules. Dark areas represent areas high in hemosiderin e.g. germinal matrix, choroid plexus and internal cerebral veins as well as late stage haemorrhage (figure 16). Earlier stages of haemorrhage can be identified as low signal intensity.



Figure 16. T2*-weighted Gradient Echo.

T2*-weighted Gradient Echo sequence of a normal control 34^{+4} weeks old fetus (transverse plane). The great cerebral vein of Galen appears darker (arrowhead).

2.3.2.5 Magnetic Resonance Spectroscopy

MRS data were acquired using the monovoxel Point Resolved Spectroscopy sequence (PRESS). A T2-weighted ssTSE was obtained prior to the ^{1}HMRS sequence to establish fetal position and guide placement of the region of interest. A 20x20x20mm voxel was placed centrally over the fetal brain therefore sampling both white and grey matter. Placement over the fetal skull or maternal tissue is avoided. The positioning of the voxel is shown in figure 17. Acquisition parameters are described in table 10 above. Data were acquired at three different echo times (42ms, 136ms, 270ms) to facilitate detection and quantification of a variety of metabolites. Thirty-two spectra were obtained at each echo time. Acquisition was performed using water suppression due to the large size of the water peak that may distort baseline and obstruct metabolite quantification. A repeat T2-weighted ssTSE scan was acquired following spectra acquisition to check for significant change in fetal position.

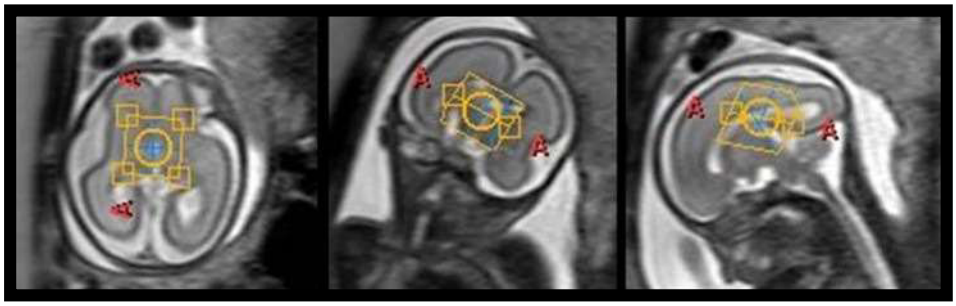


Figure 17. Voxel placement in fetal ^{1}HMRS.

The centrally placed voxel (shown in transverse, coronal and sagittal planes) samples grey and white matter in the fetal brain.

2.3.3 Identification of fetuses and study blinding

Antenatal notes and ultrasound scan reports were available for all women at the time of scanning. In cases of gross size discrepancy between co-twins, identification of individual twins is clear (see figure 18A). In the absence of an overt discrepancy the clinical notes were reviewed to determine position (in particular laterality often from illustrations on scan reports) and cord insertion. Laterality was then determined from whole uterus reference scanning which allows identification of the dividing amniotic membrane (see figure 18B). In cases imaged at early gestation or with excessive fetal movement, whole uterus reference scanning was repeated as necessary to ensure ongoing correct fetal identification over the course of image acquisition.

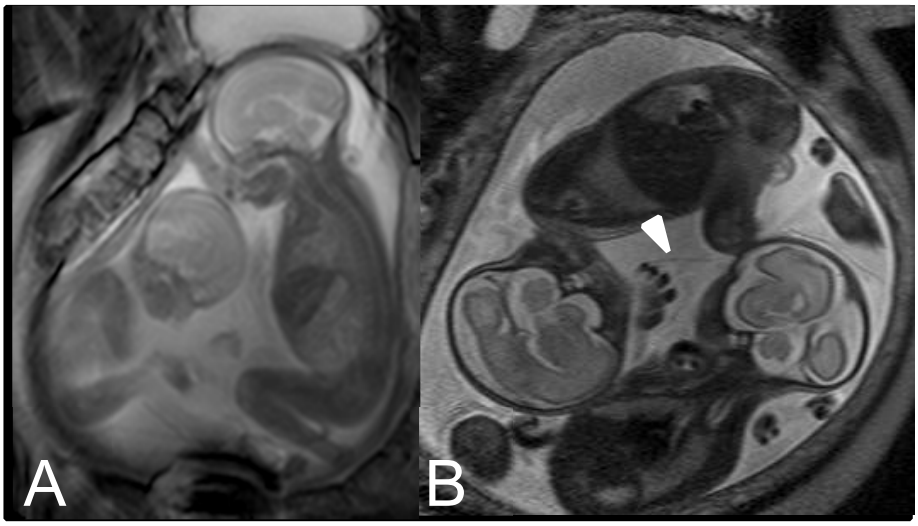


Figure 18. Identification of fetuses.

(A) Identification of ex-recipient and ex-donor twins in cases of gross size discrepancy. The smaller twin seen on the left is the ex-donor and the larger ex-recipient is seen on the right. (B) Similar sized fetuses can be distinguished according to position which is aided by identification of the separate amniotic sacs. White arrowhead indicates the dividing amniotic membrane.

Imaging files for data processing were labelled with a unique MR reference number. A separate record was kept of corresponding ex-donor or ex-recipient status. Therefore status was not known at the time of data processing and analysis. For volumetric segmentation of the lateral ventricles and supratentorial brain tissue, approximately half were performed in a single batch and subsequent segmentations performed in smaller batches of three or four. Theoretically this could have led to awareness of the ex-donor or ex-recipient status during processing due to the proximity between file labelling and processing. However all other volumetric segmentations, biometry measures, cortical folding analysis and spectroscopy were performed as a single batch on anonymised data files reducing the likelihood of bias. Furthermore lateral ventricle and supratentorial brain tissue volumes were checked for consistency across all files as a single anonymised batch.

2.3.4 Post-Processing

2.3.4.1 Clinical Examination

Images were systematically reviewed by an experienced neuroradiologist (MR) to assess gestation appropriate structure and development as well as to identify signs of acquired injury. This includes review of white matter, basal ganglia and thalami, internal capsule, corpus callosum, extra-cerebral space, lateral ventricles, 3rd ventricle, 4th ventricle, cavum septum pellucidum and vergae, orbits, cerebellum, vermis, brainstem, germinal matrix and cortex. An example of the clinical examination in an ex-recipient fetus at 28 weeks GA is shown in Figure 19.

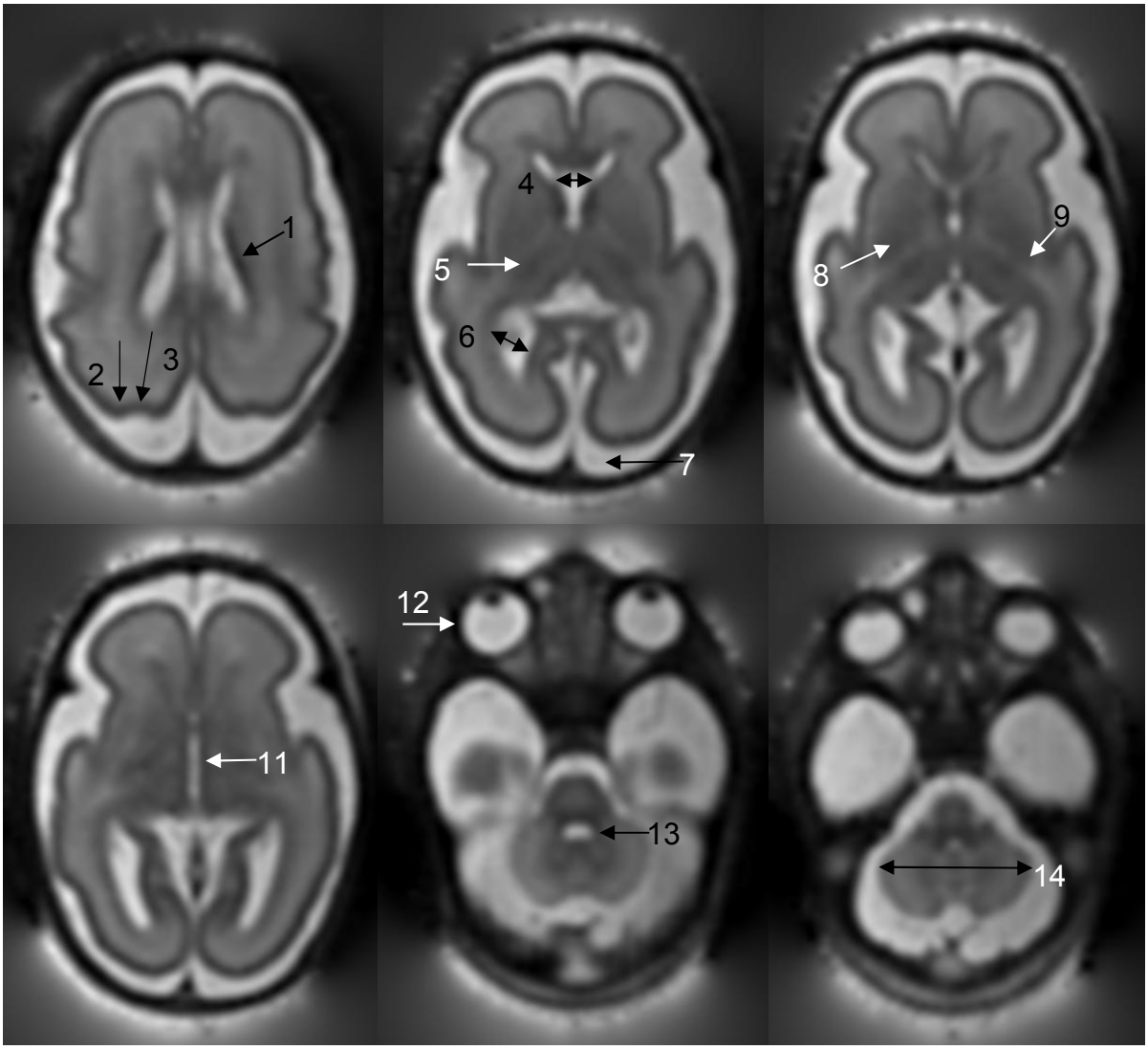


Figure 19. Normal clinical examination findings in an ex-recipient fetus at 28 weeks GA.

1) Germinal matrix 2) Normal cortical folding - gyrus 3) Normal cortical folding - sulcus 4) Cavum septum pellucidum (width shown) 5) Thalamus 6) Lateral ventricle (atrial diameter shown) 7) Extracerebral fluid 8) Basal ganglia 9) Internal capsule 10) Cell rich periventricular area 11) Third ventricle 12) Orbits 13) Fourth ventricle 14) Cerebellum (transcerebellar diameter shown).

2.3.4.2 Snapshot MRI with Volume Reconstruction (SVR)

This technique (Jiang et al 2007), designed and developed in our department, produces high resolution image datasets from multiple single shot T2 weighted images. Eight T2 weighted dynamic ssFSE brain images were acquired (4 transverse, 2 coronal, 2 sagittal) (Figure 20 panel A) as described in table 10. This ensures that the fetal brain is 'oversampled' during image acquisition. As multiple scans were performed in all three planes with overlapping slices, all parts of the brain are sampled, even with significant fetal motion. Each slice is acquired in less than one second and as fetal movement is rarely faster than this, the potential for motion artefact is reduced.

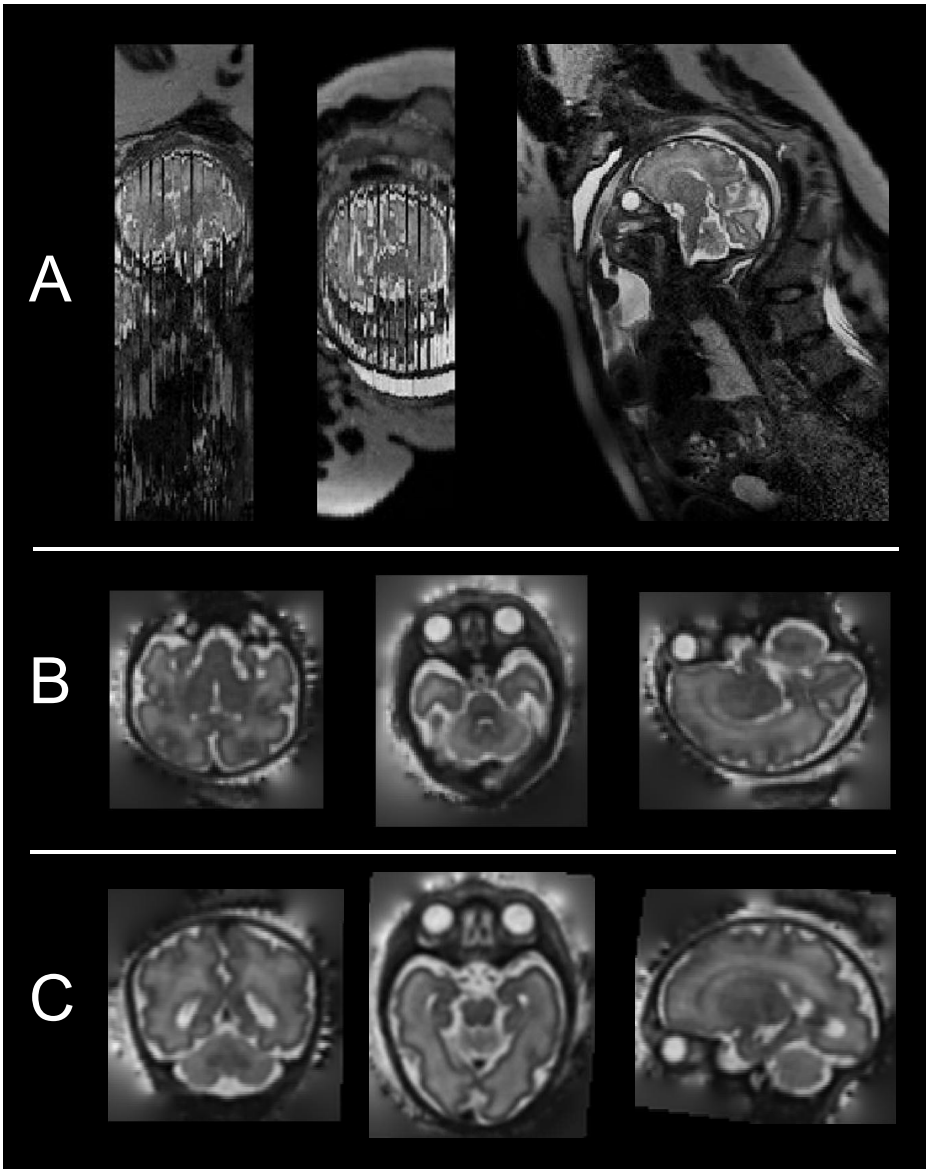


Figure 20. Post processing of MR images: Volume reconstruction and optimisation.

(A) Dynamic T2-ssFSE single loop dataset acquired in a sagittal plane. Prior to registration and reconstruction slices are corrupted due to fetal motion. Missing data are shown as blank lines in coronal and transverse planes. (B) 3D brain following registration of multiple acquisitions and reconstruction. (C) 3D brain orientated in orthogonal planes and resampled at a voxel size of 0.2x0.2x1 mm as described later.

These image files were converted from DICOM (Digital Imaging and Communications in Medicine) format to NIfTI (Neuroimaging Informatics Technology Initiative) format using an in-house DICOM to NIfTI converter. ImageJ software (http://rsb.info.nih.gov/ij/) was then used to remove the majority of maternal tissue from each slice of all eight scans (time taken approximately 60 minutes). Image intensity values were calibrated (slope = 1, intercept=0) to achieve uniformity across images using MRIcroN software (http://www.mccauslandcenter.sc.edu/mricro/). An optimal template scan (based on least motion corruption as well as full brain coverage) was selected and excess maternal tissue removed from around the fetal skull using ImageJ (time taken approximately 30 minutes). The remaining seven scans were manually transformed to match the spatial orientation of the template scan using RView software (time taken approximately 60 minutes). Each scan was then reviewed for image quality in every slice. Slices corrupted by motion artefact that resulted in loss of anatomical detail were excluded from subsequent processing (time taken approximately 30 minutes). Therefore the total time taken to prepare a single fetus' set of image files for reconstruction was three hours.

The resulting eight processed images and seven transformations were entered into a script algorithm in Matlab (MathsWorks, Natick, MA, USA) for registration into a common coordinate space in order to align the acquired slices. This is performed with the assumption that the brain may have moved but will not have changed in size or shape. The final stage of the algorithm is automatic reconstruction into a high resolution 3D brain (see figure 20 panel B) (time taken four to eight hours). This high signal-to-noise volumetric dataset can be viewed in any plane for accurate and reliable segmentation and measurement.

2.3.4.3 Data Optimisation

Consistent and accurate image orientation is important in both linear measurements and volumetric segmentation to increase homogeneity between subjects and reduce observer error. Using Rview software the reconstructed fetal brain was manually reoriented in standardized transverse, coronal and sagittal projections. The voxel size of the oriented image was converted from 1.18x1.18x1.18mm to 0.2x0.2x1mm using Rview software. This provided a higher resolution image whilst maintaining a feasible number of slices (approximately 100) for subsequent semi-automatic segmentation (figure 20 panel C). The total time taken for reorientation and resampling was about 30 minutes.

2.3.4.4 Measurement

2.3.4.4.1 Linear Measurements

Linear measurements were made using ImageJ. Biparietal diameter, head circumference and transcerebellar diameter were measured according to the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG 2007) as shown in figure 21. BPD and HC were measured at the level of the thalami. BPD was measured using the 'leading edge' or outer to inner edge technique at the widest part of the skull. HC was measured by placing the ellipse function in ImageJ around the outside of the fetal skull bone. TCD was measured at the greatest width of the cerebellum in the transverse plane.

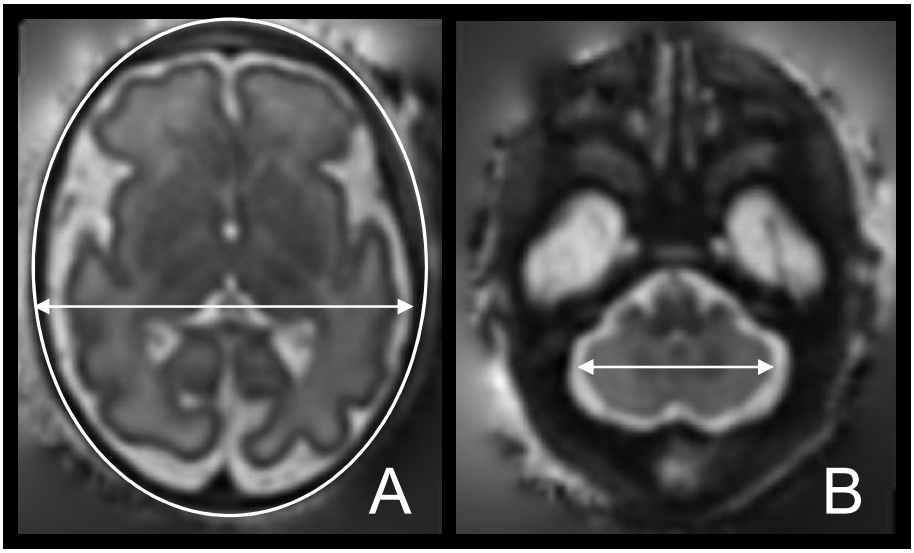


Figure 21. Example of 2D measurements.
A) Head circumference and biparietal diameter B) Transcerebellar diameter.

2.3.4.4.2 Volumetric Analysis

2.3.4.4.2.1 Semi-automatic segmentation

Semi-automatic volumetric segmentation of intracranial structures was performed using ITK-SNAP (Yushkevich et al., 2006). This user supervised imaging software allows both manual segmentation as well as the design of semi-automatic segmentation in three orthogonal planes simultaneously. Although ITK-SNAP is designed for adult MR images, it can be optimised for fetal scans with high signal-to-noise and clear structural boundaries. The "region competition" method allows image intensities to be thresholded and classified into background and foreground (region of interest).

Based on visual assessment of the image intensity, thresholds can be manually set so that a range of intensities can be classified as a region of interest (ROI). These ROIs are then filled using the "snake evolution" methodology. A snake refers to a closed curve representing a segmentation that evolves from a very rough estimate of the region of interest to a very close approximation of the structure. Snake evolution is determined by the velocity at every point of the snake which in turn depends on the shape of the snake at any given time as well as the intensities of the neighbouring pixels. The user may set upper and lower intensity thresholds, initialise the snake using spherical bubbles, set the velocities driving snake evolution and follow the process in real-time. This provides greater control over volume segmentation compared to automatic segmentation techniques. Each slice was reviewed after completion of the automatic process and manual editing of the segmentation performed. Using a drawing tablet (Intuos4, Wacom, Germany), incorrectly segmented areas were removed and areas where blurring did not allow for accurate thresholding were filled in.

Volumetric segmentation of the fetal body was performed manually. The semi-automatic technique was not appropriate for segmentation of the fetal body as the spectrum of image intensities included is too broad. It was therefore not possible to define a region of interest that could differentiate the fetal body from the surrounding uterus, amniotic fluid, umbilical cord and placenta. Furthermore twins are often touching or entwined within the uterus and in these cases there is no defined border to limit the semi-automatic segmentation. Whilst the placement of spherical bubbles to initiate the snake is well suited to regular structures such as the brain, projections such as limbs and fingers extending out from the torso present an extra challenge. These projections require placement of additional bubbles to initiate the snake. Consequently the semi-automatic method becomes more time intensive and less accurate than manual segmentation.

The time required for segmentation was variable depending on the segmentation method (semi-automatic or manual), complexity of the structure, image quality, gestation (and therefore size of the structure). For a fetus at 26 weeks gestational age, the approximate time taken for segmentations were : supratentorial brain tissue 4 hours, lateral ventricles 45 minutes, CSP and third ventricle 20 minutes, extracerebral CSF 90 minutes, cerebellum 30 minutes, cortex 4 hours, fetal body 2 hours.

2.3.4.4.2.2 Segmentation of intracranial structures

Segmentation was performed in the transverse plane and visually checked in both coronal and sagittal planes.

Supratentorial brain tissue

Segmentation of the brain parenchyma was semi-automatic and included supratentorial brain (STB) tissue. All intracerebral CSF spaces and the brainstem, medulla and pons were excluded (figure 22).

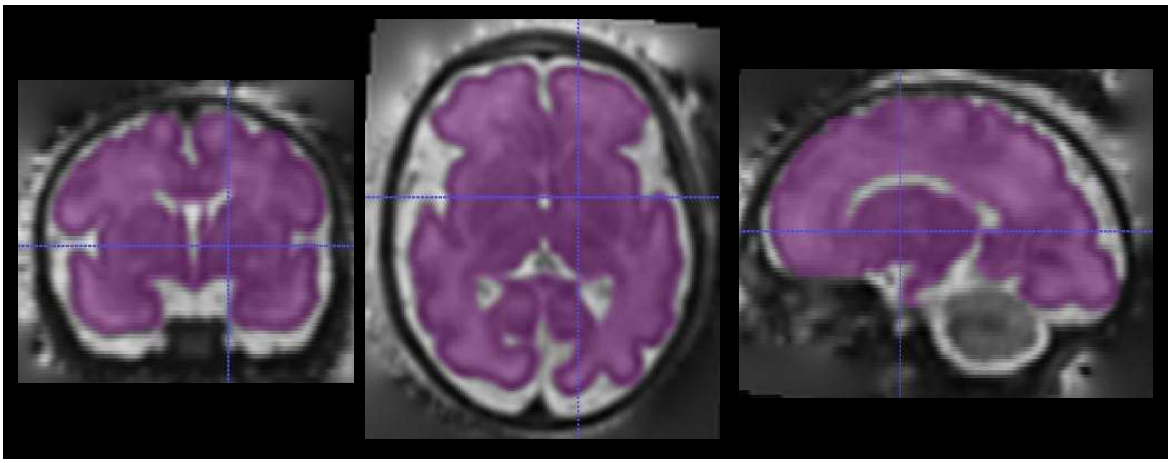


Figure 22. Segmentation of supratentorial brain tissue.

Supratentorial brain tissue segmentation shown in coronal, transverse and sagittal planes.

Lateral Ventricles

Semi-automatic segmentation included the lateral ventricles and choroid plexi and excluded the third and fourth ventricles, cavum septum pellucidae and vergae (see figure 23). The septal leaves separated the lateral ventricles and cavum.

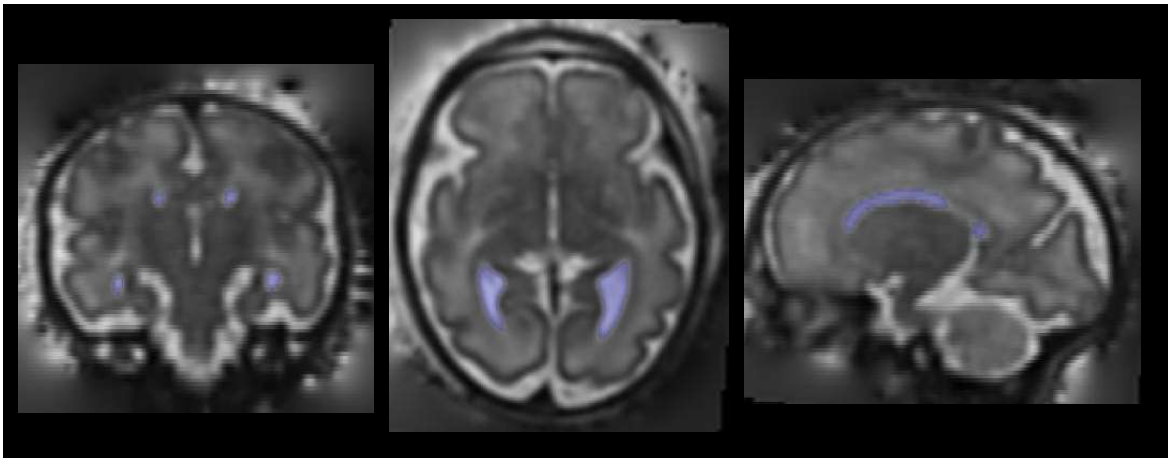


Figure 23. Segmentation of the lateral ventricles.

Lateral ventricle segmentation shown in coronal, transverse and sagittal planes.

Extracerebral CSF

Segmentation of the extracerebral CSF was semi-automatic and included all intracranial CSF space surrounding brain tissue as well as the interhemispheric fissure space. Inferiorly a boundary was defined by the differentiation of the cisterna magna (included) and the spinal cord canal. Intracranial veins were included. See figure 24.

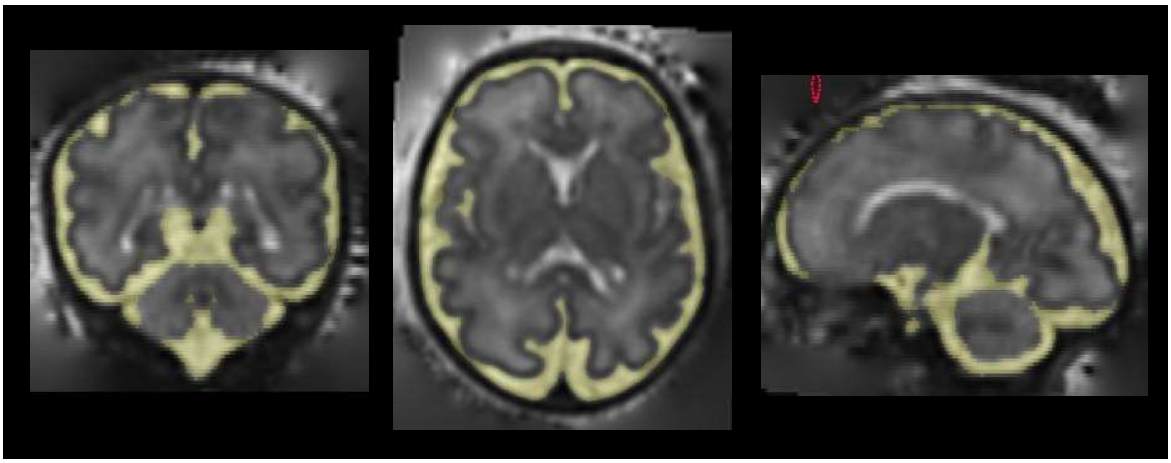


Figure 24. Segmentation of the extracerebral fluid.

Extracerebral fluid segmentation shown in coronal, transverse and sagittal planes.

Cerebellum

Cerebellar segmentation was manually performed and included both hemispheres and cerebellar vermis. The boundaries of the lateral hemispheres where distinguishable by CSF. Where the dorsal surface rested against the supratentorial membrane the boundary was defined as the lower edge of the membrane rather than by CSF. The brainstem and fourth ventricle formed the anterior boundary in the midline. See figure 25.

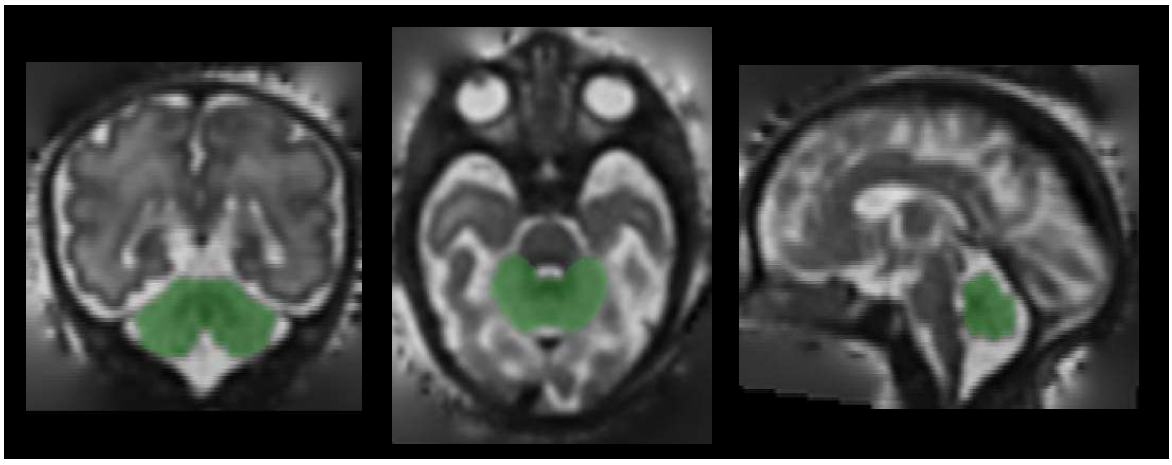


Figure 25. Segmentation of the cerebellum.

Cerebellum segmentation shown in coronal, transverse and sagittal planes.

Cavum septum pellucidum, cavum vergae and third ventricle

The CSP and cavum vergae were manually segmented and was defined superiorly by a contrast difference due to CSF content below the body of the corpus callosum. Lateral borders were defined from the lateral ventricles by the separating septal leaves. The anterior limit was defined by the genu of the corpus callosum and the posterior limit by the appearance of the internal cerebral veins. Segmentation extended inferiorly to include the third ventricle to its membranous floor at the subthalamic level inferiorly. The thalamic hemispheres composed the lateral borders. See figure 26.

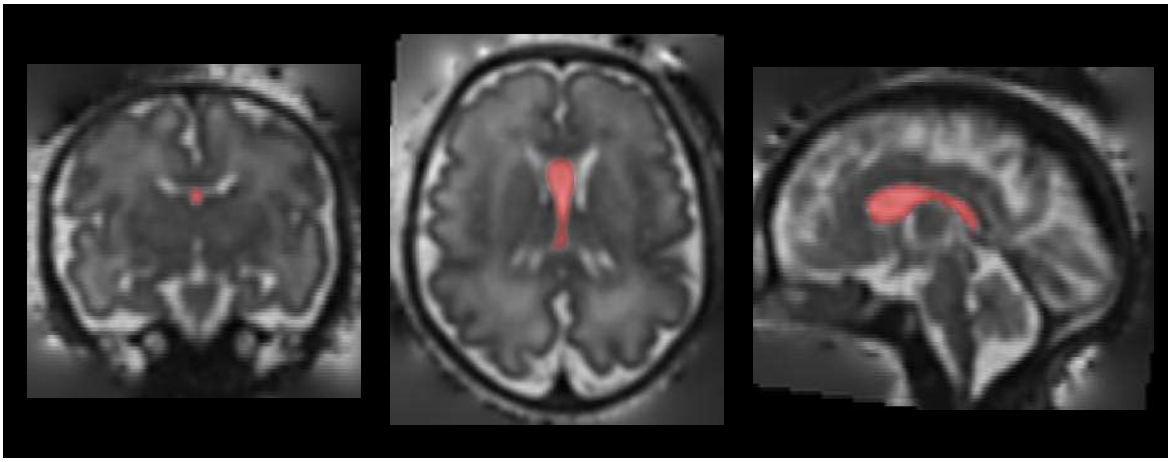


Figure 26. Segmentation of cavum septum pellucidum and cavum vergae.

CSP and cavum vergae segmentation shown in coronal, transverse and sagittal planes.

Fourth ventricle

The fourth ventricle was manually segmented and defined by the superior medullary velum superiorly and the obex inferiorly. The pons formed the anterior boundary, the middle cerebellar peduncles the lateral boundaries and the cerebellar vermis formed the posterior boundary. See figure 27.

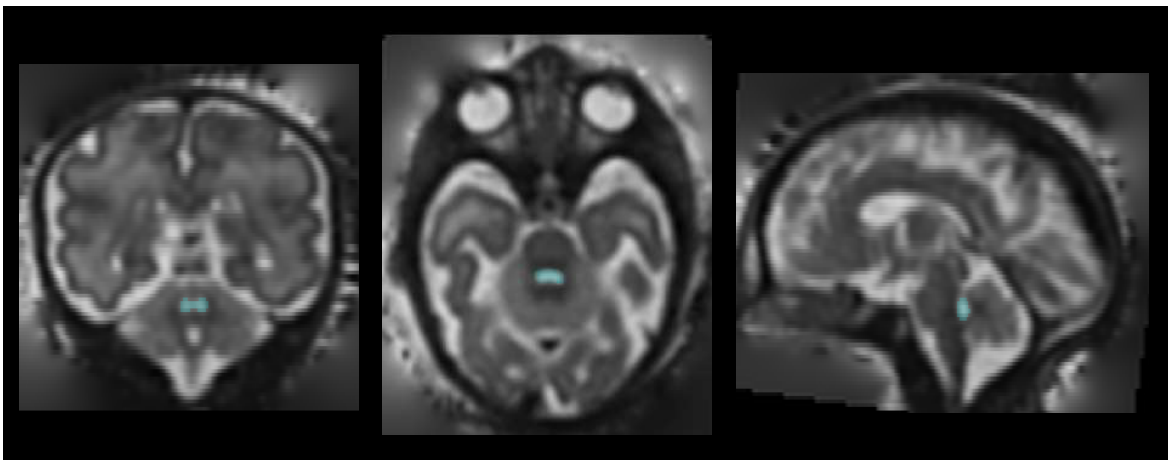


Figure 27. Segmentation of the fourth ventricle.

Fourth ventricle segmentation shown in coronal, transverse and sagittal planes.

Cortical grey matter

Cortical grey matter was manually segmented as the region between the outer cortical border (defined by contrast between CSF and cortex) and the inner cortical border (contrast between cortex and white matter) in all slices in the transverse plane (see figure 28). Around the interhemispheric fissure the cortex was excluded when corrupted by partial volume effects (figure 28A). Where the cortical boundary could be clearly visualised, the cortex was segmented including all lower signal intensity areas close to the interhemispheric fissure (figure 28B). The cingulate gyrus (figure 28C arrow) was segmented up until the first convolution from the corpus callosum (figure 28C arrow head). The parieto-occipital fissure (figure 28D arrow) was included in the segmentation up until the medial border of the hippocampus isthmus (figure 28D arrow head). The cortex surrounding the inferior part of the frontal lobes was not included in the segmentation when cortical visualization was affected by partial volume of the frontal lobe and eyes (figure 28E arrow). Regions with obvious blurring were excluded from the segmentation.

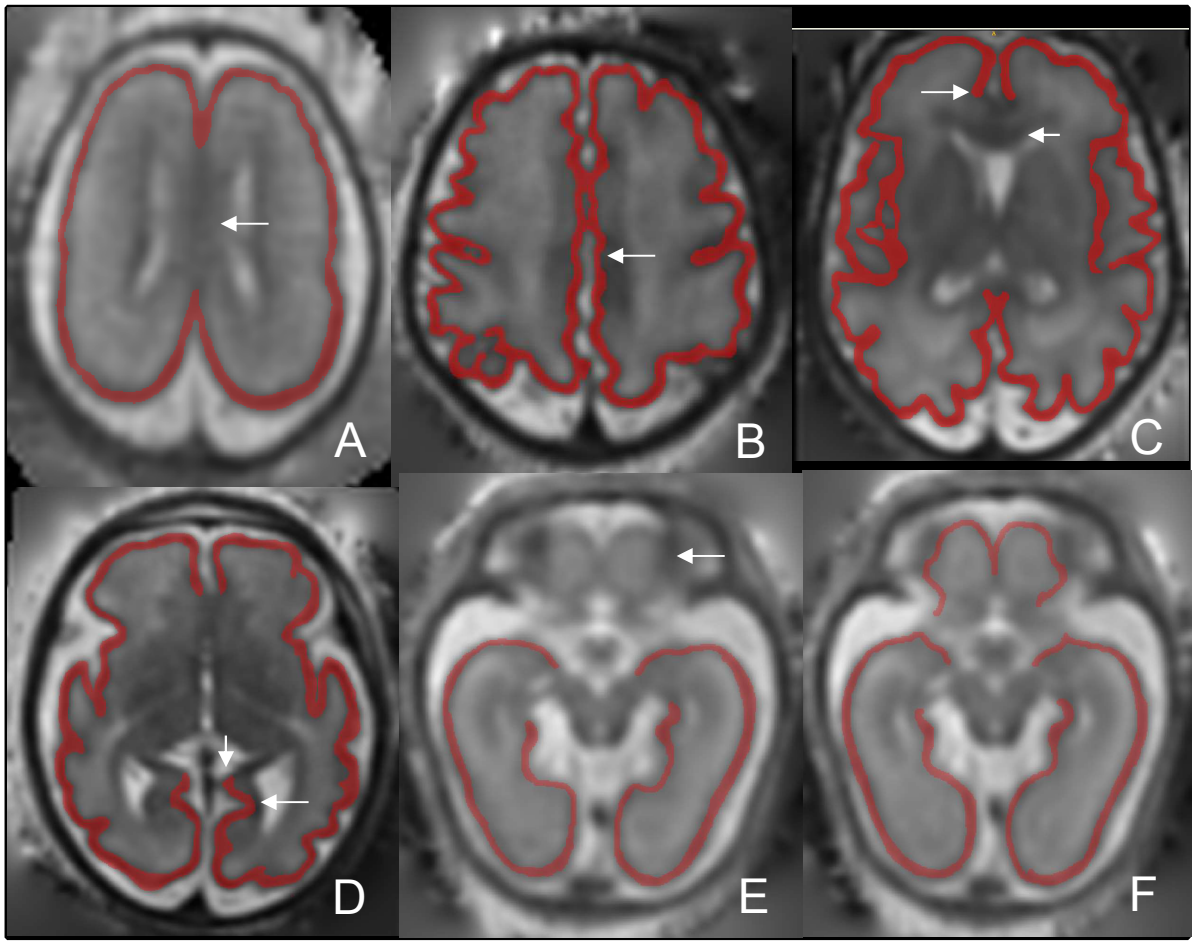


Figure 28. Segmentation of cortical grey matter.

(A) Around the interhemispheric fissure the cortex was excluded when visualization was poor due to partial volume effects (arrow). (B) Where cortical boundaries were clear, cortical segmentation included all lower signal intensity areas close to the interhemispheric fissure (arrow). (C) The cingulate gyrus (arrow) was segmented up until the first convolution from the corpus callosum (arrow head). (D) The parieto-occipital fissure (arrow) was included up until the medial border of the hippocampus isthmus (arrow head). (E) The cortex surrounding the inferior part of the frontal lobes was excluded

from the segmentation when visualization was affected by partial volume effects. (F) Slice directly above (E) was included in the segmentation.

2.3.4.4.2.3 Segmentation of the fetal body

Segmentation of the fetal body (head, neck, torso, limbs, hands and feet) was undertaken manually. The umbilical cord was excluded. Where possible borders where defined by contrast with surrounding amniotic fluid. The segmentation can be viewed in 3D which is useful when there is ambiguity in the 2D images (see figure 29).

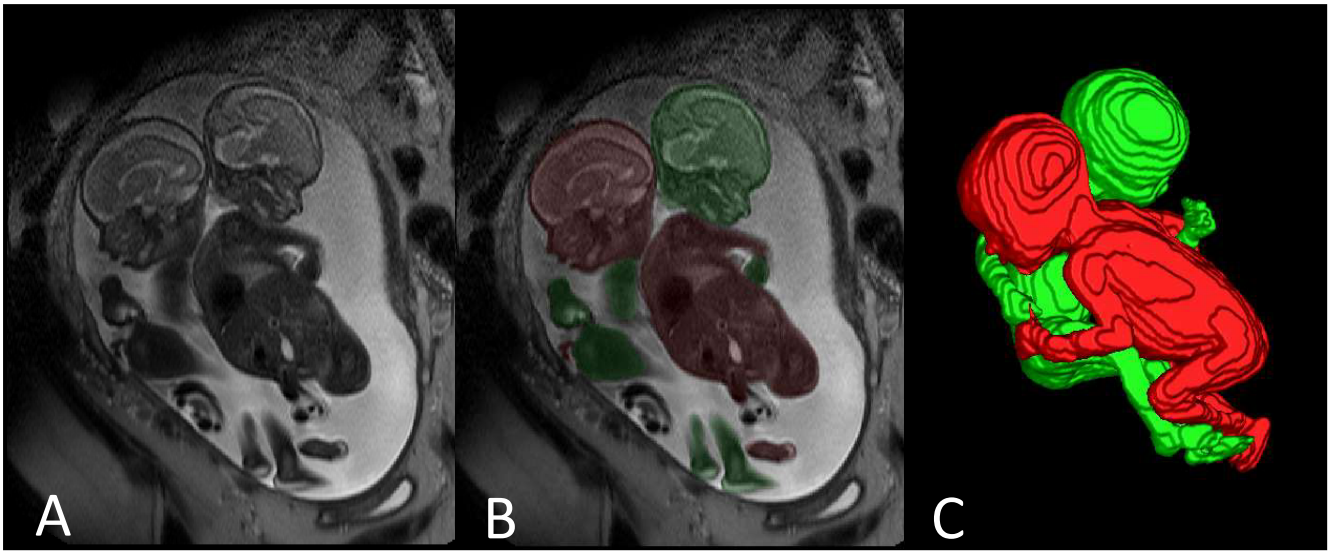


Figure 29. Segmentation of the fetal body.

A) T2 weighted ssFSE image through uterus showing both fetal heads and parts of torso and limbs. B) Segmentation of the fetuses. C) 3D view allowing confirmation that body parts are attributed to the correct twin.

Where twins body parts where next to each other or against placenta or uterine wall, review of the 3D model ensured that incorrect areas were not included or body structures excluded (see figures 30 and 31).

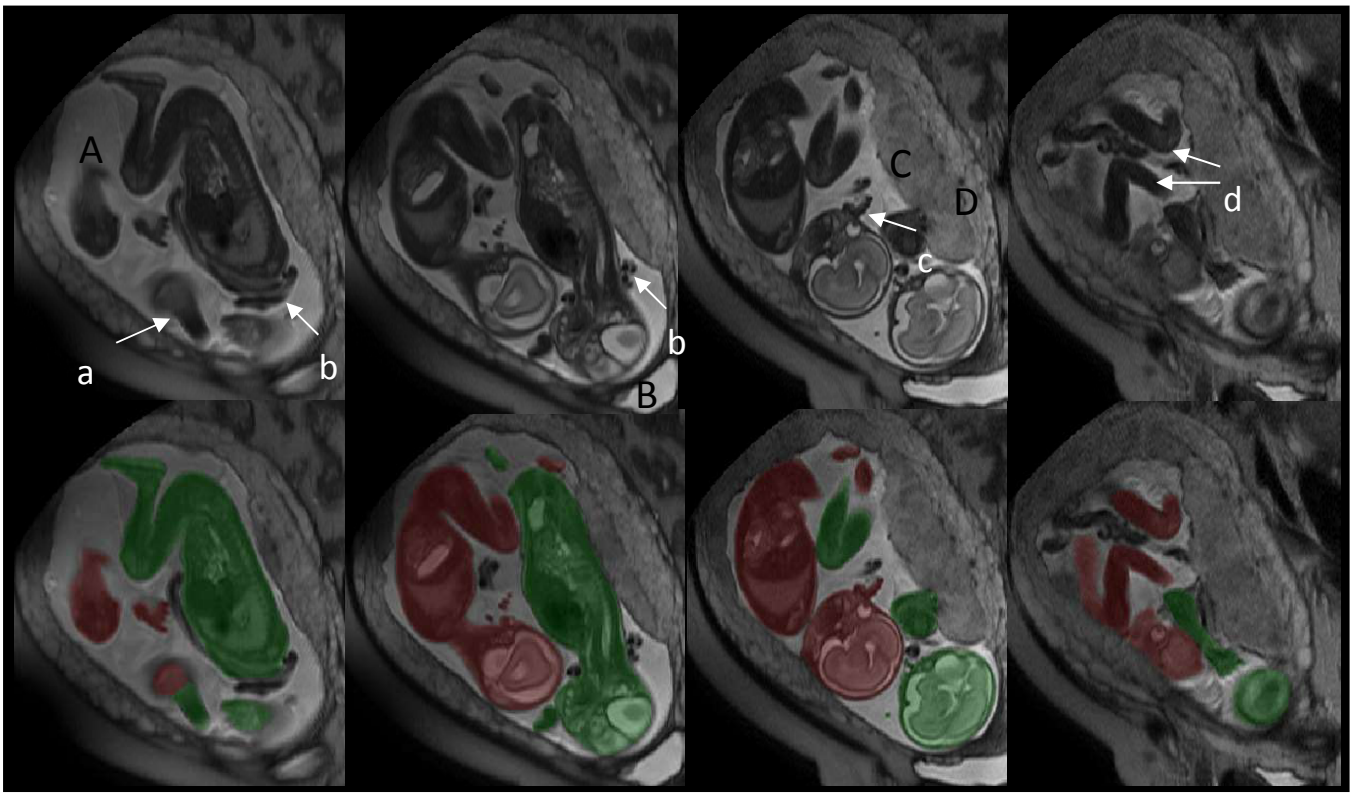


Figure 30. Segmentation of the fetal body: identification of body parts.

Raw images are shown in the upper panel. Individual fetal body segmentations are shown in the lower panel. A) amniotic fluid B) bladder C) placenta D) uterus a) adjacent fetal limbs b) nuchal umbilical cord c) fetal hand d) entwined fetal limbs.

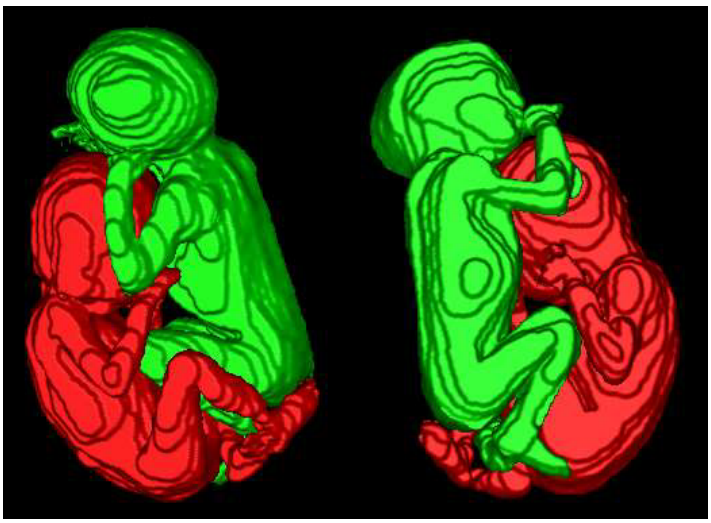


Figure 31. 3D reconstruction of the fetal body segmentations.

These reconstructions help distinguish ambiguous fetal body parts shown in figure 30.

2.3.4.4.3 Cortical development

The appearance of a range of major cortical landmarks was evaluated to assess regional brain maturation quantitatively. In anticipation of a relative delay in appearance of cortical landmarks in twins relative to singletons (Chi & Dooling 1977; Dubois et al. 2008), the earliest appearing landmarks observed in singleton populations were selected for evaluation in our twin cohort. To ensure that assessment of the brain was as comprehensive as possible, at least one major cortical landmark from each brain lobe was selected. Table 11 lists findings from major studies that have evaluated GA at which the appearance of sulci and gyri are visible on fetal imaging in singletons.

Brain region	Landmark	Kyriakopoulou	Habas	Garel	Levine
Frontal/parietal/temporal border	**Sylvian fissure**	<22			16-17
Frontal	Precentral gyrus	24-25	24-25		26-27
Frontal	**Inferior frontal gyrus**	24-25			
Frontal	Superior frontal gyrus	27			
Frontal	Middle frontal gyrus	27			
Frontal	Inferior frontal sulcus	27	23-24	27	30-31
Frontal	Superior frontal sulcus	27-28	23-24	24-25	
Frontal/parietal border	**Central sulcus**	24-25	23-24	24-25	26-27
Parietal	**Parieto-occipital fissure**	23	22-23	22-23	18-19
Parietal	Postcentral gyrus	24-25	23-24		26-27
Temporal	**Superior temporal gyrus**	24			
Temporal	Superior temporal sulcus	27-29	23-25	30	26-27
Temporal	Middle temporal gyrus	27-30	23-25		
Temporal	Inferior temporal gyrus	29-30			
Occipital	**Calcarine sulcus**	24-27	23-24	22-23	18-19

Table 11. Gestational age of first appearance of cortical landmarks in healthy singleton fetuses.
Gestational age of early appearance of cortical landmarks as detected in previous singleton fetal studies using MRI (Garel et al, 2003; Levine & Barnes, 1999; Habas et al, 2012). The Sylvian fissure was present in all fetuses. In the study by Levine & Barnes (1999), the authors quote the GA range at which at least 75% of fetuses had the landmark visible on imaging. In the other studies the GA range refers to the 50% around the median GA at which landmarks appear visible.

2.3.4.4.3.1 Quantifying maturation

Landmarks evaluated were the central sulcus, parieto-occipital fissure, Sylvian fissure, inferior frontal gyrus, superior temporal gyrus and calcarine sulcus (shown in bold in table 11). A detailed protocol was used to assess the cerebral sulcation and gyrification. The protocol was designed in our department and based on a cortical staging system by Van der Knapp et al 1996 (van der Knaap et al. 1996). The scoring system for sulci is illustrated in figure 32. Sulci received a score from 0 to 6. Gyri received a score from 0 to 4 or 6 using the same criteria. A score of 5 does not apply to gyri as the width is never zero.

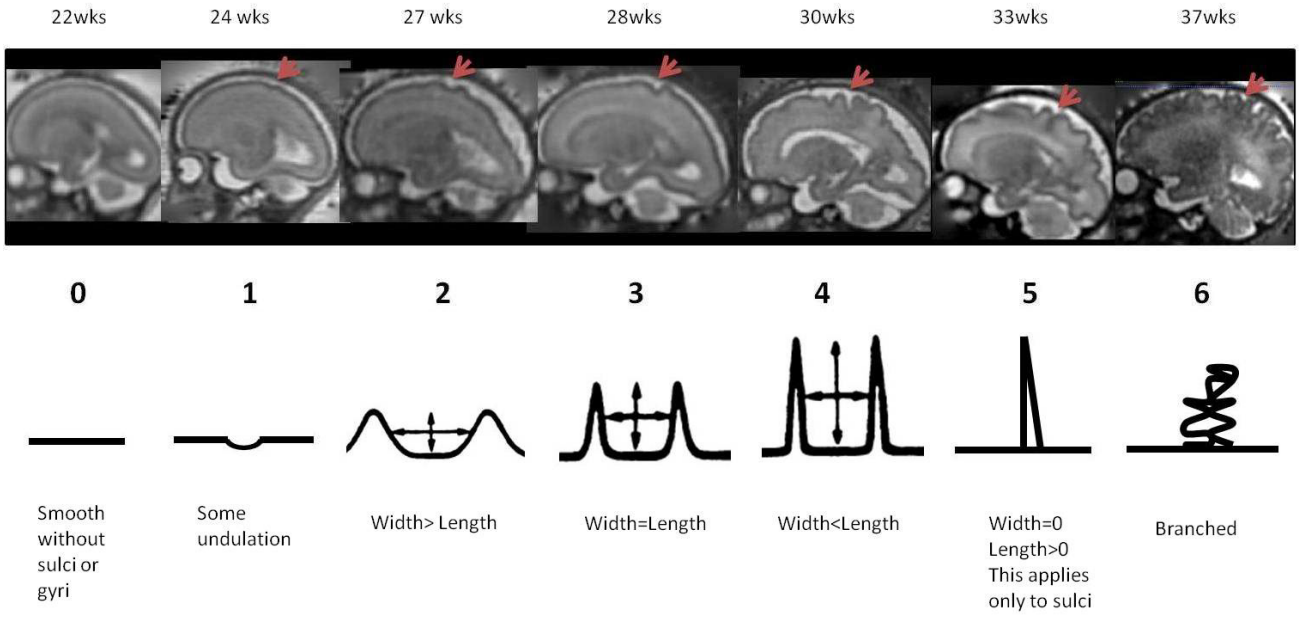


Figure 32. Cortical development scoring system.

Gyri and sulci are assigned a score from 0 to 6 according to their appearance. The development of the central sulcus in a normal singleton fetus from 22 to 37 weeks GA is shown. (Reproduced with permission from Kyriakopoulou 2014).

2.3.4.4.3.2 Image pre-processing

Standardisation between images in terms of orientation is essential for the assessment of cortical development. All images previously orientated for volumetric analysis were re-checked visually and compared to each other in order to achieve standardisation between the images. The voxel size was changed from 0.2x0.2x1.0 to 0.5x0.5x0.5 mm in order to produce uniformity in all orientations and so improve consistency in measurements. Rather than assessing all landmarks in each fetus, each landmark was evaluated separately across all fetuses. This was to maximise consistency in evaluation. Images were viewed and measurements made using ImageJ.

2.3.4.4.3.3 Scoring and measurement

Central sulcus

The central sulcus was assessed in the sagittal plane and given a score from 0 to 6 according to the scheme in figure 32. A slice where the sulcus has the maximum visible height, and when the cerebellum was present (figure 33) was selected for scoring.

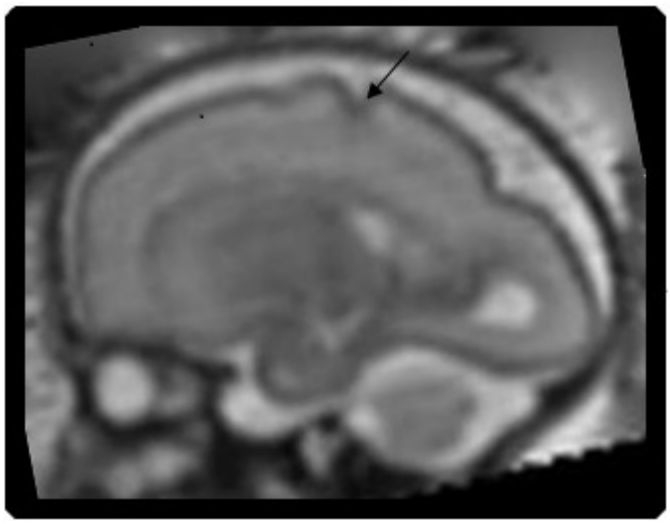


Figure 33. Assessment of the frontal-parietal border.
The central sulcus is indicated by the black arrow.

Parieto-occipital fissure

The POF was assessed in the transverse plane and given a score from 0 to 6. A slice where the sulcus "dips" into the ventricular atrium (see figure 34) was selected for scoring. This was usually on the same slice as for the measurement of the maximum distance from frontal to temporal regions along the insula-temporal cortex, i.e. the insular distance.

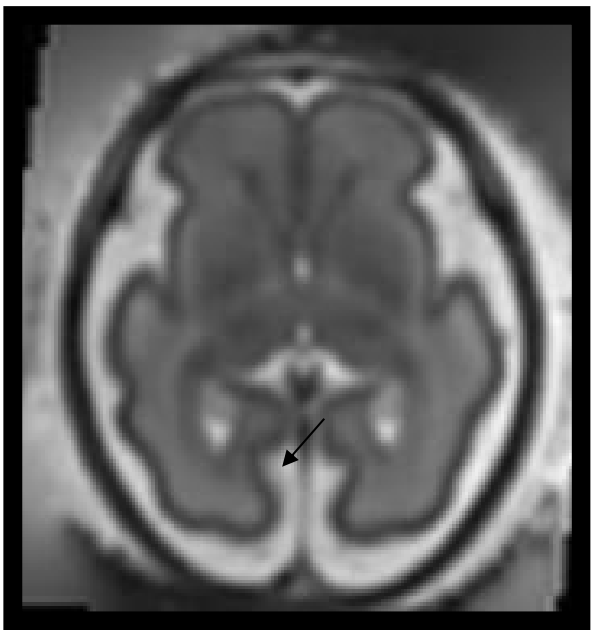


Figure 34. Assessment of the parietal lobe.
The parieto-occipital fissure is indicated by the black arrow.

Inferior frontal gyrus

The IFG was assessed in the coronal plane and given a score from 0 to 4 or 6 as gyri do not appear branched. A slice where the anterior temporal lobes were present but not the brainstem was selected for scoring (see figure 35).

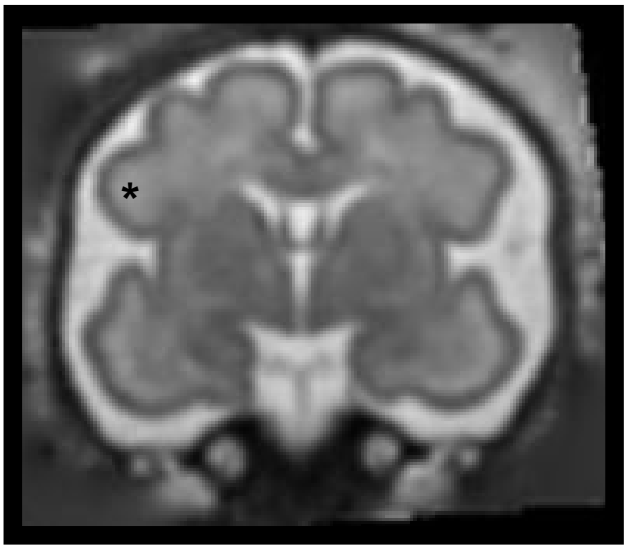


Figure 35. Assessment of the frontal lobe.
The inferior frontal gyrus is indicated by the black asterisk.

Superior temporal gyrus

The STG was assessed in the coronal plane and given a score from 0 to 4 or 6. A slice where the gyrus was visible just before the appearance of the brainstem and spinal cord was selected for scoring as shown in figure 36.

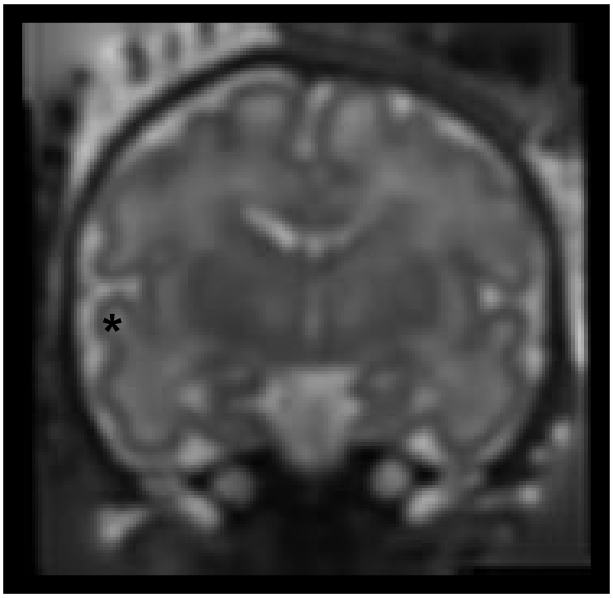


Figure 36. Assessment of the temporal lobe.
The superior temporal gyrus is indicated by the black asterisk.

Calcarine sulcus

The calcarine sulcus was assessed in the transverse plane and scored from 0 to 6. A slice was selected at eye level where the sulcus appeared deepest as shown in figure 37.

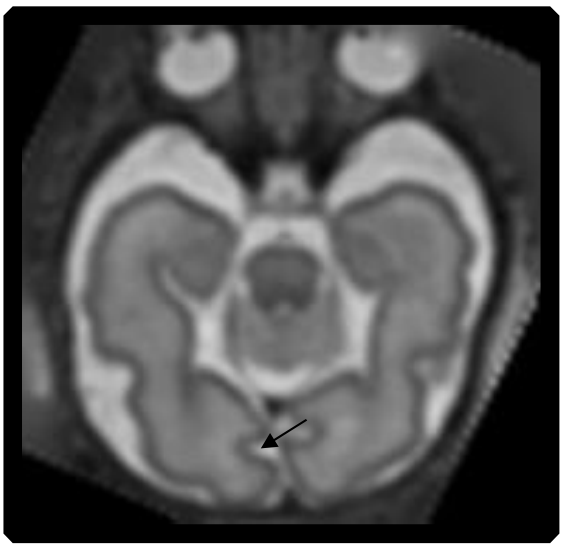


Figure 37. Assessment of the occipital lobe.
The calcarine sulcus is indicated by the black arrow.

Sylvian fissure

The Sylvian fissure was assessed in the transverse plane. The cortical development scoring system was not used as it has a much more complex configuration than other landmarks. Instead, assessment of the Sylvian fissure comprised two linear measurements and their ratio. The insular distance is the maximum distance from the frontal to temporal borders along the insula-temporal cortex (see figure 38A). The opercular distance is the minimum distance from frontal to temporal borders of the operculum (see figure 38B). The insular-opercular ratio is the insular distance divided by the opercular distance and increases with advancing maturity.

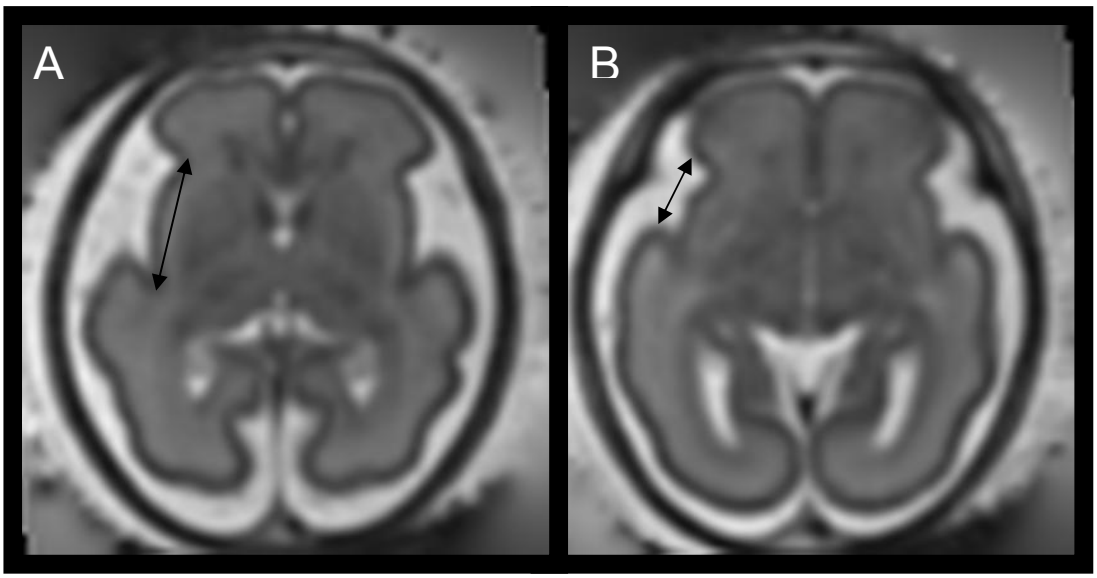


Figure 38. Assessment of the frontal-parietal-temporal border.
The Sylvian fissure was assessed by the ratio of the insular distance (indicated by the black arrow in left panel A) to the opercular distance (indicated by the black arrow in right panel B).

2.3.4.5 Spectroscopy

2.3.4.5.1 Post-processing of ^{1}HMRS spectra

Analysis of ^{1}HMRS spectra and quantification of metabolites was performed offline using jMRUI (www.mrui.uab.es/mrui/mrui_homePage.shtml, version 2.2). jMRUI (Java based MR User Interface) is a freely available graphical user interface for analysis of 1D spectra.

2.3.4.5.2 Quality assessment

Initial quality assessment of the acquired spectra was performed during scanning as the scanner software allows immediate display of the data. Repeat spectra where acquired in cases of poor quality. Individual spectra were subsequently assessed offline using jMRUI. Spectrum quality was judged according to the signal-to-noise ratio (height of metabolite peaks compared to background noise) and spectral resolution (peak width and differentiation between metabolites). Aberrant spectra indicative of fetal motion were removed. Figure 39 shows an example of a motion corrupted spectrum.

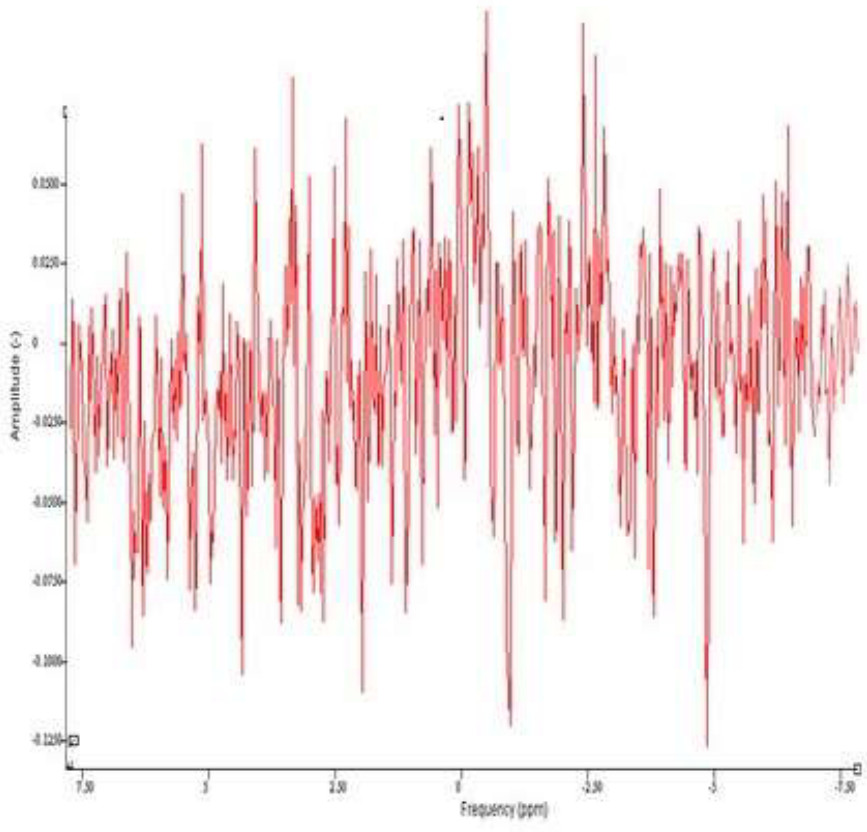


Figure 39. Example of a motion corrupted spectrum at echo time 270ms.

In this example motion corruption is evident by the absence of a water peak which should be present at 4.7ppm.

Fetal motion may also result in a shift in position of the fetal head over a region other than fetal white or grey matter. Such spectra may still have a water peak evident but are contaminated by the presence of large molecules such as lipids reflecting other fetal or maternal structures. An example is shown in figure 40 and such spectra were also removed.

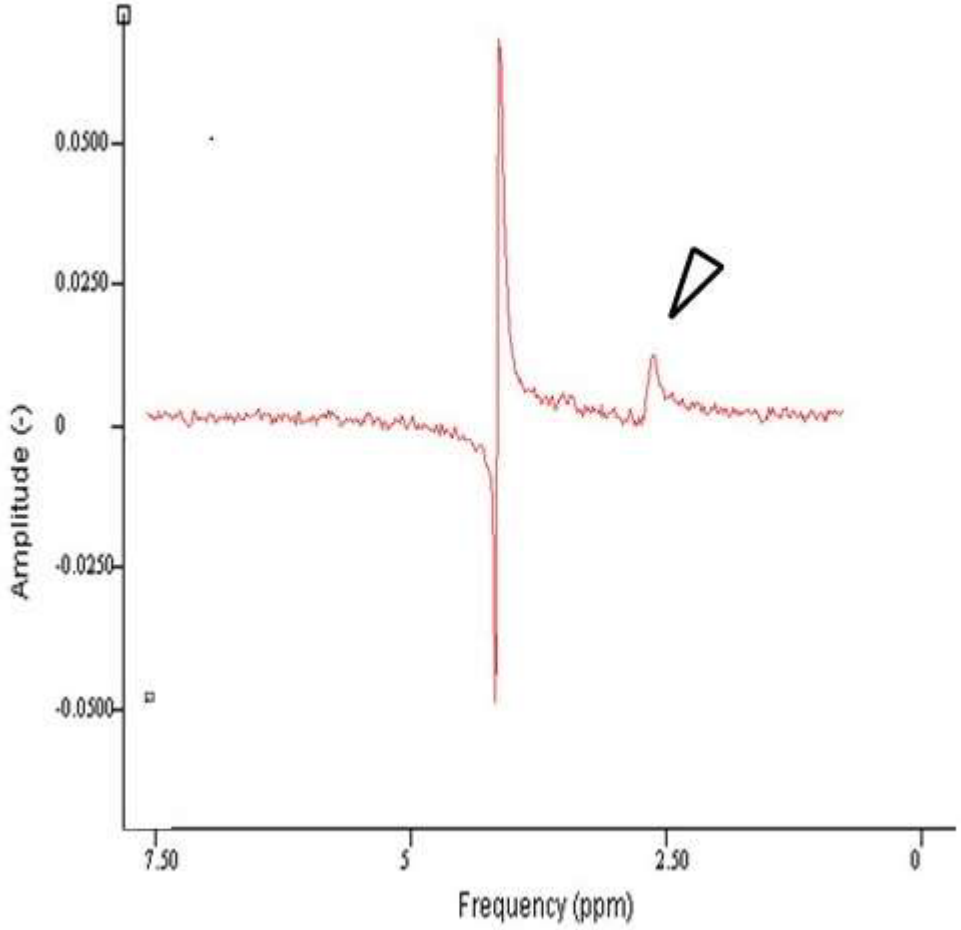


Figure 40. Example of a lipid contaminated spectrum at echo time 136ms.
In this example the water peak is present (indicated by the black arrow) but the spectrum is contaminated by a lipid peak (indicated by the black arrowhead).

The remaining spectra were summed into a final spectrum. Datasets in which greater than 50% of spectra were affected by some form of motion corruption were deemed unsuitable for analysis.

2.3.4.5.3 Noise reduction

The signal of the metabolites is affected by the noise in the detector channels. A line broadening filter can be applied which multiplies the signal by an exponentially decaying function. This increases the signal-to-noise ratio but also broadens the peaks (Drost et al. 2002). The water peak at 4.7 ppm is removed after noise reduction.

2.3.4.5.4 Phasing the spectrum

Metabolite quantification requires evaluating the area under the peaks. As distortion of the baseline may affect the accuracy of the area calculation, phase adjustment of the spectrum is manually performed to flatten the baseline.

2.3.4.5.5 Quantification

Expected peaks were identified at each echo time based on their position within the spectrum. The top and midpoint of each peak was identified and a curve generated by the software for each metabolite peak. Once curves had been fitted for all metabolites the ratios were calculated. Ratios are more robust than absolute quantification of metabolite concentrations as discussed previously in section 1.3.5. The metabolite ratios calculated at each echo time are shown in table 12. The presence of lactate was also recorded at echo time 136ms.

Echo Time (ms)	Metabolite ratios
42	MI:Cho MI:Cr Cho:Cr
136	NAA:Cho NAA:Cr Cho:Cr
270	NAA:Cho NAA:Cr Cho:Cr

Table 12. Metabolite ratios quantified at each echo time.

The presence of lactate was also recorded at echo time 136ms.

2.3.5 Data Analysis Cohort Summary

Following image acquisition and post-processing, a number of cases were not suitable for inclusion in subsequent analysis. The table below (table 13) lists the data that were included from each case in the proceeding results chapters.

Case no	Structural abnormality (Chapter 3.1)	Biometry (Chapter 3.2)	Volumetry (Chapter 3.3)	Cortical Development (Chapter 3.4)	^{1}HMRS (Chapter 3.5)
1	yes	*excluded*	*excluded*	*excluded*	acquired
2	yes	*excluded*	*excluded*	*excluded*	*not acquired*
3	yes	*excluded*	*excluded*	*excluded*	acquired
4	yes	*excluded*	*excluded*	*excluded*	acquired
5	yes	*excluded*	*excluded*	*excluded*	acquired
6	no	*excluded*	*excluded*	*excluded*	acquired
7	no	*ex-R unsuitable*	*ex-R unsuitable*	*ex-R unsuitable*	*not acquired*
8	no	*ex-R unsuitable*	*ex-R unsuitable*	*ex-R unsuitable*	*not acquired*
9	no	included	included	included	*not acquired*
10	no	included	included	included	*not acquired*
11	no	included	included	included	*not acquired*
12	no	*ex-D unsuitable*	included	included	*not acquired*
13	no	included	included	included	*not acquired*
14	no	included	included	included	*not acquired*
15	no	included	included	included	ex-D acquired
16	no	included	included	included	*not acquired*
17	no	included	included	included	*not acquired*
18	no	included	included	included	*not acquired*
19	no	included	included	included	acquired
20	no	included	included	included	*not acquired*
21	no	included	included	included	*not acquired*

22	no	included	included	included	*not acquired*
23	no	included	included	included	*not acquired*
24	no	included	included	included	acquired
25	no	included	included	included	*not acquired*
26	no	included	included	included	acquired
27	no	included	included	included	acquired
28	no	included	included	included	acquired
29	no	included	included	included	acquired
30	no	included	included	included	acquired
31	no	*unsuitable*	*unsuitable*	included	*not acquired*
32	no	included	included	included	*not acquired*
33	no	included	included	included	*not acquired*
34	no	included	included	included	*not acquired*
35	no	included	included	included	*not acquired*
36	no	included	included	included	acquired
37	no	included	included	included	*not acquired*
38	no	included	included	included	*not acquired*
39	no	included	included	included	*not acquired*

Table 13. Summary of data acquired for each case.

In four cases data from one or both fetuses within a pair were unsuitable for analysis due to artefact or incomplete coverage and the case was therefore excluded from within pair analyses. [1]HMRS data were acquired at one or more echo times in 13 twin pairs and 1 ex-donor fetus.

In case 6 (see table 9 Methods section 2.2.3), the ex-donor fetus had severe sFGR with no evidence of structural abnormality. However the appearance of the brain was generally indistinct, the significance of which was difficult to interpret, and therefore the entire case was excluded from biometric, volumetric and cortical development within pair analysis. In cases 7 and 8 (see table 9 Methods section 2.2.3) there was incomplete coverage and artefact on the images from the ex-recipients within the pair and so the case was excluded from within pair biometric, volumetric and cortical development analysis. In case 12 the reconstructed dataset of the ex-donor fetus had

incomplete coverage of the skull and was therefore not suitable for biometry but was suitable for inclusion in volumetric and cortical development analysis. In case 31 reconstructions for both fetuses had artefact over the cerebellum and were therefore unsuitable for biometric or volumetric analysis but were suitable for cortical development analysis.

2.4 Perinatal Outcome

The relevant hospital was contacted around the estimated due date for each participant and delivery summaries requested. Date of birth, gender, mode of delivery, birthweight and Apgar score data were recorded where possible. Where neonates had been admitted to the neonatal unit following delivery, contact with the unit was maintained and neonatal discharge summaries requested. Participants were also contacted by telephone following delivery.

2.5 Neurodevelopmental Assessment

Parents were invited for a neurodevelopmental assessment of their child at year one and two. On the day of the assessment the child's weight, height and head circumference were measured. The Griffiths Mental Development Scales (GMDS) (Griffiths & Huntley 1996) assessment or the Bayley Scales of Infant Development-III (BSID-III) (Bayley 2005) assessment were offered. Ages & Stages Questionnaires were provided in cases where the parents declined to attend a formal assessment at year two or when we were unable to get in contact with parents to improve collection of follow-up data (Edwards et al. 2002).

Both GMDS and BSID have been widely used in both clinical and research settings since the early 1970s (Bayley 1969; Griffiths 1970). The GMDS were developed in the UK whereas the BSID were standardised based on a US sample of 1700 children. The validity of the most recent version has been established based on a UK population sample aged between 12 and 24 months (Pearson 2012). The BSID gained in popularity following its update to BSID-II (Bayley 1993) a few years ahead of the GMDS restandardisation (Griffiths & Huntley 1996). However the most recent revision of the BSID (BSID-III) (Bayley 2005) involved a dramatic change in format; whereas earlier editions only provided a Mental Development Index (MDI) and a Psychomotor Development Index (PDI), the third edition included distinct scores for Cognitive, Language and Motor skills providing a precise assessment of these developmental domains. Although combined scores for BSID-III appear to correlate with those of BSID-II indices, performance levels appear significantly higher for BSID-III (Long et al. 2012; Moore et al. 2012; Johnson et al. 2014). This raised concern that the Bayley-III may underestimate developmental impairment (Anderson et al. 2010) reducing the number of children in clinical groups eligible for early intervention, as well as creating difficulties in categorising outcomes for research purposes.

Agreement between BSID and Griffiths scales significantly improved following the introduction of BSID-III (Chaudhary et al. 2013; Picciolini et al. 2015), although BSID-III still appears to find less impairment relative to the GMDS, particularly in the motor skills domain (Picciolini et al. 2015). Performance on BSID-III has good correlation with later performance on GMDS before school age, specifically when subscale quotients are used rather than composite scores (Milne et al. 2015).

In young children under the age of two there is generally poor agreement across serial examinations for both BSID and Griffiths scales (Chaudhary et al. 2013) and test-retest reliability is particularly poor at 12 months or less (Johnson & Marlow 2006). However assessments at one year of age allow for the detection of obvious developmental abnormalities and do help maintain parent contact which is important in maximizing follow up rates (Edwards et al. 2002). The GMDS 0-2 was preferentially used in this study for developmental assessment at one year as it is considerably shorter in duration than BSID-III and therefore more practical for younger children.

The GMDS at two years of age appears to be a reasonable predictor of IQ at school age as assessed by measures such as Wechsler scales of intelligence. This is the case for performance IQ and overall IQ but not for verbal IQ likely due to the limitations created by emerging language at two year assessment (Sutcliffe et al. 2010). In addition the two year cut-off for GMDS 0-2 may be susceptible to ceiling effects further limiting its use at 24 month assessments. BSID-III has been shown to have good predictive validity at 2 years for Wechsler IQ measures at school age (Bode et al. 2014). Although sensitivity is poor, the specificity of BSID-III performed at two years was high for detecting impairment as measured by IQ score at school age (Spencer-Smith et al. 2015) and motor delay at 4 years (Spittle et al. 2013). The BSID-III was preferentially used in this study for developmental assessment at two years.

2.5.1 Griffiths Mental Development Scales

The GMDS can be used to assess development from birth to two years (GMDS 0-2) and from two to eight years of age (GMDS-ER 2-8). The GMDS 0-2 (Huntley 1996) is composed of 5 separate scales: Locomotor, Personal-Social, Hearing and Speech, Eye- Hand Co-ordination and Performance.

Each task in the GMDS assessment is marked with 0 (failure) or 1 (success). The total raw score for each sub-scale is calculated upon completion of the assessment by adding up the total number of items passed in each sub-scale. Raw scores for each sub-scale can then be converted into

standardised scores including age equivalent, sub- and general-Quotients, and percentile equivalents of the sub-quotients (Table 14).

Sub-scale	Description	Total raw score	Age-equivalent score	Sub- and Developmental Quotient	Percentile score
Locomotor	Gross motor skills (co-ordination and balance)	+	+	+	+
Personal-Social	Social development and independence	+	+	+	+
Hearing and Language	Active listening, expressive and receptive language	+	+	+	+
Eye and hand co-ordination	Fine motor skills, visual attention and manual dexterity	+	+	+	+
Performance	Novel situations and problem solving	+	+	+	+
Total Scale		**+**	**+**	**+**	

Table 14. Griffiths Mental Development Scales.

Areas of development assessed and scoring in the GMDS 0-2.

The sum of the total scores of all subtests divided by the number of tests represents the total DQ. The sub-Quotients (SQ) and Developmental Quotients (DQ) are calculated for each sub-scale and for the total raw score. These take in to account how the child's raw scores vary around the mean score for each month of age. The mean for each developmental age represents the average score obtained by all children tested in the standardisation sample at that age. The DQ has a mean of 100 and a standard deviation of 12 while the SQ has a mean of 100 and a standard deviation of 16. DQ and SQ scores below 1SD (88 and 84 respectively) indicate developmental delay. The percentile scores are then derived from the sub-quotients.

2.5.2 Bayley Scales of Infant and Toddler Development-III

The BSID-III assesses the developmental function of infants and young children from 1 month to 42 months of age (Bayley 2005) across 3 domains; Cognition, Language (expressive and receptive) and Motor (fine and gross); see table 15 below. The BSID-III may also include a Social-Emotional scale, however this was not part of the routine examination and behaviour was informally assessed by the assessor based on observation during the testing session.

Scale or subtest	Description
Cognitive	Sensorimotor development, exploration, manipulation, object relatedness, concept formation and memory
Language	
Receptive	Vocabulary, preverbal behaviour, verbal comprehension
Expressive	Preverbal communication, morpho-synaptic development
Motor	
Fine motor	Functional hand skills (grasping, object manipulation)
Gross motor	Limbs and torso (static positioning, dynamic movement, co-ordination)

Table 15. Bayley Scales of Infant and Toddler Development-III.
Areas of development assessed in the BSID-III in this study.

Each task in the BSID-III is marked with 0 (failure) or 1 (success). Total raw scores are calculated by summing the number of completed tasks for each subtest (table 16) at the end of the assessment. Scaled scores are derived from the subtest total raw scores and range from 1-19 with a mean of 10 and a standard deviation of 3. Composite scores are generated for the Cognitive, Language and Motor scales and are derived from the sum of the various subtest scaled scores. The composite scores can be used to compare a child's performance across all 3 BSID-III scales. These are scaled to a metric with a range from 40 to 160 (mean of 100 and SD of 15). Developmental delay was classified when composite and / or scaled scores were below 1SD (85 and 7 respectively). Percentile ranks are calculated relative to performance of the child relative to children in the standardisation sample.

Scale or Subtest	Total raw score	Scaled score	Composite score	Percentile Rank	Developmental Age Equivalent
Cognitive scale	+	+	+	+	+
Language scale			+	+	
Receptive subtest	+	+			+
Expressive subtest	+	+			+
Motor scale			+	+	
Fine motor subtest	+	+			+
Gross motor subtest	+	+			+

Table 16. Scoring of Bayley Scales of Infant and Toddler Development-III.
Scoring of developmental scales and subtests of the BSID-III

.

2.5.3 Ages and Stages Questionnaires

Ages and Stages Questionnaires–III (ASQ-3) are parent-completed developmental questionnaires that assess a child's development in the areas of communication, gross motor, fine motor, problem solving and personal-social. An example of a questionnaire for a 24 month old infant is presented in the Appendix 6. Each questionnaire comprises 30 questions to assess the development domains and a series of open questions to identify parental concerns. They can be administered from 1 month to 5.5 years of age. Items required for completion of the tasks would usually be found in a family's home. The ASQ-3 takes about 15 minutes for the parent to complete and was standardized on a US sample of over 15,000 children.

The validity of the ASQ has been examined by comparing against several professionally administered standardised assessments including the BSID (Squires & Bricker 2009) and overall agreement on children's classifications was 86%. Previous studies comparing ASQ-2 and BSID-II at 2 years of age and less have shown sensitivity ranging from 73%-100% and specificity from 65%-87% (Gollenberg et al. 2010; Woodward et al. 2011). Both sensitivity and specificity are lower at younger ages. Comparison between BSID-III and ASQ-3 show modest agreement with sensitivity

and specificity of ASQ-3 above 75% (Schonhaut et al. 2013), although agreement is unsurprisingly poorer at younger ages (Veldhuizen et al. 2015).

Work done by others

The GMDS 0-2 and BSID-III assessments were performed by either a paediatrician (AC or MM) or a clinical psychologist (BH).

2.6 Statistical Analysis

Statistical analysis was carried out using StatsDirect V2.8.0 (StatsDirect statistical software. http://www.statsdirect.com. England: StatsDirect Ltd. 2013). Pearson's correlation coefficient was used to assess the linear relationship between normally distributed continuous variables. Spearman's correlation coefficient was used to assess the relationship between variables when the relationship was monotonic but not necessarily linear or when ordinal data was involved or non-normally distributed. Linear regression analysis was used to adjust for the effects of advancing gestational age in biometry and volume data. Assumptions of homoscedasticity and normality were assessed by examination of the residual plots. Analysis of covariance was used to assess group differences when corrected for gestational age. For all other data normality was assessed by Shapiro-Wilkes test. Parametric data were analysed by paired Student's t test for within twin pair comparisons. Non parametric matched data were analysed by Mann-Whitney U test. Comparison between categorical outcome variables was by Chi-squared, Fisher's Exact or Liddell's Exact test as appropriate dependent on sample size and pairing of groups. Significance was set at $p<0.05$ 2-tailed unless otherwise specified.

2.6.1 Power Calculation

The power calculation for this study was initially based on previous research in the Robert Steiner MR Unit investigating differences in brain volume in FGR and control fetuses. Following collection of preliminary data for the current study, power was recalculated using Power calculation tool from Decision Support System Ltd (http://www.dssresearch.com/toolkit/spcalc/power_a2.asp). Required sample sizes were similar. Data from a previous study (Damodaram et al. 2012) found a 5% variation in fetal volumetry when measured from different acquisition planes on the same subject. This variability in measurement was used as the test value. Preliminary analysis of fetal brain tissue

volumes from 11 cases in the current study had a within pair mean difference of 10.3% (SD 11.0). The calculated sample size to detect a difference in fetal volumetry within twin pairs treated for TTTS for a statistical power of 80% at an alpha error level of 5% was 27 twin pairs.

3. Results

3.1 Fetal brain imaging clinical examination findings

3.1.1 Background

Monochorionic twins are at higher risk of brain abnormalities compared to dichorionic twins (Adegbite et al. 2005) and singletons (Scher et al. 2002). The risk is especially increased following antenatal events that may cause cerebral vascular disturbance (Jelin et al. 2008; O'Donoghue et al. 2009). Reports of the incidence of antenatally acquired brain injury following fetoscopic laser surgery for TTTS range from 1.4% to 10% (Cincotta et al. 2009; Lenclen et al. 2007; Lopriore et al. 2006; Quarello et al. 2007; Rustico et al. 2012) depending on cohort selection, imaging modality and injury definition. A wide range of anomalies are reported although the most common appear to be IVH and white matter injuries (Lopriore et al. 2006; Quarello et al. 2007).

3.1.2 Aims

To evaluate the incidence and nature of structural abnormalities diagnosed on brain MRI in our post laser treatment cohort.

3.1.3 Methods

Women with two surviving fetuses following fetoscopic laser ablation for TTTS underwent MR imaging of the fetal brain at 1.5T. T1 and T2 weighted images were acquired for clinical examination. Images were reviewed by an expert in fetal brain imaging (MR) and structural abnormalities noted.

3.1.3.1 Classification of abnormalities

Severe abnormalities were classified as intraventricular haemorrhage (grade III or above) and periventricular leukomalacia (grade III or above). Polymicrogyria was also included as a severe finding.

3.1.3.2 Statistics

Liddell's exact test was used to compare frequency of abnormal findings between ex-recipient and ex-donor twins.

3.1.4 Results

3.1.4.1 Cohort

Seventy eight fetal MR scans were acquired from 39 women with two surviving fetuses following fetoscopic laser for TTTS (see table 9). Median gestational age at MRI was 25 weeks 5 days (range 20 to 32 weeks). Median time between treatment and MRI was 4 weeks and 6 days (range 2 to 10 weeks). Laser treatment was performed for stage 2 disease in four cases and stage 4 disease in two cases. The remaining 33 cases were treated for stage 3 disease.

3.1.4.2 Clinical findings

Structural anomalies were detected in 7/78 (9.0%) fetuses on MRI and could be classified as severe in 5 fetuses (6.4%). Abnormalities were present in 13% of ex-donor fetuses (10.3% for severe abnormality) compared to 5.1% (2.6% for severe abnormality) of ex-recipient fetuses ($F = 11.3$, $p < 0.001$; $F = 17.5$, $p < 0.001$ for severe abnormalities). The brain imaging findings and pregnancy outcomes for those fetuses with structural brain abnormality detected on MRI are shown in table 17.

Case no	Fetus	GA Laser (weeks)	MRI GA (weeks)	MR findings	Outcome
1	ex-donor	17/40	24	Polymicrogyria	Termination of pregnancy
2	ex-donor	21 1/7	23 6/7	Grade IV IVH	Confirmed on neonatal US Neonatal death at 13 days
3	ex-donor	23 5/7	26 1/7	Grade II IVH	Neonatal death at 1 day
4	ex-donor	24 1/7	27	Grade IV IVH + PVL	Termination of pregnancy
4	ex-recipient	24 1/7	27	Grade IV IVH	Termination of pregnancy
5	ex-recipient	22/40	26 2/7	Grade I IVH	Resolved on neonatal MRI NDA at 1 year normal
5	ex-donor	22/40	26 2/7	Grade IV IVH	Confirmed on neonatal MRI NDA at 1 year delayed

Table 17. Summary of cases with abnormal MR findings.

The main MR findings and pregnancy outcome are presented along with donor/recipient fetal status, GA at laser and GA at MR examination. (IVH: Intraventricular haemorrhage; PVL: Periventricular leukomalacia; NDA: Neurodevelopmental assessment)

Case 1

MR examination of the ex-donor fetus at 24 weeks GA revealed focal loss of brain tissue posteriorly, likely infarction related. The cortical appearance was suggestive of polymicrogyria (figure 41). Ultrasound examination performed eight days previously was reported as normal. No complications had been noted at the time of laser treatment. The MRI appearance of the ex-recipient twin was appropriate for gestational age. The parents opted for selective termination four days later. The surviving twin had appropriate MR appearances at 27 weeks and delivered spontaneously at 34 weeks. Neurodevelopmental function was within normal limits at two years of age.

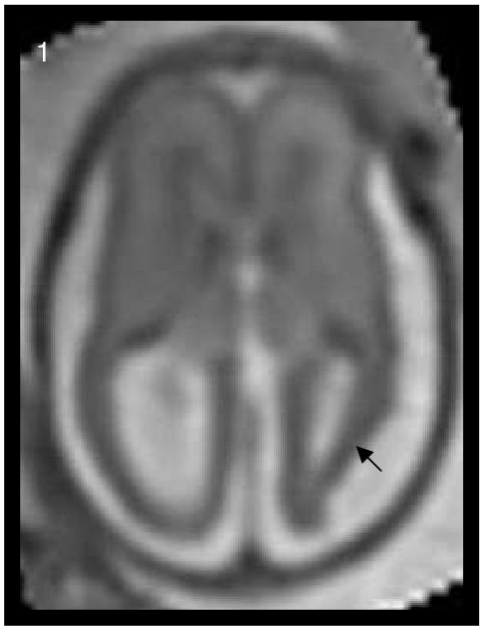


Figure 41. Antenatal brain injury in Case 1.
T2 weighted axial image at 24 weeks gestational age demonstrates asymmetrical ventricular dilatation with a thickened irregular cortical plate consistent with polymicrogyria (arrow) in the ex-donor fetus.

Case 2

The brain MR imaging of the ex-donor fetus in case 2 showed extreme abnormalities (figure 42). No complications were noted at the time of laser treatment and ultrasound at one week post procedure had been normal. However at two weeks post laser bilateral moderate ventriculomegaly with echogenic areas in the cerebral parenchyma, suggestive of haemorrhage, was seen with ultrasound. On MR imaging six days later there was severe bilateral ventriculomegaly as well as marked dilation of the third and fourth ventricles. Only a thin rim of white matter was identifiable. Minimal cerebellar tissue was visible, likely due to the effects of compression by a dilated fourth ventricle. The brainstem was displaced anteriorly. Germinal matrix haemorrhage extending into the parenchyma was evident. The parents opted to continue with the pregnancy, aware of the poor prognosis for the ex-donor twin in particular. The twins spontaneously delivered at 29 weeks GA and the ex-donor died at 13 days of age. Neonatal imaging of the ex-recipient brain showed normal appearances. The parents declined neurodevelopmental follow up but report no concerns for the surviving twin at 2 years of age.



Figure 42. Antenatal brain injury in Case 2.
T2 weighted axial image at 24 weeks gestational age demonstrates severe bilateral ventriculomegaly causing compression of cerebral hemispheres and displacement of the cerebellum (not shown) and extensive haemorrhage (arrow) in the ex-donor fetus.

Case 3

The ex-donor fetus in case 3 had evidence of bilateral grade II intraventricular haemorrhage on MRI at 26 weeks and 1 day, 17 days post uncomplicated laser treatment (see figure 43). Ultrasound examination performed the preceding day reported mild ventriculomegaly and appearances suggestive of haemorrhage. Asymmetry of the lateral ventricles was noted on MR imaging but measurements were with normal limits. Fetal brain appearances of the ex-recipient twin were appropriate for gestational age. The twins were delivered by emergency caesarean section at 28 weeks following development of reverse TTTS. Neonatal death of the ex-donor twin occurred within 24 hours of birth.

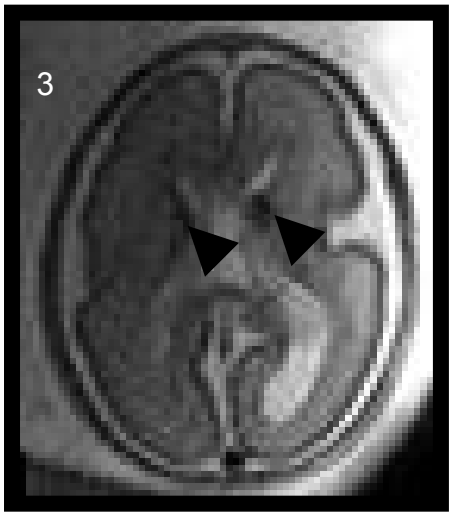


Figure 43. Antenatal brain injury in Case 3.
T2 weighted axial image at 26 weeks 1 day gestational age showing bilateral grade II IVH in the ex-donor fetus.

Case 4

Gross polyhydramnios was noted at the time of the laser treatment at 24 weeks GA with significant fluid leakage, but the procedure was otherwise uncomplicated. Ultrasound at 25 weeks GA

appeared normal. MRI was performed at 27 weeks GA, three weeks post treatment. Extensive abnormalities were apparent in the brains of both fetuses (figure 44). The ex-recipient had grade IV IVH involving the internal capsule and basal ganglia, with periventricular cystic lesions of the white matter. The ex-donor also had grade IV IVH with multicystic infarction of the white matter throughout both hemispheres. The appearance of the cortex was suggestive of developing polymicrogyria posteriorly. In view of the poor prognosis associated with these findings the parents opted for termination of the pregnancy the next day.

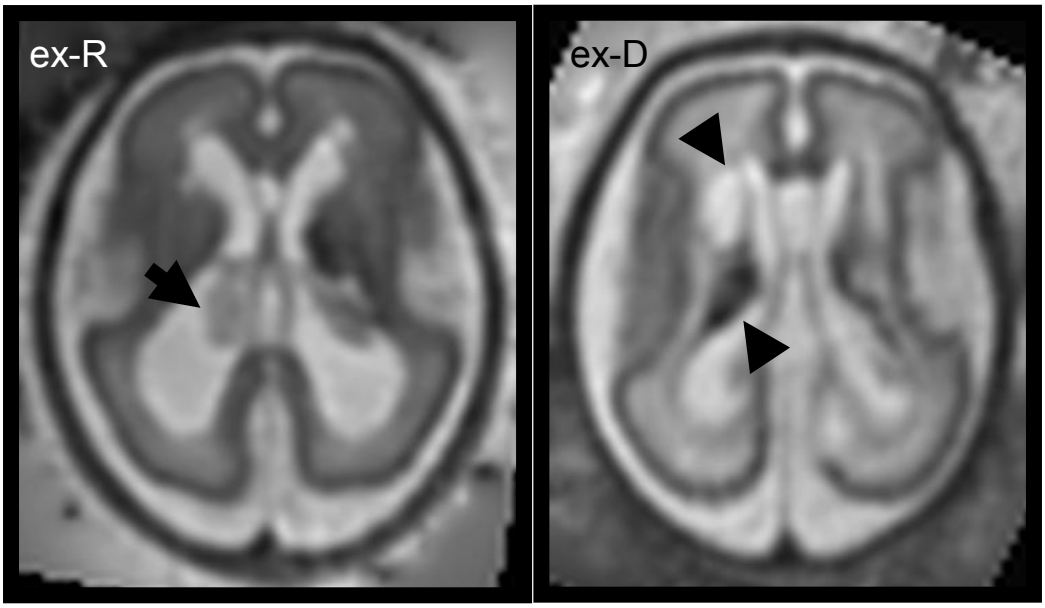


Figure 44. Antenatal brain injury in Case 4.

The left panel shows a T2-weighted image transverse image of the ex-recipient (ex-R) fetus at 27 weeks GA (three weeks post treatment). The arrow indicates grade 4 IVH. Periventricular cystic lesions are also present. The right panel shows T2-weighted image of the ex-donor (ex-D) fetus. The superior arrowhead indicates a large area of multicystic infarction of the white matter. The lower arrowhead illustrates an area of focal germinal matrix haemorrhage.

Case 5

Laser treatment was carried out at 22 weeks and MRI performed 4 weeks later. Unilateral germinal matrix haemorrhage (grade I IVH) was apparent in the ex-recipient on DWI with otherwise appropriate appearances of the brain (see figure 45a). Ultrasound examination the preceding day was suggestive of haemorrhage with mild ventriculomegaly. MRI findings in the ex-donor were grade IV IVH (see figure 45b) with involvement of the basal ganglia, thalami and internal capsule. These findings were consistent with those on ultrasound examination the preceding day. The twins delivered spontaneously at 26 weeks. Neonatal imaging at 43 weeks postmenstrual age showed resolution of injury in the ex-recipient twin (figure 45c). The ex-donor twin had unilateral ventriculomegaly with porencephaly. There was evidence of old haemorrhage in the basal ganglia and thalami. The cortex appeared immature with asymmetry of myelination in the internal capsule

and brainstem. Neurodevelopmental function at one year was within normal limits for the ex-recipient however the ex-donor co-twin was developmentally delayed.

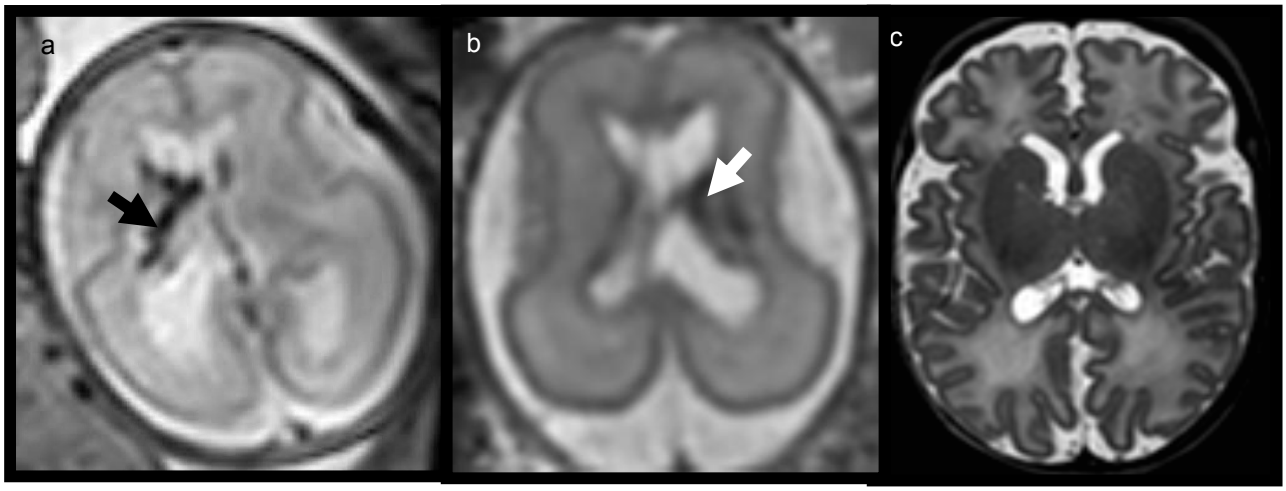


Figure 45. Antenatal brain injury in Case 5.

(a) Diffusion weighted image in axial plane of the ex-recipient fetus at 26 weeks gestational age demonstrating germinal matrix haemorrhage (black arrow). (b) T2 weighted image in the axial plane of the ex-donor fetus at 26 weeks gestational age demonstrating grade IV IVH (white arrow). c) T2 weighted imaging at 43 weeks postmenstrual age demonstrating essentially normal brain appearances in the ex-recipient.

3.1.5 Discussion

The observed incidence of all fetal brain anomalies in our cohort was 9%. Abnormal findings included intraventricular haemorrhage, white matter abnormalities and polymicrogyria. When a definition of severe injury restricted to grade III or IV PVH and cystic PVL is used in our series, the rate is 6.4%. This is comparable to the range of 1.4-10% % (Cincotta et al. 2009; Lenclen et al. 2007; Lopriore et al. 2006; Quarello et al. 2007; Rustico et al. 2012; Spruijt et al. 2012) reported in the literature.

Abnormalities were significantly more common in ex-donors compared to ex-recipients in our cohort. Two previous studies have reported similar observations (Quarello et al. 2007; Rustico et al. 2012). However meta-analysis data (Rossi & D'Addario 2009a), which included the study by Quarello et al, report no difference between ex-donors and ex-recipients. This is likely due to the reporting methods of brain injury in the studies included. Neurological abnormality in survivors was employed as a surrogate marker for brain injury in some studies or the incidence of severe injury in neonatal survivors was reported rather than antenatally diagnosed injury. Therefore the similarity observed may be confounded by a higher perinatal loss rate of ex-donors with severe cerebral injury. In the current study four out of five ex-donors diagnosed with brain injury during the antenatal period did

not survive to discharge. In two cases the prognosis was so poor that the parents opted for termination and in the remaining two cases neonatal death occurred within two weeks. Other studies have not commented on in utero brain injury differences between ex-recipient and ex-donor twins.

Multiple types of injury have been described in TTTS (Lopriore et al. 2006; Merhar et al. 2013; Quarello et al. 2007) although the most frequently reported findings are IVH and white matter injury. Intraventricular haemorrhage was the most frequently observed abnormality in our cohort. This was reported in both of the ex-recipient twins with brain injury and over half of those ex-donor twins with brain injury. Whilst there was no evidence of white matter infarction related injury in ex-recipient twins, almost half of ex-donor twins had probable infarct related pathology. The observation of both 'high flow' haemorrhagic and 'low flow' ischaemic lesions in ex-donor twins replicates findings of previous studies (Merhar et al. 2013; Quarello et al. 2007) and supports the hypothesis that, although there is a net flow of blood in TTTS from donor to recipient, fluctuations can occur in either direction (Degani et al. 2006).

Discordant activation of the renin-angiotensin system in TTTS may underlie observations of higher in utero brain injury as well as perinatal death due to severe brain injury in ex-donors compared to ex-recipients. Angiotensin II in particular appears to have a pro-inflammatory role (Suzuki et al. 2003) whilst Angiotensin I peptide forms may regulate inflammatory pathways (Moore et al. 2013). In vivo animal studies as well as in vitro human studies suggest that upregulation of the brain renin-angiotensin system appears to increase the susceptibility of brain tissue to ischaemic injury (Chen et al. 2009; Jain et al. 2015). In contrast down regulation appears to protect against ischemia and reperfusion brain injury (Panahpour & Dehghani 2010; Robbins et al. 2011; Villapol & Saavedra 2015). Given the bidirectional fluctuations in blood flow that occur between twins in TTTS, it may be that the increased renin and angiotensin II activity observed in donor fetuses (Guilherme et al. 2009; Kilby et al. 2001) increases the risk of cerebral injury.

Although polymicrogyria has multiple aetiologies, common infective and genetic causes were excluded in case 1. There are a number of other reports of polymicrogyria in cases of TTTS (Delle Urban et al. 2004; Girard et al. 2003; Van Bogaert et al. 1996). Polymicrogyria in TTTS is thought to occur as a consequence of hypoxic-ischaemic insult towards the end of the neuronal migration period and during early cortical organisation (Barkovich 2010; Ferrer & Catalá 1991). We therefore categorise this finding in an ex-donor twin as a hypoxic-ischaemic lesion.

It is noted that in six of the seven findings of in utero brain injury, the laser procedure was performed at a GA greater than the cohort median of 21 weeks GA. Laser treatment has traditionally been

limited to fetuses between 16 and 26 weeks GA and the GA characteristics of our cohort are consistent with other larger outcome studies. Gestational age at treatment does not appear to influence fetal survival (Zikulnig et al. 1999) or longer term cognitive outcomes (Vanderbilt et al. 2014). However no studies have specifically examined whether cerebral injury is impacted by GA at treatment. Other factors such as the order in which anastomoses are coagulated (Chmait et al. 2014) and pre-operative cerebral blood flow may also have an impact. Future studies may investigate how fetal Doppler parameters, such as MCA pulsatility, prior to treatment relate to the likelihood of antenatal ischaemic or haemorrhagic brain injury.

The possibility that some of the injury identified in the current study may have been acquired before laser treatment cannot be excluded. Previous studies have commented on brain injury occurring before treatment is performed (Banek et al. 2003) or have specified that the incidence reported excludes lesions diagnosed before laser (Quarello et al. 2007). One study reports a 23% incidence of brain injury acquired in early gestation pre-treatment (Merhar et al. 2013). This study was limited by small sample size and it is unclear what severity of injury was included in the observation. In our cohort all fetuses underwent regular ultrasound surveillance pre-laser and had normal brain appearances at the time of laser treatment.

Further in utero brain injury occurring after MR imaging may not have been detected in the current study. Although all fetuses underwent regular ultrasound surveillance between MR imaging and delivery, some could have been missed on subsequent ultrasound screening. The findings reported here may therefore represent a slight underestimate of the total incidence of in utero acquired injury. In addition this study was not designed to capture perinatally acquired injury. The injury attributable to prematurity and delivery that is reported in many other studies has therefore been excluded.

In the majority of abnormal cases reported here, MR imaging provided additional information to that known from ultrasound examination. In two cases the most recent ultrasound examination had been normal. It is possible that in some cases injury may have progressed during the variable interval between ultrasound examination and MR imaging. It is acknowledged that our study was not designed to directly compare detection rates between MRI and ultrasound however other studies have also observed a higher MR detection rate compared to ultrasound for brain injury in MCDA pregnancies. Kline-Fath and colleagues (Kline-Fath et al. 2007) performed an MR study of 25 twin pairs with TTTS. Cerebral ischaemia was found in two fetuses with sonographically normal brains. Both fetuses in each pair died although it is unclear whether they had received any treatment prior to imaging. In a fetal MRI study of brain injury in spontaneous co-twin death (Jelin et al. 2008), 55% of abnormalities had not been apparent on ultrasound. The authors acknowledge that inferences

about the sensitivity of MRI are limited as the interval between ultrasound examination and subsequent MR imaging varied by up to several weeks. However they also point to several cases where the character of MRI appearances make it unlikely that injury occurred in the interval between imaging examinations. Merhar et al (Merhar et al. 2013) found that only one quarter of brain injury was diagnosed on cranial ultrasound compared to postnatal MRI. Cranial ultrasound was not part of the study protocol in that report and therefore imaging interval was not systematic and definitions of brain injury were not necessarily consistent. Most recently Griffiths and colleagues (2015) (Griffiths et al. 2015) report antenatal brain ultrasound and MR findings in a series of 68 single survivors of in utero death. In two thirds of cases with brain abnormalities, new findings were detected on MR examination that had not been apparent on ultrasound. Interestingly this discordance was more common in the group of survivors of co-twin fetal death following laser for TTTS compared to survivors of spontaneous co-twin death. The actual clinical significance of the novel MR findings, either in terms of neonatal imaging or developmental follow up, is not reported however.

The use of fetal MRI as an adjunct to diagnosis for ultrasound detected brain abnormalities in singletons is well established (Pistorius et al. 2008; Trompoukis et al. 2012; Whitby et al. 2004). Agreement between prenatal and postnatal MRI findings is high (Dhouib et al. 2011). Whilst fetal MRI is increasingly used as a research tool alongside ultrasound to detect brain injury in high-risk MCDA pregnancies (Hoffmann et al. 2013; Tarui et al. 2012), formal guidance for clinical use remains limited. The Royal College of Obstetrics and Gynaecology (RCOG UK) recommend fetal MRI of the survivor following spontaneous death of a co-twin (Neilson & Kilby 2008), however there is no international formal recommendation for routine fetal MRI following interventions such as fetoscopic laser for TTTS. MRI within four weeks of laser treatment should be incorporated into routine clinical practice in order to improve early identification of acquired brain injury.

Such early identification is important because it may provide an opportunity for earlier intervention. In cases of severe abnormality this may allow for earlier termination of pregnancy as occurred in three of our cases. In scenarios where parents opt to continue conservatively, improved identification of abnormalities provides more time to prepare for impending pregnancy loss or the possibility of an infant with neurodevelopmental impairment. This may lead to enhanced developmental screening to detect delay at the earliest opportunity. Early developmental intervention can have a positive influence on outcomes for infants as well as on parents' perceived ability to cope (Pighini et al. 2014; Spittle et al. 2007). Quantifying the impact of earlier additional information provided by MR imaging is challenging. It may be argued that any abnormalities of clinical significance will eventually progress to be obvious on ultrasound examination and therefore

MRI, whilst advantageous, is not necessary. However additional information provided earlier in pregnancy by MRI clearly aids both parents and clinicians.

On occasion MR may detect lesions of uncertain significance. These may present an additional burden to parents and clinicians. An example is the clinical impact of early DWI findings which must be interpreted with caution. In our series one fetus had evidence of grade I IVH on DWI that had resolved on neonatal MRI and the infant appears neurodevelopmentally appropriate at one year. A higher incidence of findings of ambiguous significance is a consequence of increased sensitivity in screening. Large studies correlating early DWI findings with conventional fetal MRI and neonatal follow up are lacking in the literature (Hoffmann et al. 2013). In such cases of early DWI abnormalities, it is crucial that expert perinatal neurology counselling and follow up is available. This should ensure that parents and clinicians do not make inappropriate decisions based on misinterpretation of clinical significance. If such support can be provided, then the burden of uncertainty is outweighed by the benefit of early detection of significant abnormalities.

3.1.6 Conclusion

Severe brain abnormalities were identified by MRI in five fetuses, representing 6.4% of our cohort of dual survivors of laser therapy for TTTS, with ex-donors at significantly greater risk. These findings suggest that MR imaging has a complementary clinical role in screening for brain injury in all cases of TTTS, not just those limited to survivors of spontaneous co-twin death as currently recommended. This could be performed from 14 days following the event provided the fetus is more than 20 weeks gestational age. MRI may be less informative below 20 weeks of gestational age as the fetal brain is immature, and motion artefact is a greater issue. Cases in which fetal MRI findings are of unclear clinical significance should have repeat MRI in four to six weeks. This would improve antenatal detection and definition of brain abnormalities with consequences for clinical management. The importance of dedicated centres for imaging with customized radiography protocols, expert interpretation and specialist perinatal neurology support for clinicians and parents has been highlighted.

3.2 Co-twin differences in cranial biometry

3.2.1 Background

Biometric analysis of the fetal brain is clinical standard practice in both obstetric sonography (Altman & Chitty 1997) and fetal MR imaging (Tilea et al. 2009). Biparietal diameter and head circumference are commonly used and are well-established as valid methods to estimate overall cerebral growth (Garel et al. 2003). TCD has been used as a standard measurement to estimate cerebellar growth with sonography (Altman & Chitty 1997; Goldstein et al. 1987). TCD may be a more reliable measure as its intracranial position offers protection from compressive effects of uterine crowding (Goldstein & Reece 1995). There is good agreement for measures of brain biometry between both ultrasound and MRI modalities (Garel & Alberti 2006; Tilea et al. 2009).

3.2.2 Aims

To evaluate differences in BPD, HC and TCD measured from MR images between ex-recipients and ex-donors.

3.2.3 Methods

MR images were acquired, reconstructed, reoriented and resampled as described in section 2.3 to produce complete high resolution datasets of the fetal brain. Biparietal diameter, head circumference and transcerebellar diameter were measured as described in section 2.3 according to the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG 2007) using ImageJ software.

3.2.3.1 Statistics

Simple linear regression and grouped linear regression were used with Pearson's correlation coefficient, as gestational age data were normally distributed.

3.2.4 Results

3.2.4.1 Cohort

Complete image sets from 29 twin pairs were used to assess intertwin discordance measures as described in Methods section 2.3.4. Median gestational age was 25.6 weeks (range 20.7 to 32 weeks). Figure 46 shows a flowchart of reasons for exclusion from analysis. MR findings were abnormal in one or both fetuses in six cases. Five of these cases had evidence of overt brain injury and were described in Chapter 3.1. In one additional case (case 6 – see table 9 section 2.2.3 and table 13 section 2.3.5) the ex-donor fetus had severe sFGR and the appearance of the brain was difficult to interpret. Therefore the entire case was excluded. In three cases images from one twin within a pair were unsuitable for measurement due to incomplete coverage or artefact over the skull (case 7 and 8 ex-recipients, case 12 ex-donor – see table 13 Methods section 2.3.5). In one case both fetuses had artefact over the cerebellum and were unsuitable for measurement (case 31 – see table 13 Methods section 2.3.5).

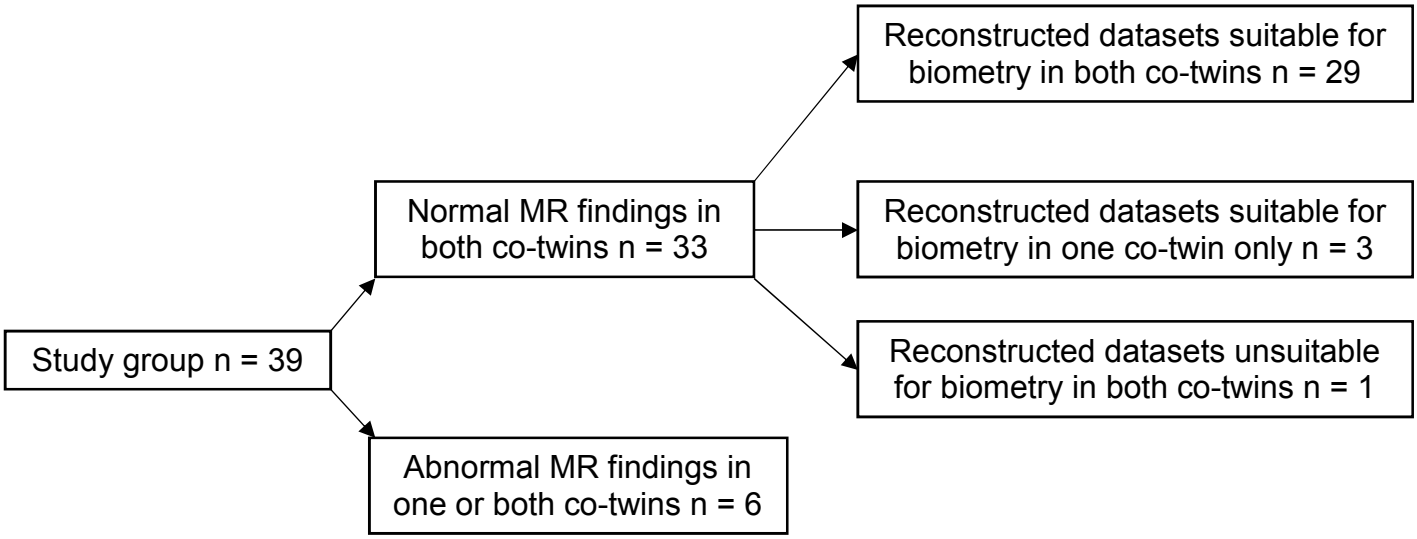


Figure 46. Flowchart of cases suitable for biometry analysis.

Twenty nine twin pairs had clinically normal MR findings and reconstructed images suitable for analysis.

3.2.4.2 Reproducibility

All measurements were performed by a single examiner (MTC) to exclude inter-observer variability. Each measurement was performed twice in ten cases to assess reproducibility. The ICC was calculated at 0.99 for all three measurements.

3.2.4.3 Correlation with gestational age

Biparietal diameter, HC and TCD all increased linearly with gestational age when assessed for all fetuses ($p < 0.001$ for all measures) with image datasets suitable for biometry. Figure 47 shows the linear regression line and r^2 values.

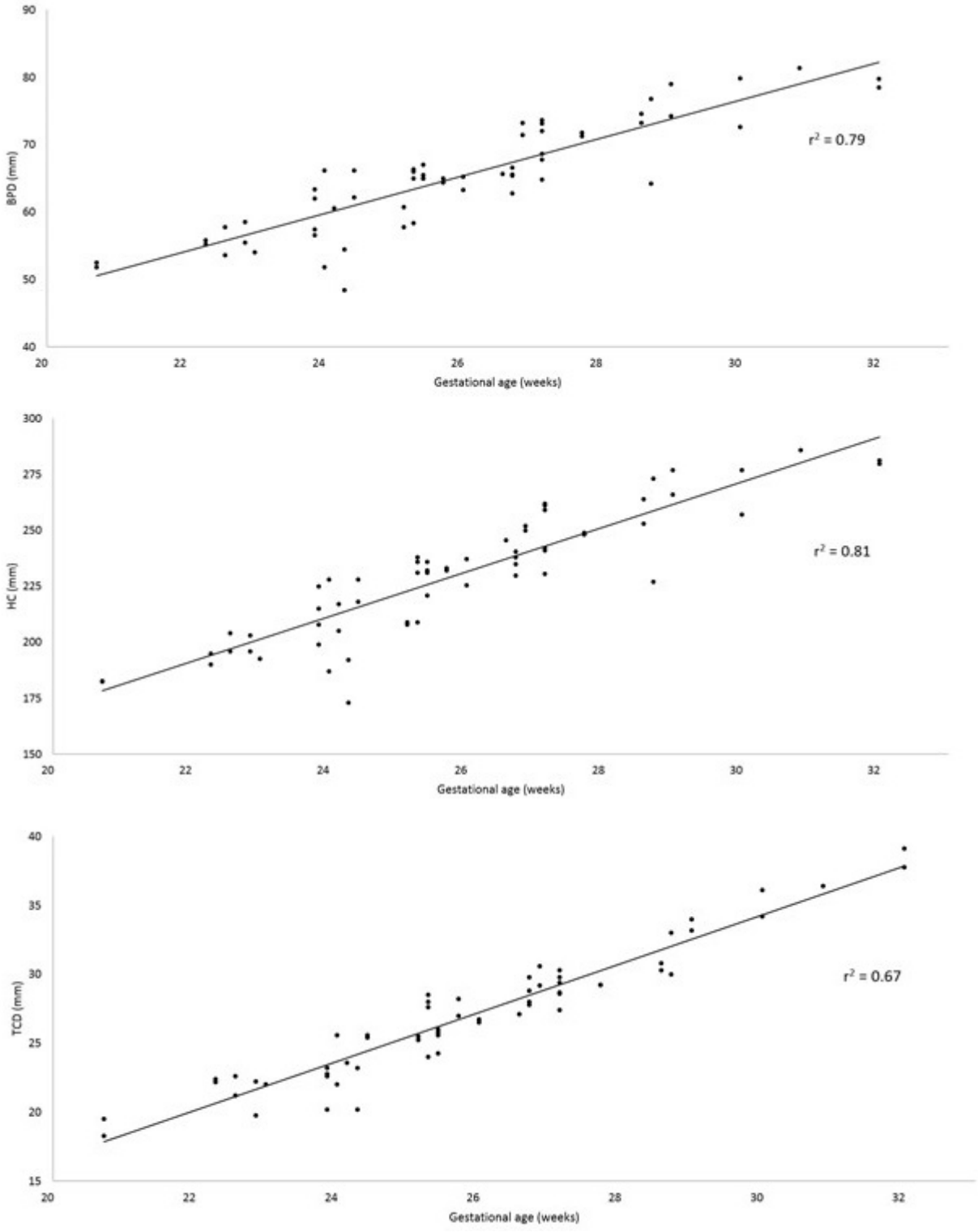


Figure 47. The relationship between linear brain measurements and GA in all twins treated for TTTS.
Biparietal diameter, HC and TCD increased significantly with gestational age ($p < 0.001$). (HC: head circumference; TCD: transcerebellar diameter).

Table 18 provides the r^2 values for BPD, HC and TCD against GA for ex-recipients and ex-donors separately. All measures showed a significant linear increase with gestational age. Ex-recipient fetuses showed less variability (higher r^2 value) than ex-donor fetuses, particularly for TCD measurements.

	BPD	HC	TCD
Ex-recipient	$r^2 = 0.87$ **	$r^2 = 0.88$ **	$r^2 = 0.94$ **
Ex-donor	$r^2 = 0.78$ **	$r^2 = 0.79$ **	$r^2 = 0.55$ **

Table 18. Correlation between linear measurements and GA for ex-recipients and ex-donors.
All measures showed a significant linear increase with gestational age. ** $p < 0.01$

3.2.4.4 Within twin pair comparisons

Figure 48 compares regression lines for BPD, HC and TCD against GA between ex-recipients and ex-donors. The rate of increase was similar between ex-recipients and ex-donors for all measures (BPD p = 0.9, HC p = 0.8, TCD p = 0.9). Biometry values were significantly lower in ex-donors compared to ex-recipient co-twins for all measures ($p<0.01$).

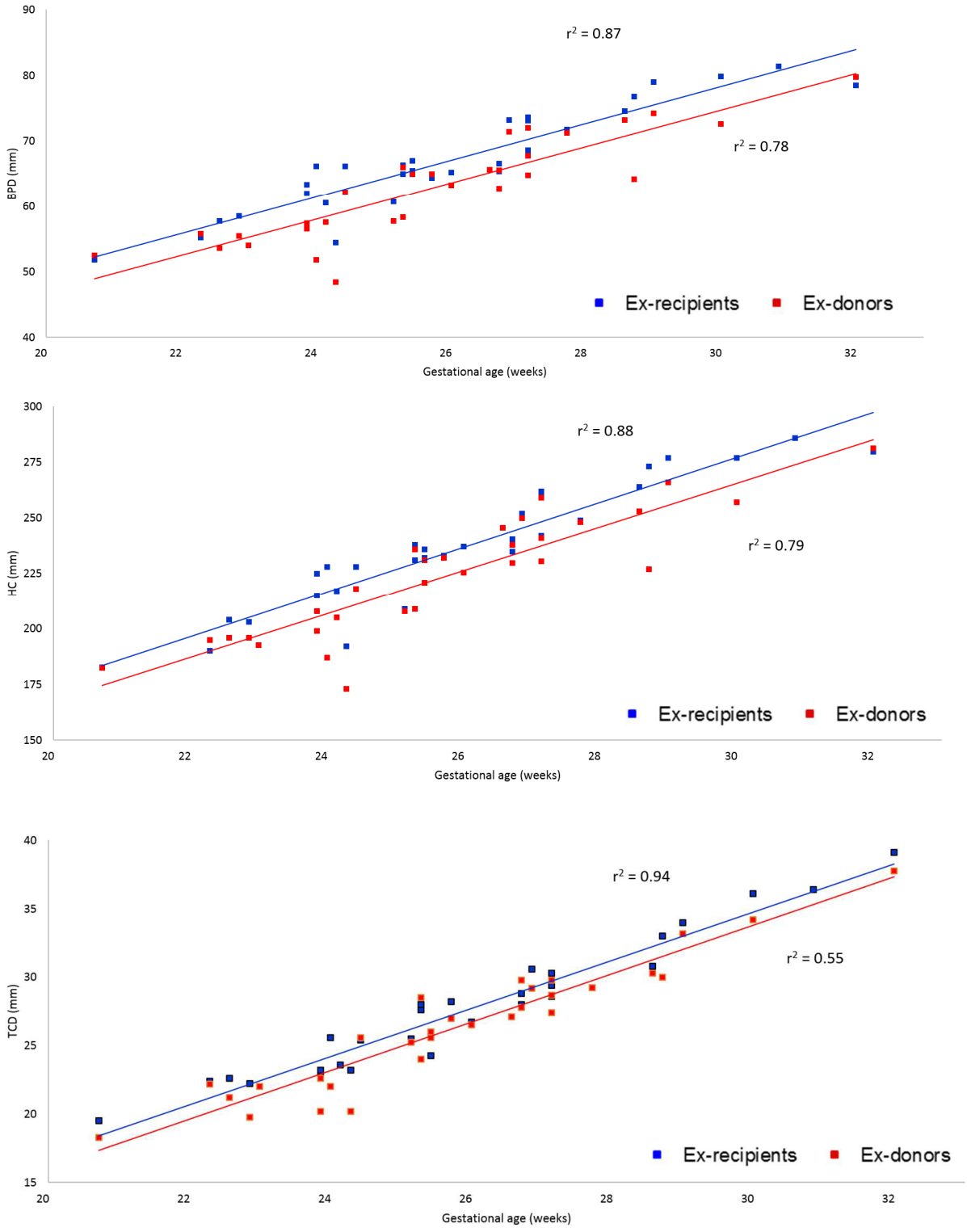


Figure 48. The relationship between linear brain measurements and GA in ex-recipients and ex-donors. Biparietal diameter, HC and TCD were significantly smaller in ex-donors compared to ex-recipients ($p < 0.01$).

There was strong correlation between individual linear measurements for all fetuses as shown in table 19.

	BPD and TCD	HC and TCD	BPD and HC
r^2	0.87 **	0.88 **	0.98 **

Table 19. Correlation coefficients between linear measures of BPD, HC and TCD.
All measures correlated well with each other (** $p < 0.001$).

Transcerebellar diameter was significantly greater in ex-donors compared to ex-recipients when corrected for BPD ($p<0.05$) as shown in figure 49. This difference in TCD was not statistically significant when corrected for HC ($p = 0.15$).

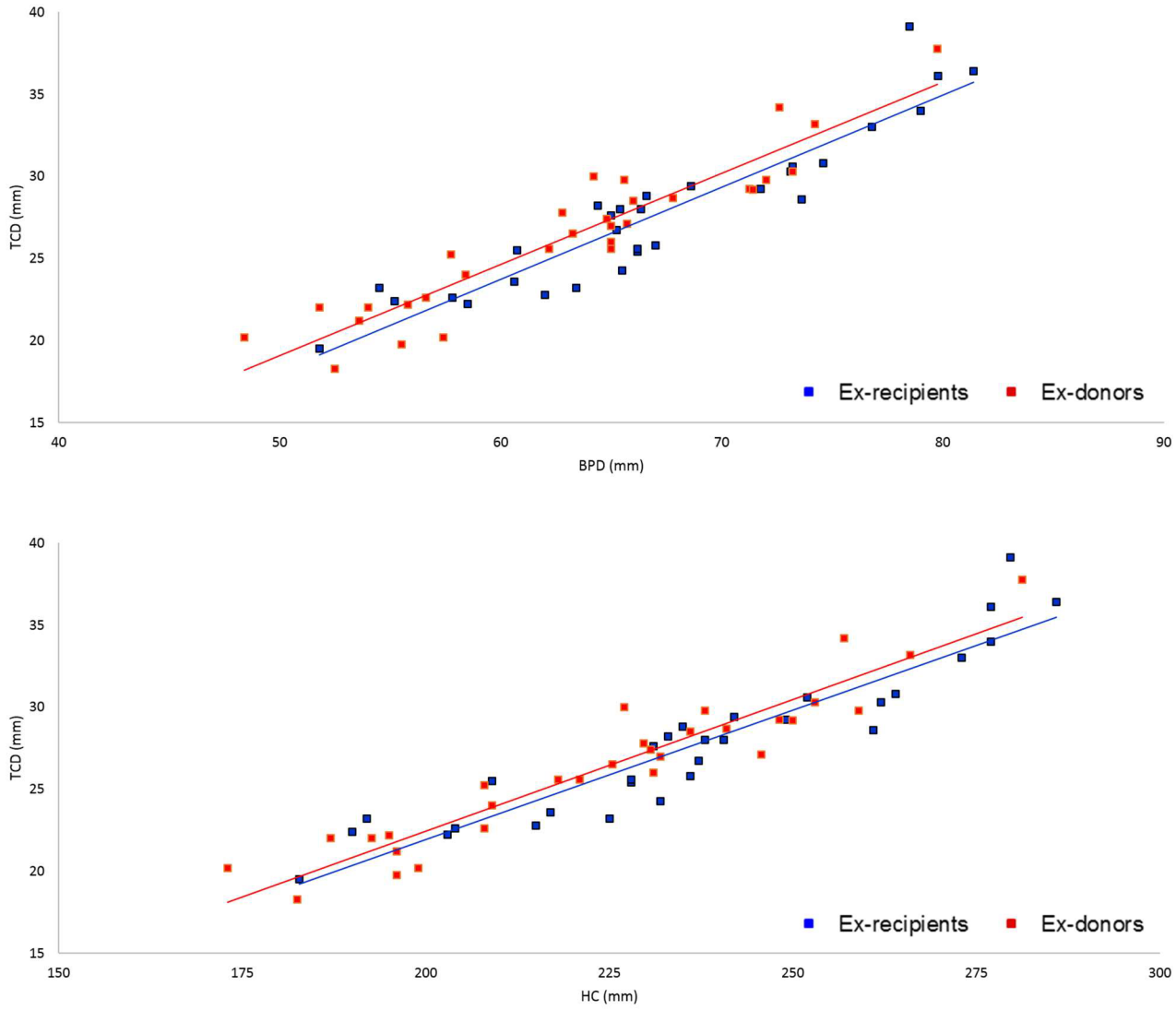


Figure 49. Differences in BPD-corrected and HC-corrected TCD between ex-recipients and ex-donors.
TCD was significantly larger in ex-donors compared to ex-recipients when corrected for BPD ($p < 0.05$) (shown in upper panel) but not significantly different when corrected by HC ($p = 0.15$) (shown in lower panel).

3.2.5 Discussion

In this chapter conventional biometric measures of brain growth have been shown to correlate well with gestational age in this cohort. All measures were significantly smaller in ex-donor twins relative to their ex-recipient co-twins, although growth pattern was similarly linear between 22 and 32 weeks GA for both twins. Transcerebellar diameter, when corrected for BPD, appeared greater in ex-donor twins relative to the ex-recipient co-twin, but not when corrected by HC.

As one might expect based on the frequency of growth restriction in donor twins (Chmait et al. 2008; Moreira de Sa et al. 2005), ex-donors had consistently smaller BPDs and HCs than ex-recipients. This finding is consistent with the only other report in the literature of differences in MRI measured cranial biometry in TTTS (Tarui et al. 2012). In this study both bone BPD and bitemporal diameter measured on a coronal slice were reported in 17 twins with TTTS with grossly normal brain appearances. Linear growth patterns in the second trimester in both twins were observed in line with our observations.

Biparietal diameter, head circumference and transcerebellar diameter correlated well with gestational age. There was greater variability compared to published reports of ultrasound based biometry in twins although this may be accounted for by the smaller sample size and pathology in this cohort. Correlation coefficients for all measures were similar to those reported in one other study of MRI brain biometry in TTTS (Tarui et al. 2012).

When analysed separately it became apparent that the majority of variability was seen in ex-donor co-twins. Variability was greater in ex-donors relative to ex-recipients across all measures, although the effect was most marked for TCD. The majority of donor twins are growth restricted prior to laser therapy and over half will have caught up by delivery implying accelerated growth relative to their co-twins (Chmait et al. 2008). Therefore our data will have represented ex-donor twins across a range of phases of catch up growth.

The observation of greatest variability in transcerebellar measurements is surprising. Previous reports of TCD correlation with gestational age in twin pregnancies report much higher r^2 values, even when selective growth restriction is present (Goldstein & Reece 1995; Goldstein et al. 2002). Whilst small TCD differences have been observed in growth discordant twins in some ultrasound based studies (Charlemaine et al. 2000; Lettieri et al. 1992), most report concordance within twin pairs irrespective of chorionicity or growth discordance (Goldstein & Reece 1995; Shimizu et al. 1992).

This has led some to suggest that TCD may be a more reliable measure for estimating gestational age later in pregnancy. Aside from its relatively protected position from the compressive effects of uterine crowding, it may also be more robust developmentally. The cerebellum has a well-established evolutionary status. Similar cerebellar circuits are seen in all classes of vertebrates (Bell et al. 2008) and whilst it is certainly important in motor control, its full role is yet to be fully understood. It demonstrates unusual plasticity and whilst the neocortex has reduced in size over recent human evolution, the cerebellum has increased (Weaver 2005). Furthermore, in growth restricted fetuses regional blood flow redistribution appears to favour the cerebellum over other cortical areas (Hernandez-Andrade et al. 2008) implying developmental prioritisation.

In the only existing study of cerebellar growth in TTTS, Tarui and colleagues found that TCD was smaller in ex-donor twins compared to co-twins, even when corrected by BTD. Based on growth rate analysis across the second trimester and in comparison with singleton control growth rates, these authors hypothesise that this discrepancy reflects accelerated growth in ex-recipient twin TCD relative to ex-donors and controls. This seems difficult to explain theoretically especially given the relatively stable growth observed in ex-recipients overall (Chmait et al. 2008). Their study had a small sample size and the authors do not comment on stage of disease or treatment status in their cohort which may have confounded some results. In addition their measurements were not performed on motion corrected images and so a small number of errors due to fetal motion with a small sample size may have had a significant impact on perceived linearity of relationships. In the current study, TCDs in ex-donors were larger than in ex-recipients when corrected for BPD but not significantly different when corrected for HC. Analysis of cerebellar volumes corrected by brain parenchymal volume may clarify the relationship between cerebellar and overall brain growth in TTTS. This question will be addressed as part of the next chapter.

3.2.6 Conclusion

In this chapter it has been shown that conventional brain biometry measured on MR images correlates well with gestational age in TTTS although variability is particularly high in ex-donors. BPD and HC were smaller in ex-donors relative to ex-recipients although it is unclear how the size of the cerebellum relates to overall brain growth. Further volumetric assessment may help clarify this relationship. A larger cerebellum relative to overall intracranial growth would support a theory of preferential protection of cerebellar development.

3.3 Measurement of intracranial volumes

3.3.1 Background

The second and third trimesters are a period of rapid fetal growth and development. Whilst much is controlled by genetic programming, alterations in the in utero environment may have far-reaching effects. Twin-twin transfusion syndrome provides a unique opportunity to examine the nature and magnitude of differences that can occur when the in utero environment is discordant between identical twins. Fetal brain volumetry is a novel technique that has not been applied to this population and will be used in this chapter to quantify within twin pair differences.

3.3.2 Aims

To establish whether there are differences in the growth and development of intracranial structures (supratentorial brain (STB) parenchyma, cerebellum, cortex, intracerebral and extracerebral fluid) within twin pairs treated for TTTS and compared to singletons.

3.3.3 Methods

MR images were acquired, reconstructed, reoriented and resampled as described in section 2.3. Semi-automatic volumetric segmentation of intracranial structures including STB tissue, lateral ventricles, cerebellum, cortical grey matter, intracerebral fluid, extracerebral fluid and intracranial volume was performed. Values from the post treatment TTTS cohort were compared within twin pairs as well as to values from singleton controls.

3.3.3.1 Statistics

Correlation between variables was assessed with Spearman's rank coefficient. Analysis of covariance was used to compare volumetric results between groups when corrected for GA. Wilcoxon signed ranks test was used to compare volumes within twin pairs.

3.3.4 Results

3.3.4.1 Singleton Control Cohort

The singleton control group data are part of an ongoing wider study establishing normal fetal intracranial volumetry reference data. A total of 72 fetuses imaged at less than 32 weeks were identified from the database. All had normal brain appearances on MRI. Fifty six were healthy volunteers and 16 had a previous fetus or infant diagnosed with a CNS or non-CNS abnormality. The reasons for referral in these 16 cases are detailed in table 20.

Polymicrogyria	Neonatal encephalopathy
Agenesis of the corpus callosum (2)	Schizencephaly
Pontocerebellar or cerebellar hypoplasia (4)	Otohara syndrome
Pontine cap dysplasia	Diaphragmatic hernia
Antenatal haemorrhage	Unexplained neonatal death
Lissencephaly	Aicardi syndrome

Table 20. Referral reasons in cases subsequently included as healthy controls.
Sixteen cases were originally referred for having had an abnormality previously diagnosed in a fetus or infant.

The median GA of the singleton control group was 26.5 weeks (range 21.5 to 32 weeks). The distribution is shown with the TTTS cohort for comparison in figure 50.

Volumetry data for brain parenchyma, cerebellum, intracerebral fluid and extracerebral fluid were available for all fetuses. Data were available for cortical volume analysis in a subset of 35 singleton control fetuses. The median GA in this sub-group was 26.9 weeks (range 22.4 to 32 weeks) and did not differ significantly to main cohort ($p = 0.417$).

There was no significant difference in GA at MRI between the singleton control and TTTS cohorts for either the complete groups ($p = 0.58$) or cortical volume subsets ($p = 0.21$).

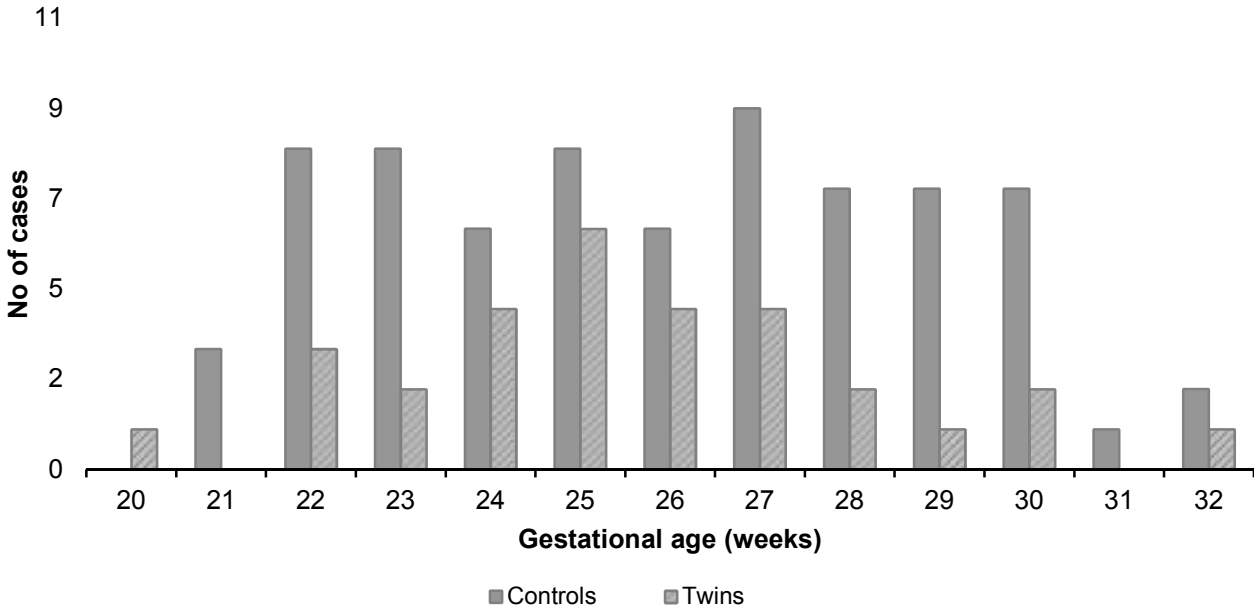


Figure 50. Histogram of GA distribution in singleton controls and twin pairs.
Gestational age was determined at first trimester dating ultrasound scan.

3.3.4.2 TTTS Cohort

During the study period 2008 to 2012 a total of 39 twin pairs were recruited. As shown in the flowchart below (figure 51), images from one or both twins within a pair were unsuitable for volumetric segmentation due to motion or other artefact following dataset reconstruction in three cases (case 7 and 8 ex-recipients, case 31 both fetuses artefact over cerebellum – see table 13 Methods section 2.3.5). Volumetric segmentation data from six twin pairs with abnormal MR findings in one or both co-twins were excluded (see figure 51) from within pair discordance statistical analysis but are illustrated for interest. Cases 1-5 were described in table 19 (Chapter 3.2.4) and case 6 was excluded as described in table 13 (Chapter 2.3.5). Only data for the ex-recipient is available in case 3 as the ex-donor images were too motion corrupted for reconstruction.

The median gestational age at MRI of the remaining 30 pairs was 25.6 weeks (range 20.7 to 32 weeks), see figure 50 for gestational age distribution. Median gestational age at laser treatment was 20.7 weeks (range 16 to 25 weeks). Median maternal age was 33.7 years (range 24.2 to 43.7 years).

Images were unsuitable for cortical volume segmentation in two fetuses (cases 12 and 35). Median GA of the remaining 28 pairs of TTTS twins was 25.6 weeks (range 22.3 to 32). There was no significant difference in GA between the subset and complete cohort (p = 0.3).

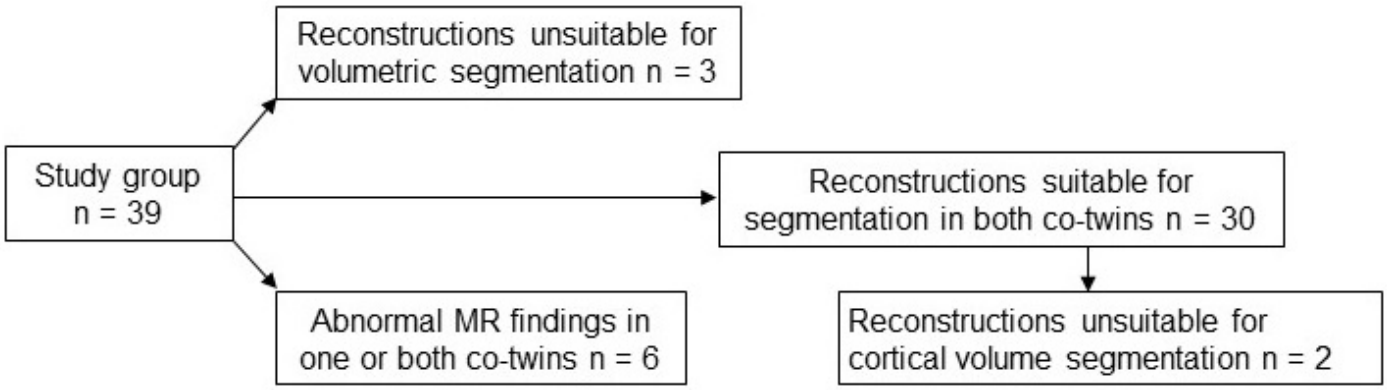


Figure 51. Flowchart of cases excluded from volumetric segmentation analysis.

Volumetric segmentations from cases with abnormal MR findings in one or both co-twins were excluded from analysis but are shown on graphs for interest.

3.3.4.3 Inter-rater Reliability

Inter-rater reliability was assessed between MTC, DV and VK for the reproducibility of 15 lateral ventricle volumes for the singleton control cohort. The mean difference and limits of agreement between examiners are shown in figure 52 below.

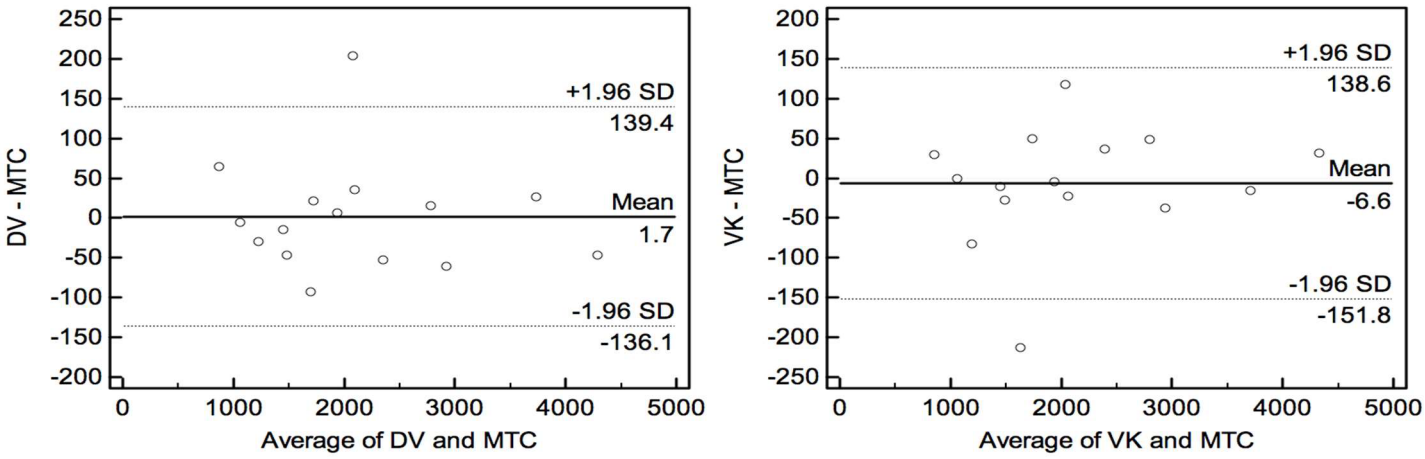


Figure 52. Bland-Altman plots for inter-rater reliability.

There was good agreement between both MTC and DV and MTC and VK.

3.3.4.4 Supratentorial brain tissue volume

Singleton controls

Total STB volume increased linearly with gestational age (Spearman's r=0.97, p<0.001) (Figure 53) from 42.34 cm^3 at week 22 to 172.45 cm^3 at week 32 at a rate of 13.0cm^3 per week (13.1%) (Table 21).

Comparison between ex-recipients and singletons

Total STB volume increased linearly with gestational age (Spearman's r=0.96, p<0.001) (Figure 53) from 41.79 cm^3 at week 22 to 172.36 cm^3 at week 32 at a rate of 13.1cm^3 per week (13.4%) (Table 21). Relative growth rate was not significantly different compared to singleton controls (13.4% vs 13.1% p = 0.95) and STB tissue volumes were similar (p = 0.85).

Comparison between ex-donors and singletons

Total STB volume increased linearly with gestational age (Spearman's r=0.95, p<0.001) (Figure 53) from 36.38 cm^3 at week 22 to 160.89 cm^3 at week 32 at a rate of 12.5cm^3 per week (13.9%) (Table 21). Relative growth rate was not significantly different compared to singleton controls (13.9% vs 13.1% p = 0.95) although ex-donors had significantly smaller GA corrected STB volumes compared to singleton controls (8.4%, p < 0.001).

Comparison within twin pairs

Relative growth rate was similar between co-twins (13.4% vs 13.9%; p = 0.54). Ex-donors had significantly smaller STB volumes compared to ex-recipient co-twins (7.84cm^3, 8.3%, p < 0.001) as shown in figure 53.

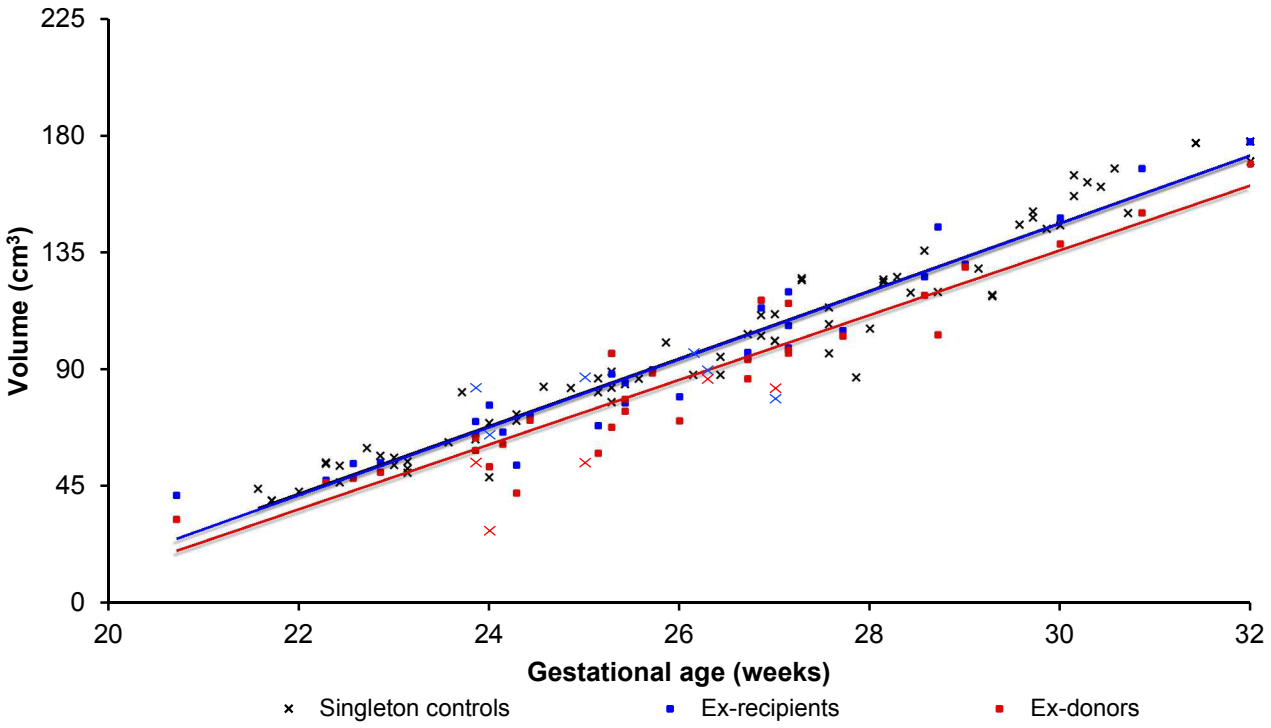


Figure 53. Supratentorial brain tissue volume in ex-recipient / ex-donor co-twins and singleton controls.
Ex-donor fetuses had significantly smaller STB volumes than both ex-recipient co-twins and singleton controls ($p<0.001$). Coloured crosses represent fetuses from cases with abnormal brain MR findings in one or both twins (blue : ex-recipient; red : ex-donor). From left to right these points represent data from twin pairs in cases 2,1,6,3 (ex-recipient only),5 and 4.

Correlation with biometry

BPD showed a strong correlation with STB volume in ex-recipients (Spearman's $r = 0.9$, $p<0.001$) and ex-donors (Spearman's $r = 0.91$, $p<0.001$). This linear relationship is shown in figure 54. Total STB volume was associated with higher BPDs in ex-recipient twins compared to ex-donors ($p < 0.05$).

Head circumference showed a strong correlation with STB volume in ex-recipients (Spearman's $r = 0.92$, $p<0.001$) and ex-donors (Spearman's $r = 0.92$, $p<0.001$). This linear relationship is shown in figure 55. Total STB volume was higher in ex-donors compared to ex-recipients for a given HC but this difference did not reach statistical significance ($p = 0.08$).

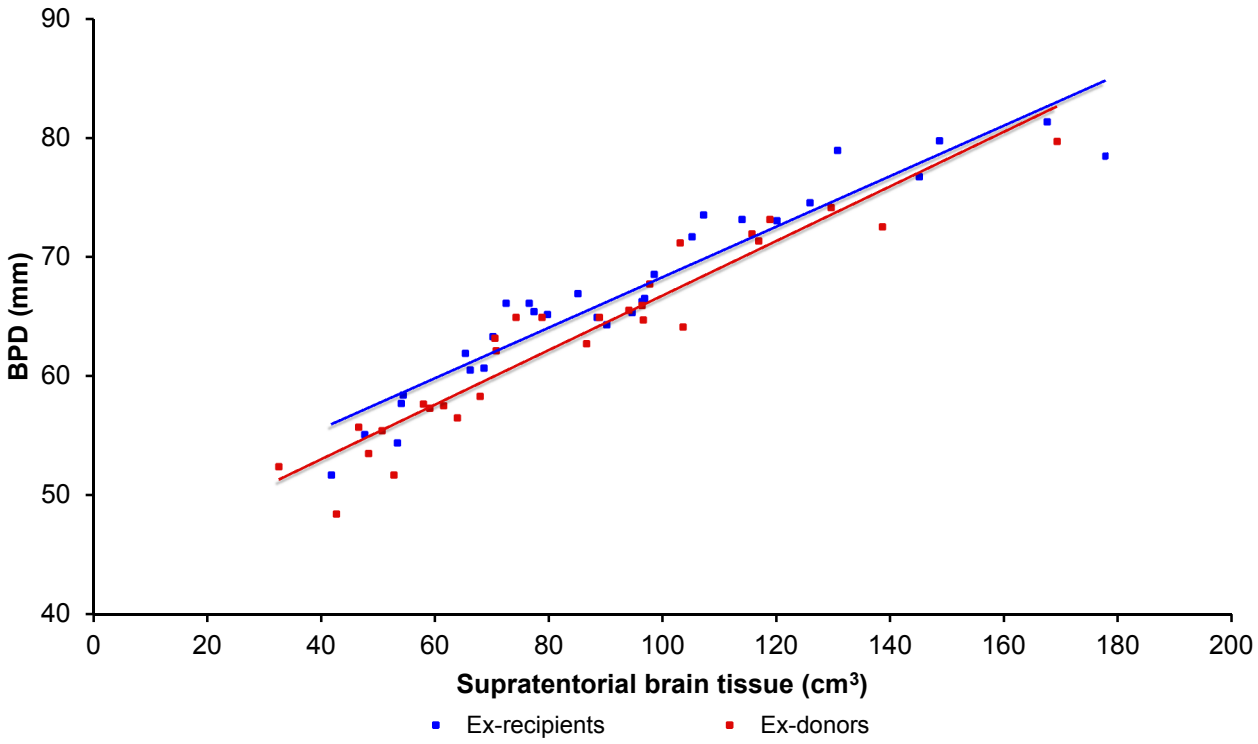


Figure 54. Relationship between biparietal diameter and total supratentorial brain tissue volume.
Biparietal diameter and total STB volume correlated well. STB volume was greater in ex-donors compared with ex-recipients at any given BPD ($p<0.05$).

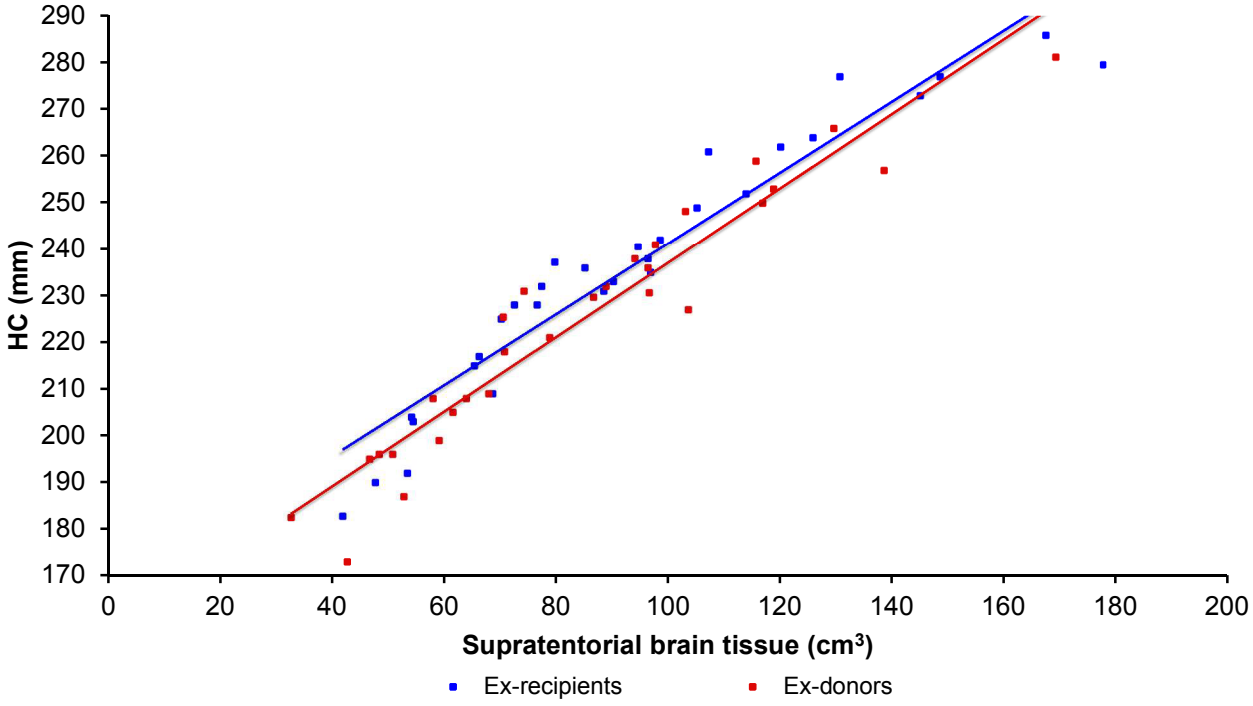


Figure 55. Relationship between head circumference and total supratentorial brain tissue volume.
Head circumference and total STB correlated well. There was no significant difference between ex-recipients and ex-donors ($p=0.08$).

3.3.4.5 Cerebellum

Singleton controls

Total cerebellar volume increased with gestational age (Spearman's r=0.97, p<0.001) (Figure 56) from 1.09 cm^3 at week 22 to 8.85 cm^3 at week 32 at a rate of 0.78cm^3 per week (17.1%) (Table 21). Goodness of fit was similar for linear, exponential and geometric functions so an exponential curve was selected based on visual fit.

Comparison between ex-recipients and singletons

Total cerebellar volume increased with gestational age (Spearman's r=0.96, p<0.001) (Figure 56) from 0.66 cm^3 at week 22 to 7.3 cm^3 at week 32 at a rate of 0.66cm^3 per week (18.9%) (Table 21). Goodness of fit was similar for linear, exponential and geometric functions so an exponential curve was selected based on visual fit. Ex-recipients had significantly smaller cerebellar tissue volumes than singleton controls across the age range studied, even when corrected for STB volume (0.89cm^3, 20.2% $p < 0.001$) (figure 57).

Comparison between ex-donors and singletons

Total cerebellar tissue volume increased with gestational age (Spearman's r=0.92, p<0.001) (Figure 56) from 0.43 cm^3 at week 22 to 6.69 cm^3 at week 32 at a rate of 0.63cm^3 per week (20.1%) (Table 21). Goodness of fit was similar for linear, exponential and geometric functions so an exponential curve was selected based on visual fit. Ex-donors had significantly smaller cerebellar volumes across the GA range studied compared to singleton controls, even when corrected for STB volume (1.27cm^3, 28.9%, $p < 0.001$) (figure 57).

Comparison within twin pairs

Ex-donors had significantly smaller cerebellar volumes compared to ex-recipient co-twins as shown in figure 56 (0.38cm^3, 11.5%, $p < 0.01$). When corrected for STB volume, cerebellar volumes were similar between ex-recipients and ex-donor twins ($p = 0.73$) (figure 57).

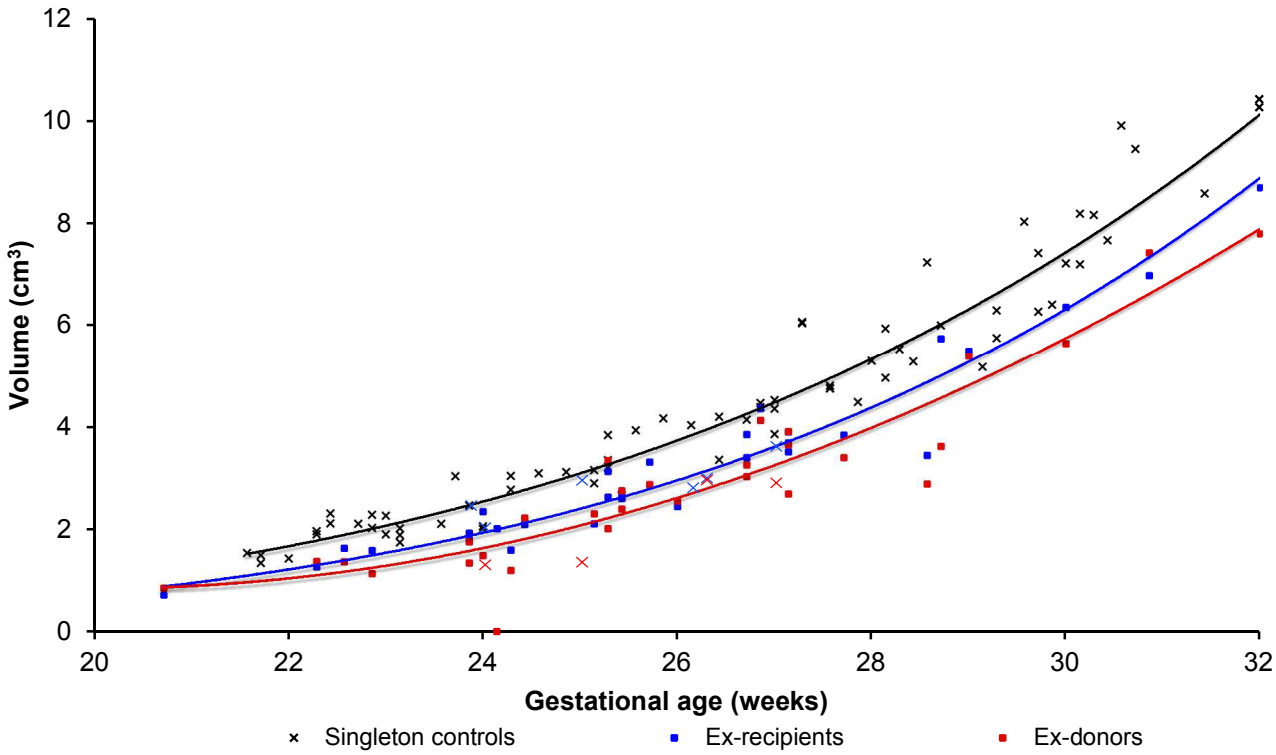


Figure 56. Cerebellar volume and GA in ex-recipient and ex-donor co-twins and singleton controls.
Cerebellar volume increased exponentially with gestational age for all fetuses ($p < 0.01$). Coloured crosses represent fetuses from cases with abnormal brain MR findings in one or both twins (blue : ex-recipient; red : ex-donor). From left to right these points represent data from twin pairs in cases 2 (ex-recipient only as ex-donor had no visible cerebellar tissue),1,6,3,5 and 4.

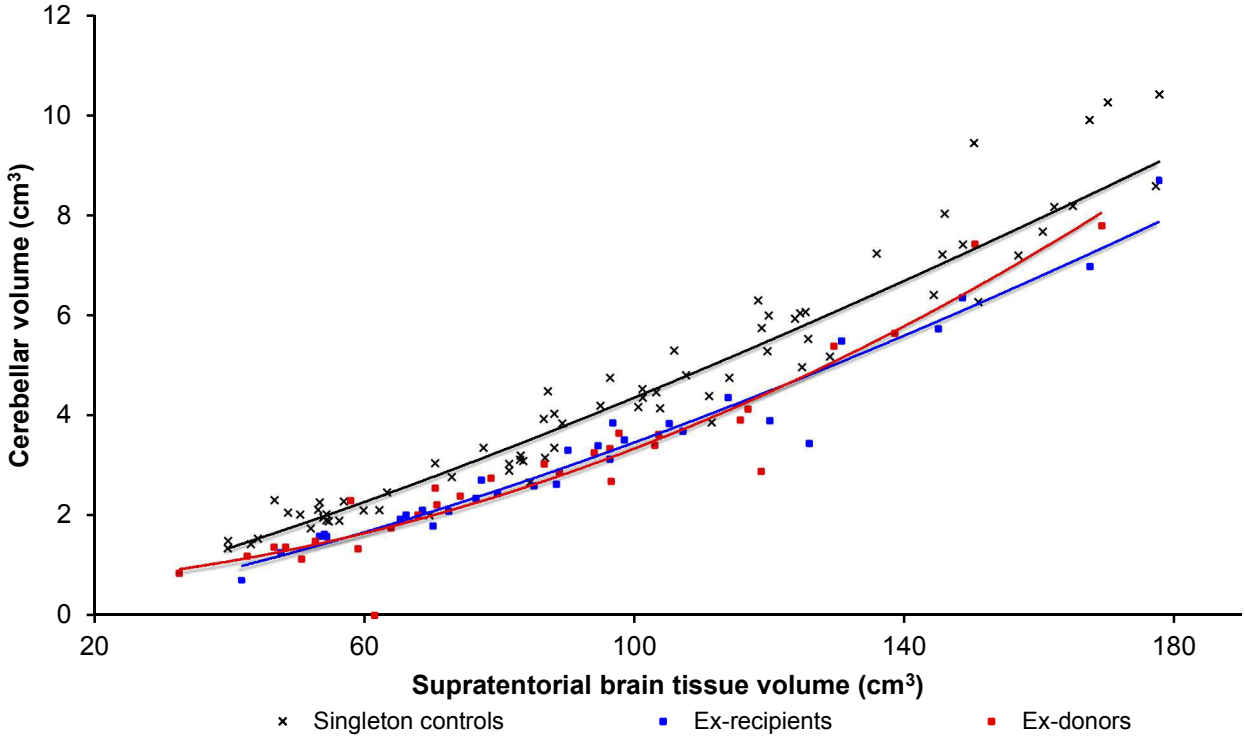


Figure 57. Cerebellar volume corrected by supratentorial brain tissue volume.
Corrected cerebellar volume was significantly smaller in twins treated for TTTS compared to singleton controls ($p<0.001$). There was no significant difference in corrected cerebellar volume between co-twins ($p = 0.73$).

Correlation with biometry

Cerebellar volume correlated strongly with TCD in both ex-recipients (Spearman's r = 0.94, p < 0.001) and ex-donors (Spearman's r = 0.91, p < 0.001). There was no significant difference in slope or separations between ex-recipient and ex-donor twins (p = 0.9) (see figure 58).

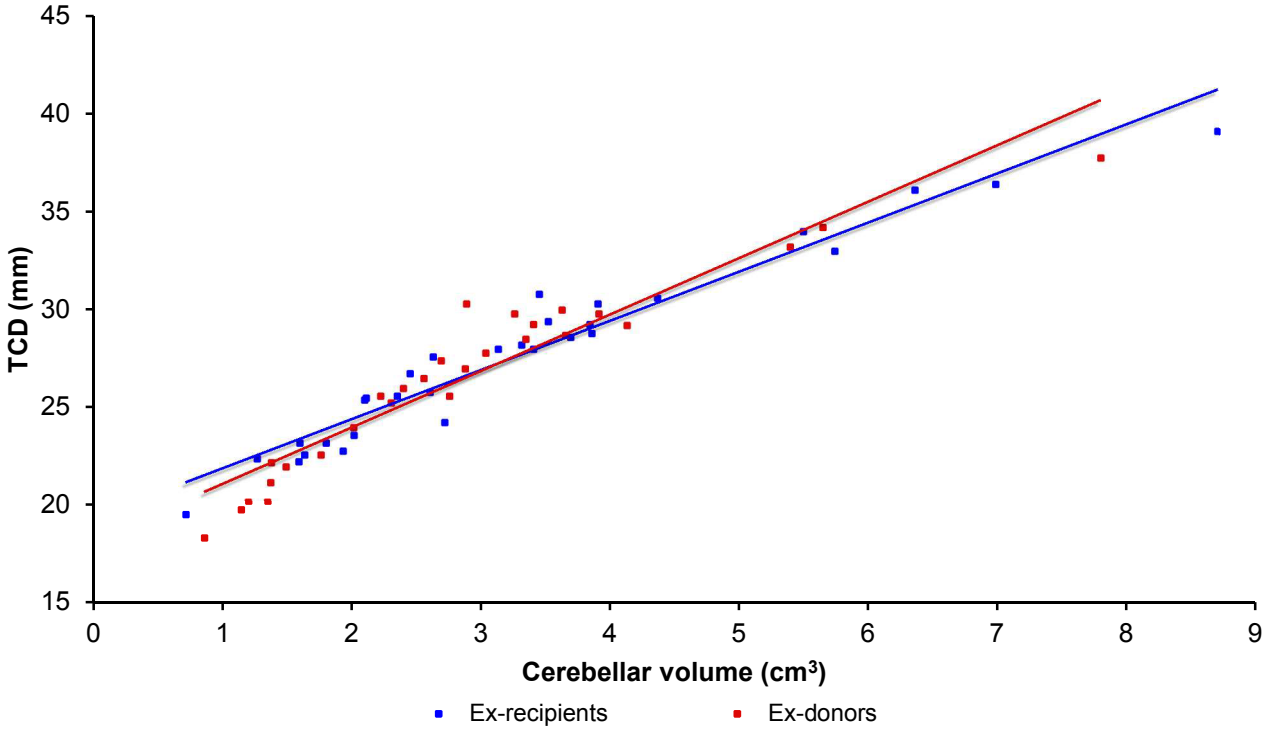


Figure 58. Relationship between transcerebellar diameter and cerebellar volume.

There was a strong correlation between TCD and cerebellar volume and no significant difference between ex-recipients and ex-donors.

3.3.4.6 Lateral Ventricles

Singleton controls

Total lateral ventricular volume increased with gestational age (Spearman's r=0.22, p<0.05) (Figure 59) from 2.68 cm^3 at week 22 to 3.64 cm^3 at week 32 at a rate of 0.10cm^3 per week (3.2%) (Table 21).

Comparison between ex-recipients and singletons

Total lateral ventricular volume showed a weak increase with gestational age that did not reach statistical significance (Spearman's r=0.31, p = 0.1) (Figure 59) (Table 21). There was no significant difference in total lateral ventricular volume between ex-recipients and singleton controls across the age range studied (p = 0.89).

Comparison between ex-donors and singletons

Total lateral ventricular volume increased with gestational age (Spearman's r=0.51, p<0.01) (Figure 59) from 2.12 cm^3 at week 22 to 3.96 cm^3 at week 32 at a rate of 0.18cm^3 per week (6.2%) (Table 21). There was no significant difference in total lateral ventricular volume between ex-donors and singleton controls across the age range studied (p = 0.39).

Comparison within twin pairs

Total lateral ventricular volume was not significantly different between ex-recipients and ex-donors within twin pairs (p = 0.43).

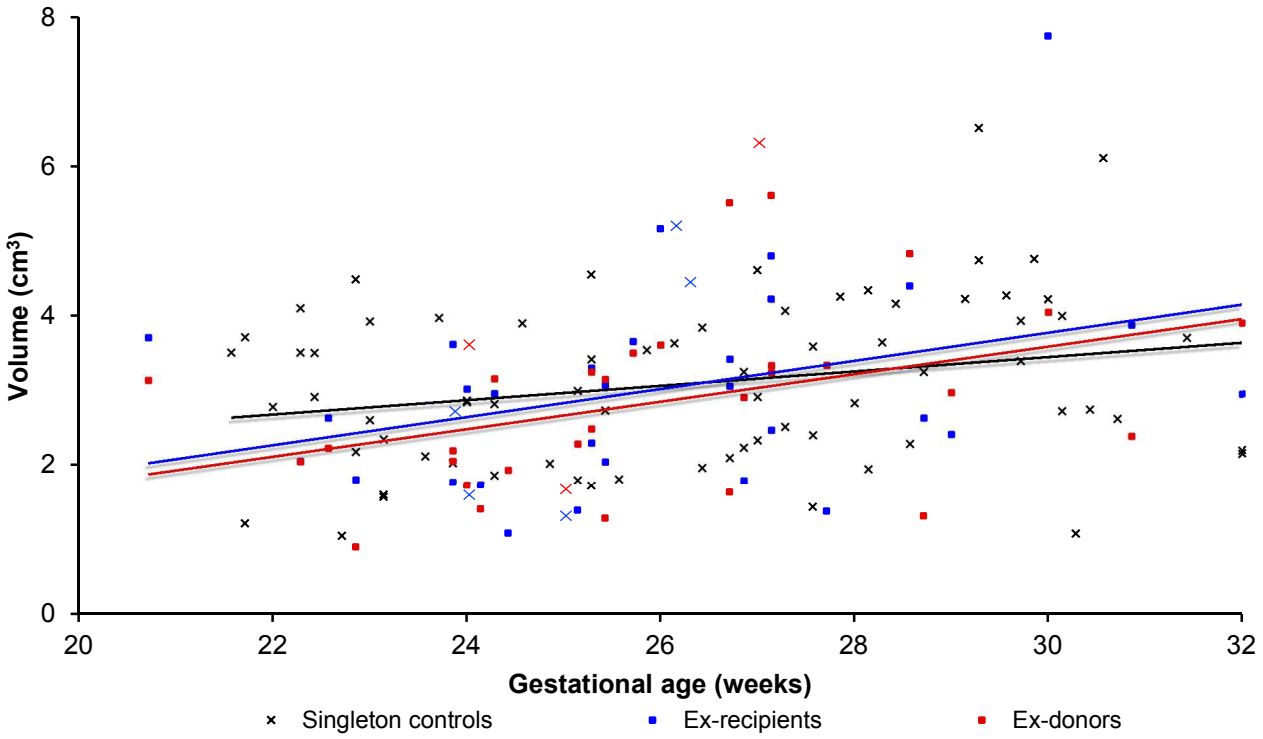


Figure 59. Lateral ventricle volume in ex-recipients, ex-donors and singleton control fetuses.

Total lateral ventricle volume increased with gestational age but was not significantly different between ex-recipients, ex-donors and singleton controls. Coloured crosses represent fetuses from cases with abnormal brain MR findings in one or both twins (blue : ex-recipient; red : ex-donor). From left to right these points represent data from twin pairs in case 2 (ex-recipient only as ex-donor had ventriculomegaly >10cm^3), 1, 6, 3 (ex-recipient only), 5 (ex-recipient only as ex-donor had ventriculomegaly >10cm^3) and case 4 (ex-donor only as ex-recipient had ventriculomegaly >10cm^3).

3.3.4.7 Cortical grey matter

Singleton controls

Total cortical grey matter volume increased with gestational age (Spearman's r=0.90, p<0.001) (Figure 60) from 7.55 cm^3 at week 22 to 35.70 cm^3 at week 32 at a rate of 2.82cm^3 per week (14.0%) (Table 21). Although the goodness of fit was similar for both linear and exponential curves, an exponential curve was selected as it appeared to fit the data better visually.

Comparison between ex-recipients and singletons

Total cortical grey matter volume increased with gestational age (Spearman's r=0.88, p<0.001) (Figure 60) from 3.84 cm^3 at week 22 to 32.83 cm^3 at week 32 at a rate of 2.90cm^3 per week (17.2%) (Table 21). The goodness of fit was similar for both linear and exponential functions however an exponential curve was chosen as it appeared to fit the data better visually. Ex-recipients had significantly smaller cortices than singleton controls across the age range studied (3.34cm^3, 16.5% $p < 0.001$), even when corrected for STB volume (3.38cm^3, 16.7%, $p < 0.001$) (figure 61).

Comparison between ex-donors and singletons

Total cortical tissue volume increased with gestational age (Spearman's r=0.94, p<0.001) (Figure 60) from 4.42 cm^3 at week 22 to 29.55 cm^3 at week 32 at a rate of 2.51cm^3 per week (15.9%) (Table 21). An exponential curve appeared to fit the data best. Ex-donors had significantly smaller cortical volumes across the GA range studied compared to singleton controls (4.43cm^3 , 21.9%, $p < 0.001$), even when corrected for STB volume (2.93cm^3 , 14.9%, $p < 0.001$) (figure 61).

Comparison within twin pairs

Ex-donors had significantly smaller cortical volumes compared to ex-recipient co-twins (0.98cm^3, 6.3%, $p < 0.01$). When corrected for STB tissue volume (figure 61), cortical volumes were similar between ex-recipients and ex-donor twins ($p = 0.45$).

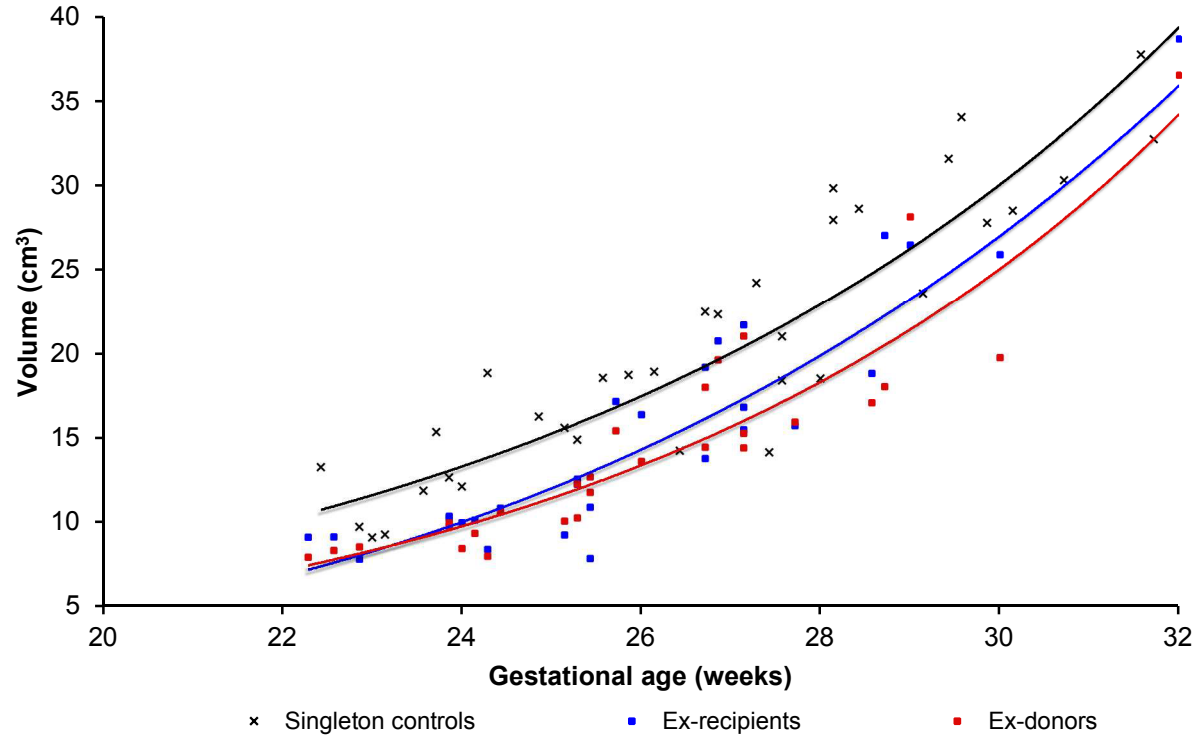


Figure 60. Cortical grey matter volume in ex-recipients, ex-donors and singleton controls.

Cortical grey matter volumes were smaller in twins treated for TTTS compared to singleton controls (p<0.001).

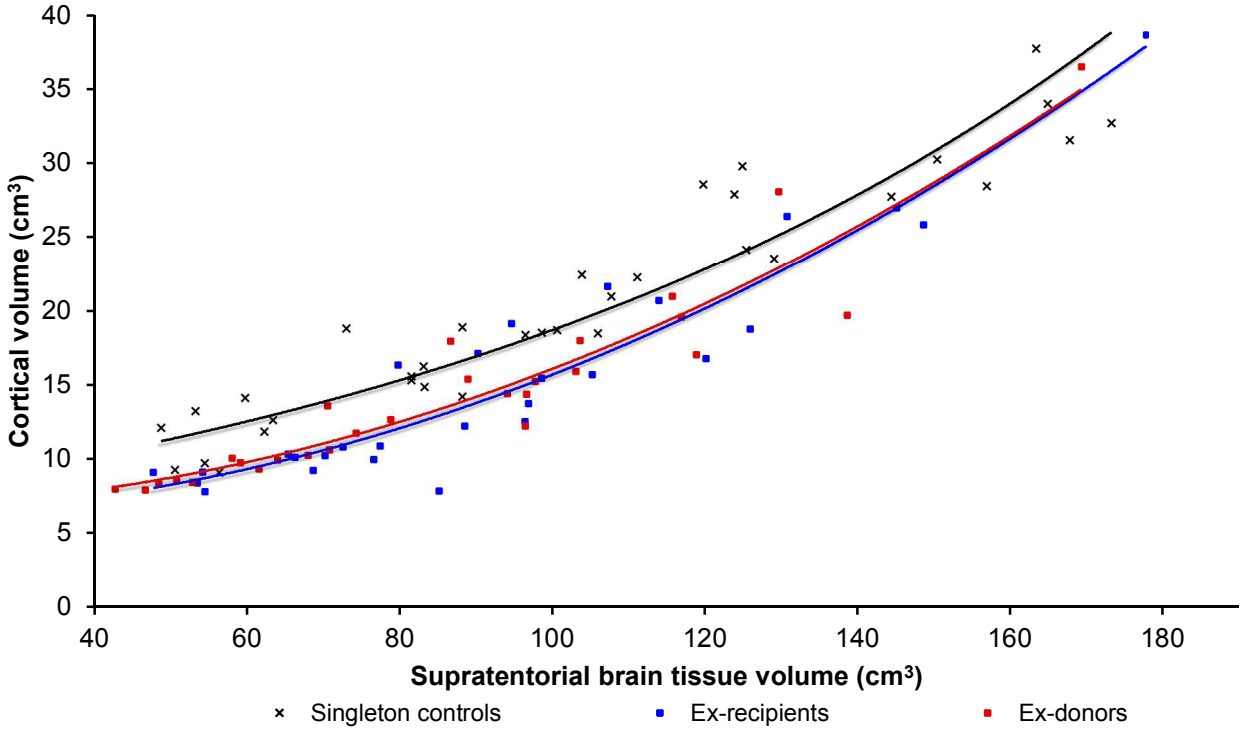


Figure 61. Cortical grey matter volume corrected by supratentorial brain tissue volume.

Corrected cortical grey matter volume was significantly smaller in twins treated for TTTS compared to singleton controls (p<0.001). There was no significant difference in corrected cortical grey matter volume between co-twins (p=0.45).

3.3.4.8 Intracerebral fluid

Singleton controls

Total ICF volume increased with gestational age (Spearman's r=0.52, p<0.01) (Figure 62) from 2.62 cm^3 at week 22 to 5.02 cm^3 at week 32 at a rate of 0.24cm^3 per week (6.5%) (Table 21).

Comparison between ex-recipients and singletons

Total ICF volume increased with gestational age (Spearman's r=0.46, p < 0.05) (Figure 62) from 2.27cm^3 at week 22 to 4.15cm^3 at week 32 at a rate of 0.19cm^3 per week (5.1%) (Table 21). There was no significant difference in ICF volume between ex-recipients and singleton controls across the age range studied (p = 0.94).

Comparison between ex-donors and singletons

Total ICF volume increased with gestational age (Spearman's r=0.60, p<0.01) (Figure 62) from 2.34 cm^3 at week 22 to 4.94 cm^3 at week 32 at a rate of 0.26cm^3 per week (7.4%) (Table 21). There was no significant difference in total ICF volume between ex-donors and singleton controls across the age range studied (p = 0.51).

Comparison within twin pairs

Total ICF volume was not significantly different between ex-recipients and ex-donors within twin pairs (p = 0.42).

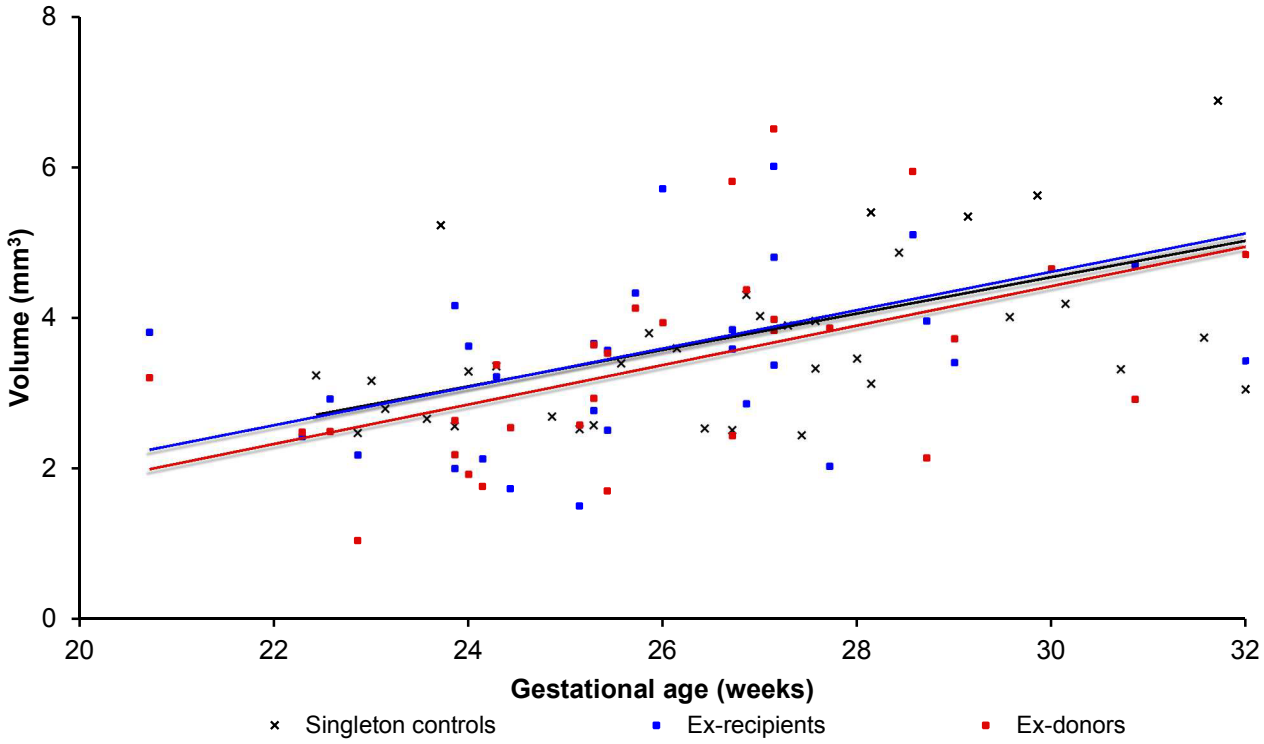


Figure 62. Total intracerebral fluid volume in ex-recipients, ex-donors and singleton control fetuses.
Total ICF volume increased with gestational age but was not significantly different between ex-recipient, ex-donor and singleton control fetuses.

3.3.4.9 Extracerebral fluid

Singleton controls

Total ECF volume increased linearly with gestational age (Spearman's r=0.92, p<0.001) (Figure 63) from 30.51 cm^3 at week 22 to 98.88 cm^3 at week 32 at a rate of 6.83cm^3 per week (11.3%) (Table 21).

Comparison between ex-recipients and singletons

Total ECF volume increased with gestational age (Spearman's r=0.91, p < 0.001) (Figure 63) from 28.64cm^3 at week 22 to 97.78cm^3 at week 32 at a rate of 6.90cm^3 per week (11.9%) (Table 21). There was no significant difference in ECF volume between ex-recipients and singleton controls across the age range studied (p = 0.43).

Comparison between ex-donors and singletons

Total ECF volume increased with gestational age (Spearman's r=0.80, p<0.01) (Figure 63) from 26.92 cm^3 at week 22 to 75.61 cm^3 at week 32 at a rate of 4.87cm^3 per week (10.1%) (Table 21).

ECF volumes were significantly lower in ex-donors compared to singleton controls across the age range studied ($p < 0.001$).

Comparison within twin pairs

Extracerebral fluid volume was significantly lower in ex-donors compared to ex-recipients within twin pairs ($p < 0.001$) by an average of 9.89cm^3 (17.6%).

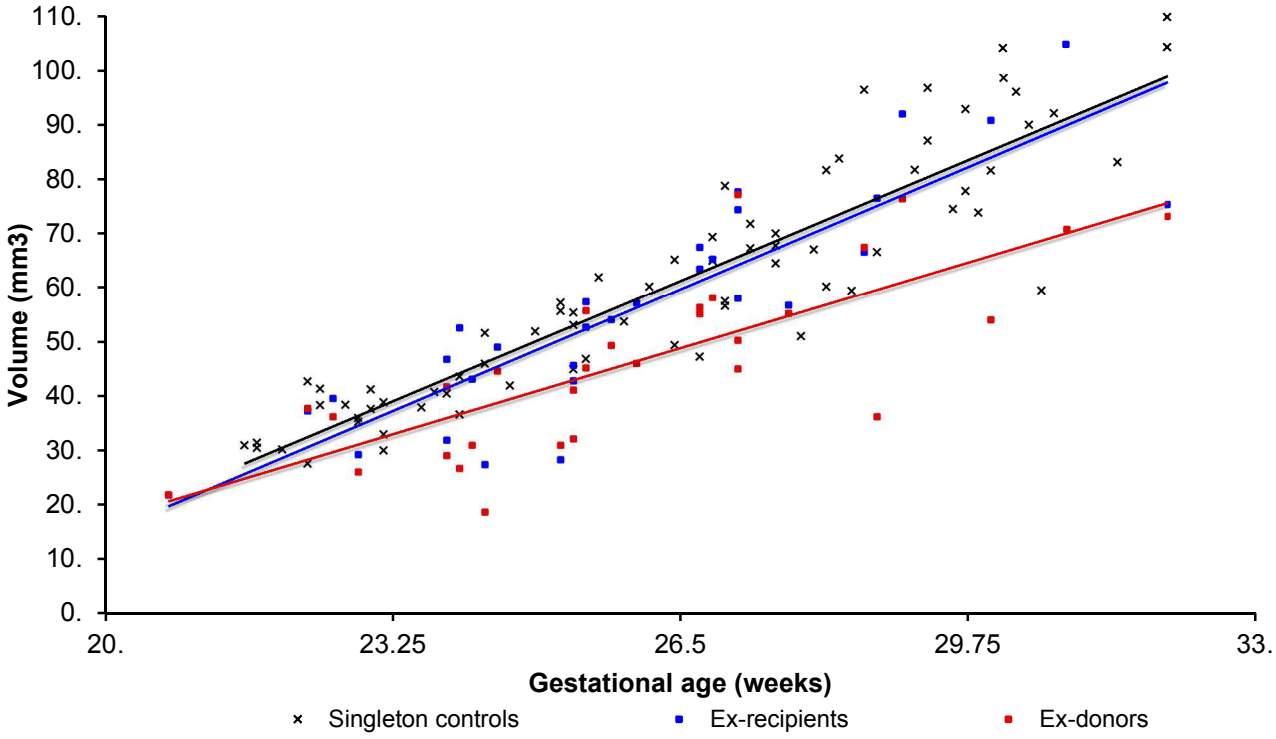


Figure 63. Total extracerebral fluid in ex-recipients, ex-donors and singleton control fetuses.

Total ECF increased with gestational age ($p<0.01$ for all groups). Total ECF was significantly lower in ex-donors compared to ex-recipients and singleton controls ($p<0.01$).

Singleton controls	STB	LV	Cerebellum	Cortical GM	ICF	ECF
Spearman's r	0.97**	0.22*	0.97**	0.90**	0.52**	0.92**
Relative growth rate (% per week)	13.1	3.2	17.1	14.0	6.5	11.3
Average volume (cm^3)						
At 22 weeks	42.35	2.68	1.09	7.55	2.62	30.51
At 26 weeks	94.39	3.07	4.19	18.81	3.58	57.87
At 30 weeks	146.43	3.45	7.29	30.07	4.54	85.20

Ex-recipients	STB	LV	Cerebellum	Cortical GM	ICF	ECF
Spearman's r	0.96**	0.31	0.96**	0.88**	0.46*	0.91**
Relative growth rate (% per week)	13.4	6.2	18.9	17.2	5.1	11.9
Average volume (cm^3)						
At 22 weeks	41.79	2.27	0.66	3.84	2.27	28.64
At 26 weeks	94.02	3.02	3.31	15.44	3.02	56.29
At 30 weeks	146.25	3.77	5.97	27.04	3.77	83.91

Ex-donors	STB	LV	Cerebellum	Cortical GM	ICF	ECF
Spearman's r	0.95**	0.51**	0.92**	0.94**	0.60**	0.80**
Relative growth rate (% per week)	13.9	6.2	20.1	15.9	7.4	10.1
Average volume (cm^3)						
At 22 weeks	36.38	2.12	0.43	4.42	2.34	26.92
At 26 weeks	86.18	2.85	2.93	14.47	3.38	46.41
At 30 weeks	135.98	3.59	5.44	24.52	4.42	65.87

Table 21. Correlation between GA and intracranial volumes in singleton controls, ex-recipients and ex-donors. (STB supratentorial brain, LV lateral ventricle, ICF intracerebral fluid, ECF extracerebral fluid) * $p < 0.05 > 0.01$; ** $p < 0.01$

3.3.4.10 Total fetal volumetry in post treatment TTTS twins

3.3.4.10.1 Cohort

Images were suitable for segmentation of the whole fetal body in both twins within a pair in 21 cases. Median GA at MRI was 26 weeks (range 22.3 to 32 weeks). The median GA at treatment was 20.6 weeks (range 16 to 25 weeks). Median maternal age was 33.2 years (range 24.2 to 43.7 years). There was no significant difference between the whole fetal body sub-group and the main TTTS cohort in gestational age at MRI (p =0.77), gestational age at treatment (p = 0.79) or maternal age (p = 0.71).

3.3.4.10.2 Correlation with gestational age and within pair differences

Whole fetal volume increased with gestational age in both ex-recipients and ex-donors. The goodness of fit was similar for both linear and exponential curves (both r^2 = 0.88) in ex-recipients. Goodness of fit for ex-donors was slightly better for a linear compared to exponential function (exponential r2 = 0.64; linear r2 = 0.67). Mean growth rate was 103ml/week in ex-recipients and 91ml/week in ex-donors (p = 0.5). Ex-donor fetuses had significantly smaller whole fetal volumes than their ex-recipient co-twins (mean difference 160cm^3 se mean 34cm^3; p < 0.001) (figure 64).

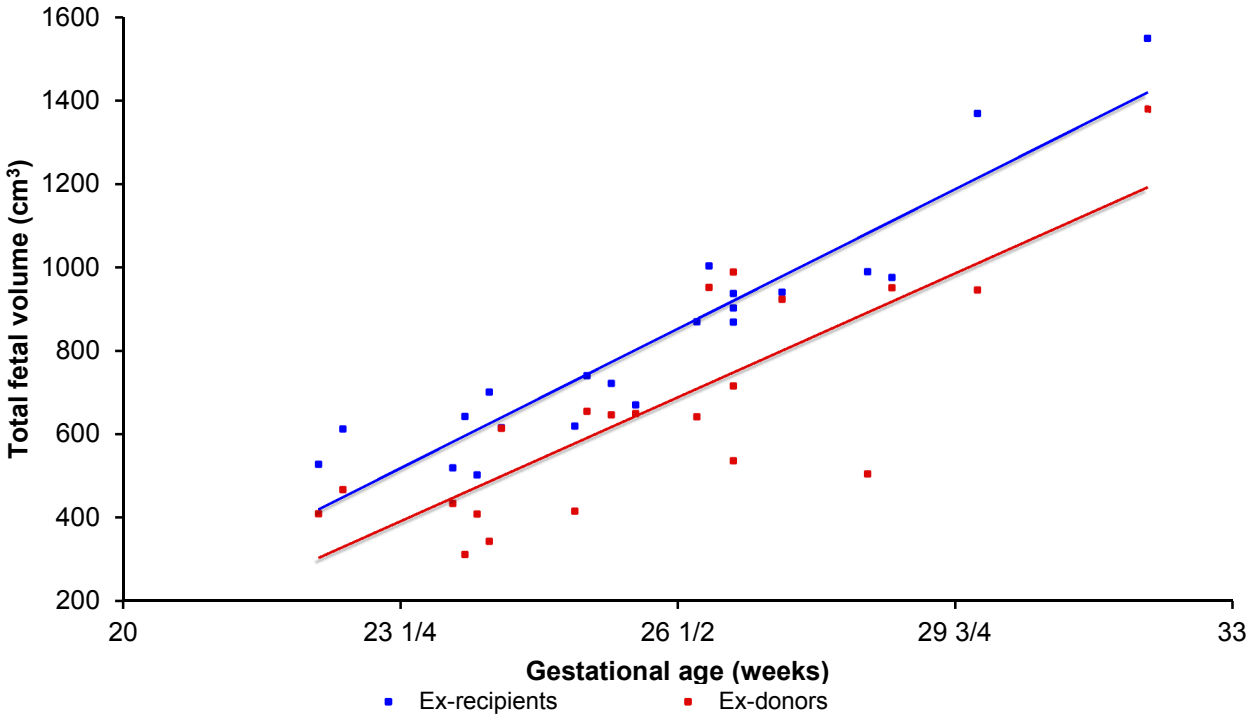


Figure 64. Relationship between total fetal volumetry and GA in ex-recipient and ex-donor fetuses.

Whole fetal volume was significantly smaller in ex-donor fetuses compared to their ex-recipient co-twins (p<0.001).

3.3.4.10.3 Intracranial volumetry relative to total fetal volume

Intracranial volume increased linearly with gestational age in ex-recipient fetuses ($r^2 = 0.91$, $p < 0.001$) and in ex-donor fetuses ($r^2 = 0.85$, $p < 0.001$). Mean rate of increase was 20.3ml/week in ex-recipients and 17.5ml/week in ex-donors with no significant difference in growth trajectory between co-twins ($p = 0.13$). Ex-recipients had significantly larger intracranial volumes than ex-donors within twin pairs ($p < 0.001$).

Intracranial volume correlated strongly with total fetal volume in both ex-recipient twins ($r^2 = 0.82$, $p <0.001$) and ex-donor twins ($r^2 = 0.90$, $p <0.001$) (see figure 65). As a proportion of total fetal volume, the intracranial volume was significantly greater in ex-donors (21.3%) compared to ex-recipient co-twins (19.3%, $p<0.001$). This proportion did not appear to vary systematically with gestational age (ex-recipients $r^2 = 0.03$, p 0.4 and ex-donors $r^2 = 0.0001$, $p = 0.97$), interval between treatment and MRI or gestational age at treatment.

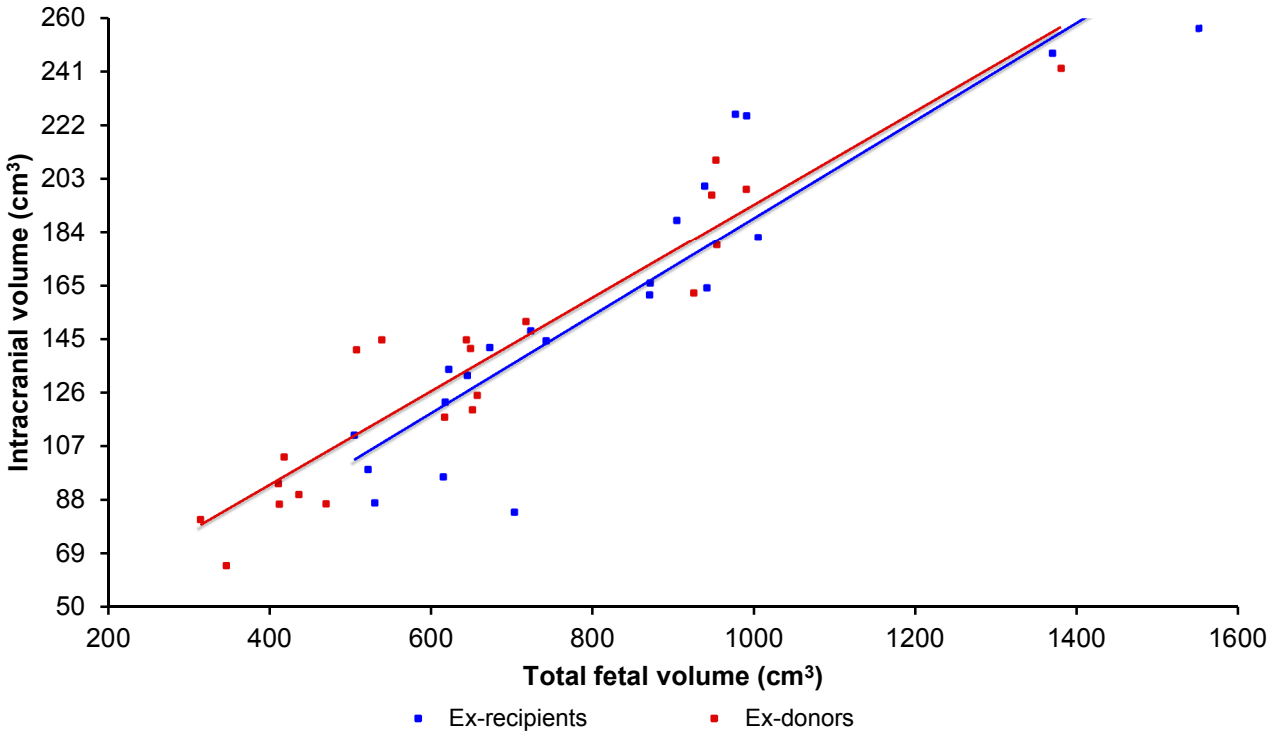


Figure 65. Relationship between intracranial volume and total fetal volume in ex-donors and ex-recipients. As a proportion of total fetal volume, the intracranial volume was significantly greater in ex-donors compared to ex-recipient co-twins.

Supratentorial brain tissue volume (STB parenchyma excluding all intracerebral fluid) was significantly greater in ex-donors compared to ex-recipient co-twins when corrected by intracranial

volume ($p < 0.05$), as shown in figure 66. In contrast corrected extracerebral fluid volume remained relatively reduced ($p < 0.05$) (see figure 67).

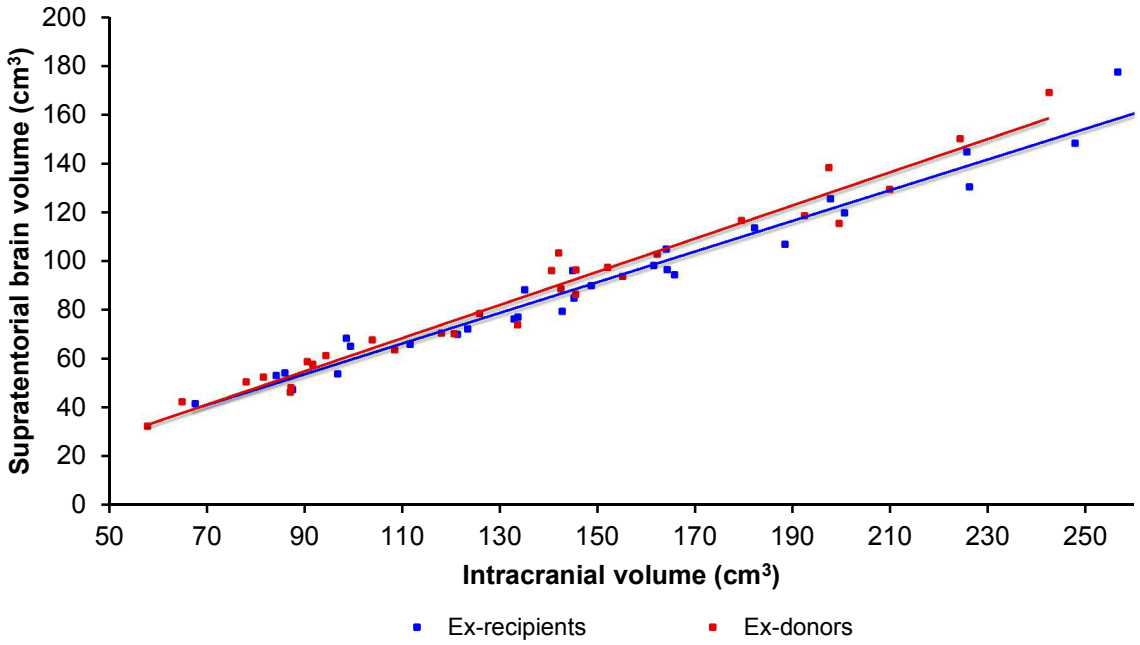


Figure 66. Relationship between supratentorial brain tissue and intracranial volume in ex-recipients and ex-donors.

Supratentorial brain tissue formed a greater proportion of the intracranial volume in ex-donors compared to ex-recipient co-twins ($p < 0.05$).

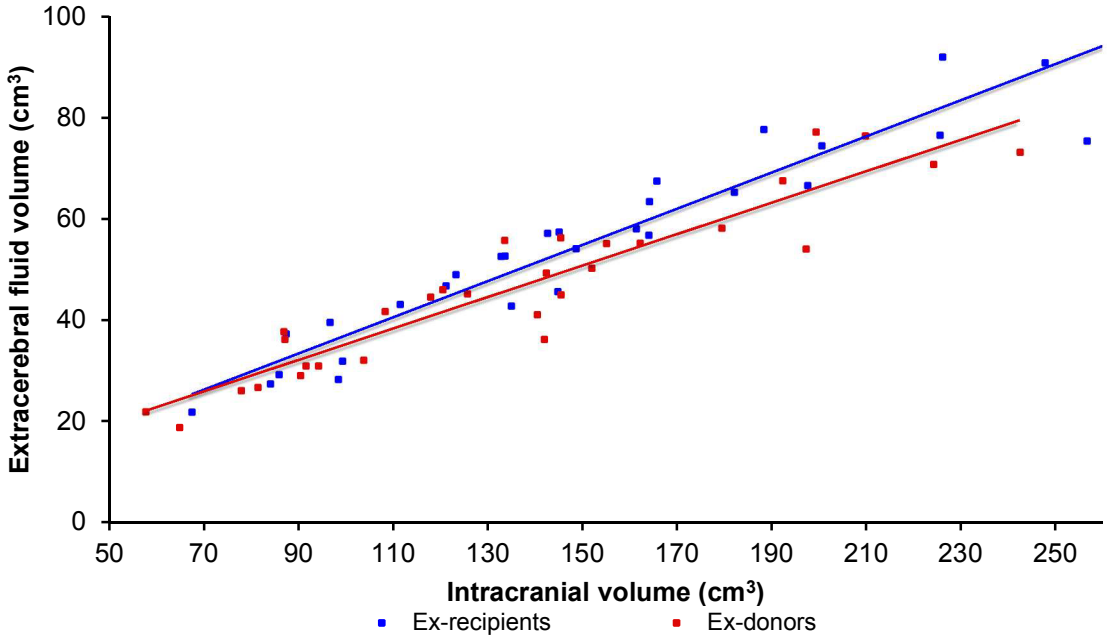


Figure 67. Relationship between extracerebral fluid volume and intracranial volume in ex-recipients and ex-donors.

Extracerebral fluid formed a smaller proportion of the intracranial volume in ex-donors compared to ex-recipient co-twins ($p < 0.05$).

3.3.5 Discussion

In this chapter the successful application of 3D image reconstruction with motion correction to produce volumetric datasets in an abnormal twin population has been demonstrated. Both STB volume and ECF volume were smaller in ex-donor fetuses compared to both ex-recipient co-twins and age matched singleton controls, which had similar volumes. When corrected for overall fetal body volume however, ex-donor fetuses had proportionately larger intracranial volumes than their co-twin. This was accounted for by significantly greater corrected STB volumes. Corrected ECF volumes remained smaller in ex-donors compared to their co-twins. Corrected cortical grey matter and cerebellar volumes were similar within twin pairs and significantly smaller in both ex-recipients and ex-donors compared to singleton controls.

Supratentorial brain tissue volume

In the singleton control group STB volume increased at a relative growth rate of 13.1% per week. This is comparable to other growth rates of 10 to 15% reported in the literature (Clouchoux et al. 2011; Kyriakopoulou et al. 2014; Scott et al. 2011). The estimated STB volume at 26 weeks of 94cm^3 in this study is consistent with other estimates of 77 - 88 cm^3 at 25 weeks (Clouchoux et al. 2011; Rajagopalan et al. 2011) and 85-104 cm^3 at 26 weeks (Scott et al. 2011; Kyriakopoulou et al. 2014). Other studies have reported total brain volume, which included the cerebellum and brainstem, of 104cm^3 at 26 weeks (Limperopoulos et al. 2011) and 85-90cm^3 at 25 weeks (Clouchoux et al. 2011; Gholipour et al. 2011).

Growth rates were similarly linear and correlated strongly with gestational age across normal singletons, ex-recipients and ex-donors. No significant difference in STB tissue volume was detected between ex-recipient twins and normal singletons. However STB tissue volumes were 8% smaller in ex-donors compared to their ex-recipient co-twins. Whilst no studies have reported STB tissue volume differences in utero between twins and singletons, total cortical volume has been shown to be similar in the first month of life between healthy MZ twins and singletons (Knickmeyer et al. 2011), despite significantly lower birthweights in MZ twins. The finding of a reduction in brain volume in ex-donors relative to their co-twin in this chapter suggests that the pathophysiology of TTTS has a disproportionate effect on brain growth in the donor twin, preventing it from achieving its genetic growth potential.

When adjusted for whole fetal body volume, the ex-donor STB was in fact proportionately larger than its co-twin in this cohort. The proportion of the fetal body occupied by STB tissue was 13.5%

in ex-donors and 11.6% in ex-recipients and this difference was statistically significant within twin pairs. This may reflect a brain sparing mechanism. One other study has measured fetal body and STB tissue volume ratios from MRI datasets in growth restricted fetuses compared to normal singletons (Damodaram et al. 2012). These authors found no significant difference in absolute brain tissue volumes between groups. Supratentorial brain tissue volume contributed 11% of fetal body volume in normal singletons in that study and this is similar to the proportion that was measured in ex-recipients in the current cohort. Interestingly STB tissue volume formed 18% of fetal body volume in growth restricted fetuses, which was much higher than the 13.5% proportion that we found in our ex-donor twins. In addition a higher fetal brain to body volume ratio predicted poor perinatal outcome in these growth restricted fetuses.

One other study reports total brain volume comparisons in late onset FGR relative to AGA fetuses (Egana et al 2013) with smaller absolute and weight corrected volumes in the pathology group. However the methodology makes the findings hard to interpret. The diagnostic criteria for the late onset FGR population were an estimated and postnatally confirmed weight below the 10th centile with normal UA pulsatility index findings. The control group were normal term fetuses above the 10th centile. This suggests that the study therefore was actually a comparison between otherwise healthy SGA and AGA fetuses. However reference is made in a sub-analysis to positive and negative FGR within the SGA group where positive FGR includes fetuses with abnormal CPR or uterine artery Dopplers. Sample size is not reported for subgroups and the figure and text are contradictory with respect to findings of significant differences. This is important as one might expect otherwise normal SGA fetuses to have constitutionally smaller brain volumes. Furthermore the authors report that total brain volumes were significantly smaller when adjusted for birthweight centile implying disproportionately small volumes in the abnormal population. This is hard to understand, particularly as reported differences in absolute brain volumes between groups are in the order of 15% whereas the mean birthweight centile difference between groups was over 50%. Finally, total brain volume in this study included intracerebral fluid as well as the cerebellum and brainstem. Inclusion of lateral ventricular volume contributes significant variability as will be discussed later. This study also used 3.5mm thick image slices without motion correction introducing further error into measurements.

A relative reduction in brain volume with increasing gestational age has been reported in one other abnormal population (Limperopoulos et al. 2011). In fetuses with congenital heart disease, total brain volume (including cerebellum and brainstem but excluding intracerebral fluid) was similar to age matched healthy controls at 25 weeks GA but became progressively smaller over the third trimester. Brain volumes were not corrected for fetal weight but there was no significant difference in estimated fetal weight percentiles between groups. Smaller total brain volume was independently

associated with low ventricular aortic output. The abnormal cardiovascular system appears unable to meet the metabolic demands of the rapid brain growth and maturation that occurs in the third trimester.

The first half of gestation is characterized by cellular proliferation and migration. In contrast the second half is concerned with synaptogenesis and myelination. By 20 weeks GA the fetal brain typically weighs about 20g but is highly cellular and densely packed. Rapid expansion in size and weight occurs at an increasing rate over during the latter second trimester, third trimester and into the first 2 years of life. The smaller absolute STB volumes that we observed in ex-donors compared to ex-recipients may reflect the effects of chronic hypoperfusion (Becker et al. 2006; Gratacós et al. 2002; Ishii et al. 2004; Yamamoto et al. 2007) on cerebral cellular proliferation during the crucial first half of gestation. Our observation that fetal body corrected STB volumes were greater in ex-donors compared to their co-twin, suggests a brain sparing mechanism in ex-donor fetuses. However this was not sufficient for the ex-donor brain to achieve its full genetic growth potential. This effect may become more or less marked at later gestations. Future studies could follow patterns of discordant brain growth into the third trimester.

Cerebellar volumetry findings in TTTS and singletons

In this study cerebellar volume in normal singletons increased at a relative rate of 17% per week, increasing by a factor of 6.6 between 22 and 30 weeks gestational age. Scott and colleagues (Scott et al. 2012) measured a similar 7-fold increase between 22 and 32 weeks gestational age with comparable estimated volumes. These authors also commented on an accelerating growth trajectory with increasing gestational age. Only one other study has quantified cerebellar volumes in normal singletons and these authors report a slightly lower relative growth rate of 13% (Clouchoux et al. 2011). Of note the gestational age range described in that study was later from 25 to 36 weeks. Furthermore segmentation was performed using reconstructed datasets with 2mm thick slices. In the current study, and that of Scott and colleagues (2011), segmentations were performed using reconstructed datasets with 1mm or less slice thickness. Given the relatively small size of the cerebellum, the use of thicker slices for automatic segmentation may have led to volume underestimation. In both studies the authors observed that the cerebellum increased in size at a faster rate than the cerebrum and this was replicated in our group.

Cerebellar volume was significantly smaller in both TTTS fetuses in our cohort compared to singleton controls. The majority of ultrasound based biometry studies have found no difference in

TCD between healthy age matched twins and singletons, regardless of zygosity (Dias et al. 2010; Goldstein & Reece 1995; Goldstein et al. 2002; Shimizu et al. 1992). A single MRI based brain volume study in neonates found no difference in cerebellar volumes between healthy twins and singletons irrespective of zygosity (Knickmeyer et al. 2011).

Ultrasound based biometry studies of FGR in twins have found a similar preservation of cerebellar growth. With the exception of one study (Lettieri et al. 1992), TCDs appear generally unaffected by selective fetal growth restriction, both within twin pairs and compared to singletons (Goldstein & Reece 1995; Shimizu et al. 1992;). Similarly in singletons, although TCD may be more likely to be reduced in severe (Campbell et al. 1994; Snijders et al. 1994) or symmetrical FGR (Lee et al. 1991), TCD is not significantly different between growth restricted fetuses and age matched healthy controls (Campbell et al. 1994; Dilmen et al. 1996; Lee et al. 1991; Meyer et al. 1993). One MRI based study of cerebellar volume at 37 weeks (Sanz-Cortes et al. 2014) suggests that growth is maintained in proportion to gestational age in SGA fetuses compared to AGA fetuses. This study did not use motion corrected data, image acquisition was of thick 3.5mm slices and cerebellar volume was corrected by intracranial volume rather than by STB volume. The observation of reduced cerebellar volume in TTTS in the current study suggests a unique pathophysiological impact of the disease on cerebellar development.

A single MRI biometry study in TTTS reports a different pattern of results to our findings (Tarui et al. 2012). These authors comment that whilst similar growth trajectories are seen in donors and singletons, recipients appear to have steeper growth trajectories. Bitemporal diameter corrected TCDs were smaller than singletons in early gestation but eventually caught up to and surpassed singletons by 24 weeks gestational age. One must be cautious about drawing conclusions regarding growth trajectories in this study as the sample size was small and serial imaging was not performed. In addition it is not clear what stage of disease was included nor when or whether treatment for TTTS was performed. There is therefore the potential for significant variability in the data arising from disease and treatment factors. These authors also reported smaller TCDs (corrected by bitemporal diameter) in donors compared to both recipient twins and singletons, in direct contrast to our findings.

The cerebellum is critically involved in motor coordination and balance (Bastian & Thach 2002) but has also been implicated in a variety of other cognitive roles including executive functioning, abstract reasoning, language, working memory, behavioural-affective modulation and visual-spatial regulation (Baillieux et al. 2008; Schmahmann & Sherman 1998). Aberrant development of the cerebellum is consequently associated with a wide range of deficits (Bolduc et al. 2011; Manto &

Jissendi 2012). In children with cerebellar malformations, decreased total cerebellar volume is associated with delays in global development, expressive language, cognition, as well as gross and fine motor function (Bolduc et al. 2012). A reduction in cerebellar volume has been shown to associate with cognitive impairment in preterm children (Peterson 2000) and with lower motor development scores in SGA fetuses (Sanz-Cortes et al. 2014). Furthermore there may be white and grey matter differences in volume reduction as well as regional variation that impacts outcome (Bolduc et al. 2012; Courchesne et al. 2001; Padilla et al. 2011). Additional volumetric analysis in our TTTS cohort could include differentiation of white and grey matter volumes in the cerebellum.

Cortical grey matter volume

Cortical grey matter volume increased at a relative rate of 14% per week between 22 and 32 weeks GA in normal singletons in our study. Only two studies in fetal volumetry have quantified cortical volume and report comparable growth rates of 13% and 18% in normal singletons (Kyriakopoulou et al. 2014; Scott et al. 2011). In this chapter it was demonstrated that cortical volumes were similar between ex-recipients and ex-donors, when corrected for STB tissue volume. This may reflect consistently proportionate cortical growth within twin pairs or stabilization of any intertwin differences following successful treatment. However in comparison to singleton controls, cortical volumes were smaller in both ex-recipient and ex-donor twins, even when corrected for STB volume.

No studies could be identified that compared cortical grey matter volumes between twins and singletons in utero. However one study has assessed differences between groups during the first month of life (Knickmeyer et al. 2011). These authors found that growth of the cortical grey matter was accelerated in MZ twins compared to singletons and DZ twins. They hypothesise that this rapid increase in volume during the neonatal period reflects catch up growth following delayed in utero neocortical development. One cannot therefore conclude whether the observed reduction in cortical grey matter volumes in this chapter represent pathology specific to TTTS or a more general effect on monozygosity.

Regional variation in cortical volume and thickness, specifically a reduction in insular cortex, has been reported in a fetal MR study in late onset FGR compared to normally grown singletons (Egaña-Ugrinovic et al. 2014). However these results are difficult to interpret for a number of reasons. The data were acquired with 3.5mm slice thickness and were not motion corrected. Insular cortical volume was corrected by a TBV which included intra- and extracerebral fluid. Insular cortical thickness was reported as a ratio over insular depth, with smaller ratios found in FGR. The same

authors have previously reported that insular depth is increased in FGR relative to normally grown fetuses (Egaña-Ugrinovic et al. 2013), although why insular depth should necessarily relate to cortical thickness is unclear. Furthermore, in that study (Egaña-Ugrinovic et al. 2013) increased insula depth was only reported as a ratio over BPD, although BPD alone was shown to be significantly smaller in their FGR population. There are several factors which may influence BPD in FGR including reduced intracranial fluid volumes and more prominent compressive effects of the uterus secondary to oligohydramnios. Whilst development and growth are obviously related, the appropriateness of correcting fissure depth by BPD is questionable. These issues make it hard to draw conclusions about the true nature of differences in cortical volume between groups.

MR studies in growth restricted neonates (Tolsa et al. 2004) have reported reduced cortical grey matter volumes compared to age matched appropriately grown preterm neonates that persists at term. In a preterm cohort follow up study, cortical grey matter volume was found to be smaller when assessed at school age relative to term born children (Peterson 2000). Studies using optical fractionation in pathological specimens have reported reduced cortical grey matter cell number in growth restricted fetuses and fetuses with Down Syndrome compared to healthy controls (Larsen et al. 2008; Samuelsen et al. 2007).

Reduced cortical grey matter volume has been shown to correlate with cognitive impairment in a number of populations including growth restricted neonates and school aged preterms (Peterson et al. 2000; Tolsa et al. 2004). In contrast other studies have demonstrated relative cortical overgrowth associated with poorer outcomes and neurodevelopmental delay, particularly in ventriculomegaly. In autistic spectrum disorders larger total brain volumes are reported however there appears to be global cortical enlargement as opposed to grey matter overgrowth (Palmen et al. 2004; Palmen et al. 2005). These contrasting findings suggest that it is not volume in itself that determines cognitive function. Deviations from normal volume may associate with abnormal structure and reflect aberrant development.

It is noted that both the cerebellum and cortical grey matter demonstrated a similar pattern of volume reduction in TTTS relative to singletons and that this reduction was proportionate to STB tissue volume between co-twins for both structures. One possibility is that cerebellar and cortical grey matter growth is linked. Both structures demonstrate an acceleration in growth that exceeds overall cerebral growth rates by around 24-25 weeks (Scott et al. 2011). This is characterized by proliferation and outward migration of fibres that increase both cerebellar and cortical surface area and thickness coupled with regression of ventricular zones. The cerebellum has multiple reciprocal projections with frontal, parietal, and occipital cortices (Thach 2007) and it has been suggested that

the extremely rapid growth of the cerebellum in the first year may be a prerequisite for specific aspects of later cortical development (Knickmeyer et al. 2008). Furthermore, isolated cerebellar malformations have been found to affect cerebral regional development, in particular associating with reduced mid-temporal and parietal-occipital grey matter volume reductions (Bolduc et al. 2012). Growth mechanisms or developmental links between cerebellum and cortical grey matter may be selectively impaired in twins compared to singletons. Growth differences may be distinct from developmental maturity and cortical maturation will be addressed in a later chapter.

Cerebral fluid

Lateral ventricle volume showed a weak increase with GA in the singleton control group at a rate of 3.2% per week, with no significant differences either between singletons and TTTS fetuses or within TTTS twin pairs. Intracerebral fluid volumes were also similar for all comparisons. The growth of the total lateral ventricle volume in utero has been studied with variable results. One study has observed a relative decrease in volume of 4% per week between 25 and 36 weeks GA (Clouchoux et al. 2011). Other studies report relative increases in volume of between 3% and 9% (Kyriakopoulou et al. 2014; Scott et al. 2011), although the latter figure included the third ventricle. Reported intersubject variability was high in all studies and only a relatively small proportion is accounted for by increasing gestational age. Intracerebral fluid volume increased at a rate of 6% per week in our singleton group which is comparable to the figure reported by Scott and colleagues (Scott et al. 2011).

Volumetry studies that include extracerebral fluid report equally variable findings, although few have looked at ECF volume in isolation (Kyriakopoulou et al. 2014). Other studies have reported total cerebral fluid which includes the lateral ventricles. Clouchoux and colleagues (Clouchoux et al. 2011) found that total cerebral fluid increased by 5% per week although intersubject variability was high. In contrast Limperopoulos and colleagues (Limperopoulos et al. 2011) found that total cerebral fluid decreased until 30 weeks gestational age, followed by an increase. These authors also comment on high intersubject variability. This lack of agreement between studies may be accounted for by the inclusion of the lateral ventricles which show wide variation in measurements as previously described.

Volumetry in neonates imaged within the first four weeks of life suggest that healthy MZ twins have relatively higher total cerebral fluid volumes than either DZ twins or singletons (Knickmeyer et al. 2011). Lateral ventricle volume did not differ significantly between groups implying that the additional

fluid in MZ twins reflects an increase in the extracerebral fluid volume. In the current study there was no significant difference between ECF volume in singletons and ex-recipients. However ECF volumes were 17.6% lower in ex-donors compared to their ex-recipient co-twins, even when corrected for total intracranial volume. A reduction in ECF volume in ex-donor twins is consistent with the overall volume depletion that is characteristic of donor twin status in TTTS. Whilst laser treatment for TTTS separates co-twin placental circulations, it does directly modify RAS activation discordance between twins. The within pair difference in ECF volume that we have observed in laser treated co-twins may simply relate to slow fluid stabilization following treatment. Alternatively it may reflect subtle effects of ongoing discordant RAS activation.

Limitations

Growth rates and trajectories are cautiously commented upon in this study. The study design did not include serial scanning and therefore the data represent an estimation from single points across the cohort rather than observed change over time within individuals. This is particularly relevant with respect to ex-donors. It is noted that ex-donors showed greater variability than ex-recipients or singletons and this most likely reflects catch-up growth post laser treatment. The other consequence of performing imaging at a single time point post treatment is that one must be restrained in interpreting intertwin differences. The findings represent observations at a particular point following apparently successful treatment. As has been mentioned at various points in the preceding discussion, one cannot comment on whether the findings reflect general consequences of the pathophysiology of TTTS or relate to stabilization following treatment. Pre and post treatment imaging would be necessary to distinguish this.

Given the nature of conducting research within a clinical setting there were a number of variables that could not be controlled for. In particular gestational age at treatment varied across the TTTS cohort (as treatment tends to be guided by severity) and this important variable may influence brain development. The interval between treatment and MR imaging was also variable. This is partly due to the practical considerations of mother's availability. However the main issue in fixing the interval relates to the gestational age at which treatment usually occurs. Whilst MR imaging before 22 weeks GA may identify overt brain injury, the brain is still relatively immature and it is therefore more difficult to comment in detail on development. However, whilst fixing the MR imaging interval to 6 weeks would be appropriate for those fetuses treated at 16 or 17 weeks GA, it would be unreasonably long to expect a fetus treated at 22 weeks to wait. One of the strengths of the study is that despite these variations across the group, performing within pair comparisons means that these factors are

identical between co-twins. Within pair comparisons also inherently control for maternal characteristics and genetic variation.

Unfortunately complete fetal body or cortical grey matter volumes could not be acquired for the entire cohort. However the cases that were not included in these analyses did not have significantly different outcomes to those that were included. Therefore it can be assumed that the fetal body and cortical grey matter analyses are representative of the entire cohort.

Healthy MCDA twins would have been an alternative control group and may be considered more appropriate. The use of singletons meant that there was some ambiguity in interpreting selected results as parameters such as cortical grey matter volume are thought to show inherently different patterns of development compared to singletons (Knickmeyer et al. 2011).

3.3.6 Conclusion

In this chapter significant intracranial differences within twin pairs treated for TTTS have been demonstrated. There is evidence of brain sparing in ex-donors with relatively less extracerebral fluid compared to ex-recipient co-twins. Smaller cortical grey matter and cerebellar volumes relative to STB tissue volume suggest aberrant development in the twin cohort compared to singleton controls but this does not appear to be influenced by ex-recipient or ex-donor status. Alterations in cerebral growth may be secondary to cerebral blood flow disturbances occurring before treatment for TTTS. This may relate to later neurodevelopmental impairment in children without evidence of overt brain injury.

3.4 Assessment of cortical development

3.4.1 Background

Brain maturation is characterized by both morphological and compositional changes. Much of our knowledge regarding early development is derived from histopathological and imaging autopsy studies (Brody et al. 1987; Chi & Dooling 1977; Dolman 1977; Dorovini-Zis & Naidich et al. 1994; Kinney et al. 1988; Martin et al. 1988). However advancement of MR imaging techniques have made it increasingly possible to study fetal cerebral maturation in vivo (Garel et al. 2001; Huang 2010; Levine & Barnes 1999; Rutherford et al. 2008). Abnormal patterns of cortical folding have been associated with a variety of neurocognitive deficits (Casanova et al. 2004; Jackson & Plante 1996; Sallet et al. 2003) and therefore cortical development has received particular focus.

Cellular proliferation and migration is the first step in cortical development during the first half of gestation. Organization and differentiation (synaptogenesis) occurs in the second half of gestation. It is in this period that folding of the cerebral cortex takes place following a highly orchestrated sequence of gyral and sulcal formation. Progress of sulcation follows an invariable temporospatial schedule. As such it provides a reliable estimate of gestational age and is a good marker of cortical maturation (Dorovini-Zis & Dolman 1977; Garel et al. 2001). The final stage in development is myelination of the intracortical fibres. This begins in the fetal period and continues into adult life (Fogliarini et al. 2005).

A number of MR studies have attempted to quantify the maturation of the cerebral cortex in the normal fetus using different techniques. The majority of earlier studies used an observational approach by generating a timeline of development of different cortical landmarks (Fogliarini et al. 2005; Garel et al. 2001; Glenn 2009; Levine & Barnes 1999). Recent advances in post-processing techniques have also allowed for the use of computational methods with the use of algorithms for the delineation of the sulcal patterns during development (Clouchoux et al. 2012; Habas et al. 2012; Scott et al. 2012; Wright et al. 2014) but lack standardisation and further validation. Approaches such as deformation tensor based morphometry (Rajagopalan et al. 2011) are promising but do not currently allow for detection of abnormalities if sulci are developing at a rate similar to average brain growth. Other authors have developed scoring systems such as ‘fetal total maturation score’, which incorporates sulcation, myelination and germinal matrix evolution, and the gyrification index

(calculated as the ratio of whole gyral contour length to the outer exposed surface) (Armstrong et al. 1995; Dubois et al. 2008; Kesler et al. 2006; Limperopoulos et al. 2013; Zilles et al. 1988). Whilst these measures may be useful for global assessment of maturation, they cannot localise regional abnormalities.

Investigating cortical development in utero may provide a useful means for identifying intertwin differences in TTTS, including more subtle regional variations. Such differences could prove to be of value in predicting neurocognitive outcome. In this chapter the developmental stages of the cortex in TTTS compared to singleton controls is characterised as well as quantifying within twin pair differences.

3.4.2 Aims

To assess whether ex-donor fetuses show delayed cortical maturation relative to their ex-recipient co-twins.

3.4.3 Methods

3.4.3.1 Cohort

Thirty nine cases with two surviving fetuses underwent fetal MRI following fetoscopic laser for TTTS. A total of eight cases were excluded due to abnormal findings on clinical MR examination (6) or images unsuitable for assessment of all cortical landmarks (2) as described in section 2.3.5 table 9.

3.4.3.2 Assessment of cortical development

Cortical landmarks were assessed from the reconstructed datasets which were reoriented and resampled for accuracy as described in section 2.3. Six landmarks were selected for analysis and are listed in table 22. Landmarks were chosen across a range of lobes to ensure global representation of the brain. Gestational age at first presentation (based on normal fetal data) was also a factor in selection to maximize the likelihood that the landmark would be visible in the cohort.

Brain region	Landmark
Frontal/parietal/temporal border	Sylvian fissure
Frontal	Inferior frontal gyrus
Frontal/parietal border	Central sulcus
Parietal	Parieto-occipital fissure
Temporal	Superior temporal gyrus
Occipital	Calcarine sulcus

Table 22. Cortical landmarks chosen for assessment and corresponding brain region.

Cortical sulcation and gyrification were scored according to a specific protocol as described in section 2.3. The insular-opercular ratio was calculated for the Sylvian fissure on each side of the brain as described in section 2.3 and the mean ratio recorded for statistical analysis.

3.4.3.3 Statistics

The Spearman correlation coefficient (r) was used to assess the strength of the association between development score and GA. Comparisons were performed using Mann-Whitney U test for group comparisons between singleton controls and the twin cohort in gestational age at score 1. Wilcoxon Signed Ranks test was used for within twin pair comparisons in development score. No correction for multiple comparisons was necessary as all comparisons were pairwise and planned.

3.4.3.4 Intra-rater reliability

All measurements were performed by a single examiner (MTC) to exclude inter-observer variability. Twenty linear measurements (opercular and insular distance of the Sylvian fissure) were performed twice to assess intra-observer variability. The ICC was calculated at 0.94 ($p<0.001$).

3.4.4 Results

3.4.4.1 Cohort

Thirty one cases (62 fetuses) were included in the final analysis (see table 13 Methods section 2.3.5). Of the 39 cases recruited, six cases were excluded due to abnormal MR findings. These were the same cases excluded from biometric and volumetric analysis. Two cases (case 7 and case 8) were excluded due to motion or other artefact resulting in inadequate visualisation of brain tissue following reconstruction in one or both fetuses. These were the same cases excluded from biometric and volumetric analysis. Median gestational age at MRI was 25.7 weeks (range 20.7 to 32 weeks) in the remaining 31 twin pairs. Median time between treatment and MRI was 5.1 weeks (range 3 to 10 weeks). Laser treatment was performed for stage 2 disease in four cases and stage 4 disease in two cases. The remaining 25 cases were treated for stage 3 disease.

Singleton control reference data were obtained from 72 fetuses that underwent imaging at a median gestational age of 29.4 weeks (range 22.4 to 38.9 weeks).

3.4.4.2 Gestational age of early sulcation

The gestational age for fetuses that were given a score of 1 (some undulation) is presented compared to departmental singleton control reference data in a whisker box plot in figure 68.

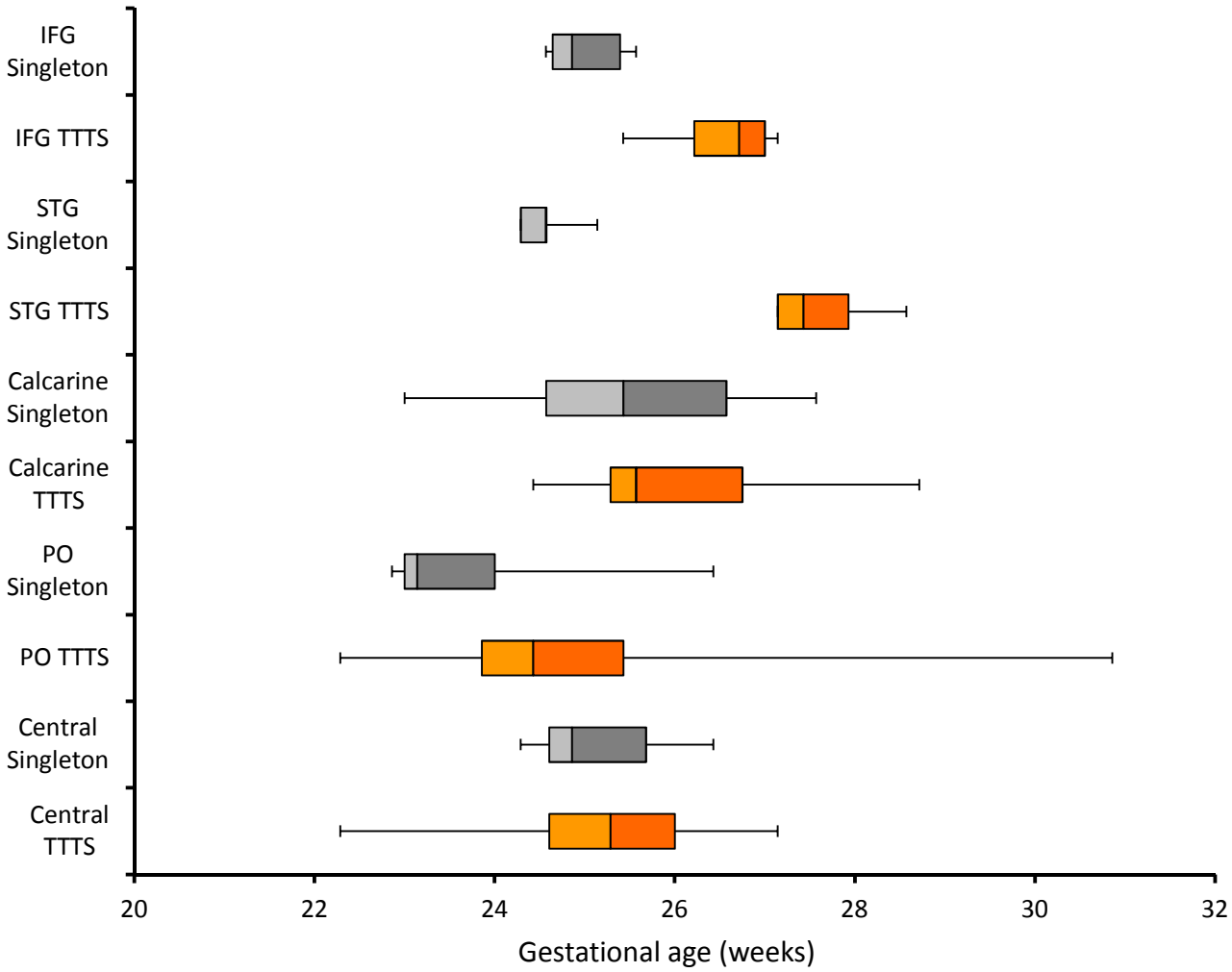


Figure 68. Gestational age of early sulcation.

The data represent all fetuses with a cortical development score of 1. The twin cohort is shown in orange and singleton controls in grey. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. (Central: central sulcus, PO: parieto-occipital fissure, Cal: calcarine, STG: superior temporal gyrus, IFG: inferior frontal gyrus).

The Sylvian fissure was present in all fetuses. In the twin TTTS cohort the PO fissure was visualised at around 24-25 weeks followed by the central sulcus at 25-26 weeks. The calcarine sulcus was visualised at around 26 weeks, followed by the IFG at around 27 weeks and finally the STG at around 28. There was a delay in visualisation of all the cortical landmarks assessed of between one to three weeks in the twin TTTS cohort compared to singleton controls. This was statistically

significant for the parieto-occipital fissure ($p<0.05$), STG ($p<0.01$) and IFG ($p<0.01$). This difference did not reach statistical significance for the calcarine sulcus ($p = 0.1$) or the central sulcus ($p = 0.3$).

The GA range at which each landmark has a score of 1 in 25-75% of ex-recipient and ex-donor fetuses is presented in Table 23 alongside the departmental singleton control population data.

Landmark	Ex-recipient	Ex-donor	Singleton
POF	24.1 (22.3-24.4)	25.3 (24-26)	23.1 (23-24)
Central	25.3 (24.4-25.7)	25.3 (25.1-26.7)	24.9 (24.6-25.7)
IFG	26.7 (25.7-26.9)	26.9 (26.7-27.1)	24.9 (24.6-25.4)
STG	27.7 (27.7-28.7)	27.7 (27.1-28.6)	24.6 (24.3-24.7)
Calcarine	25.4 (25.3-26.7)	26 (25.4-27.1)	25.4 (24.6-26.6)

Table 23. GA of early sulcation in ex-recipient twins, ex-donor twins and singleton controls.
Median GA at which landmarks receiving a score of 1 were observed. Interquartile range is presented in brackets. (POF: Parieto-occipital fissure; Central: Central sulcus; IFG: Inferior frontal gyrus; STG: Superior temporal gyrus; Calcarine: Calcarine sulcus)

Figure 69 shows a whisker box plot comparison between ex-recipients and ex-donors of the gestational age range at which cortical landmarks received a score of 1. A later gestational age was observed in ex-donors for the parieto-occipital fissure ($p<0.05$). A trend towards later gestational age in visualisation of the calcarine sulcus ($p = 0.12$) was observed but this did not reach statistical significance.

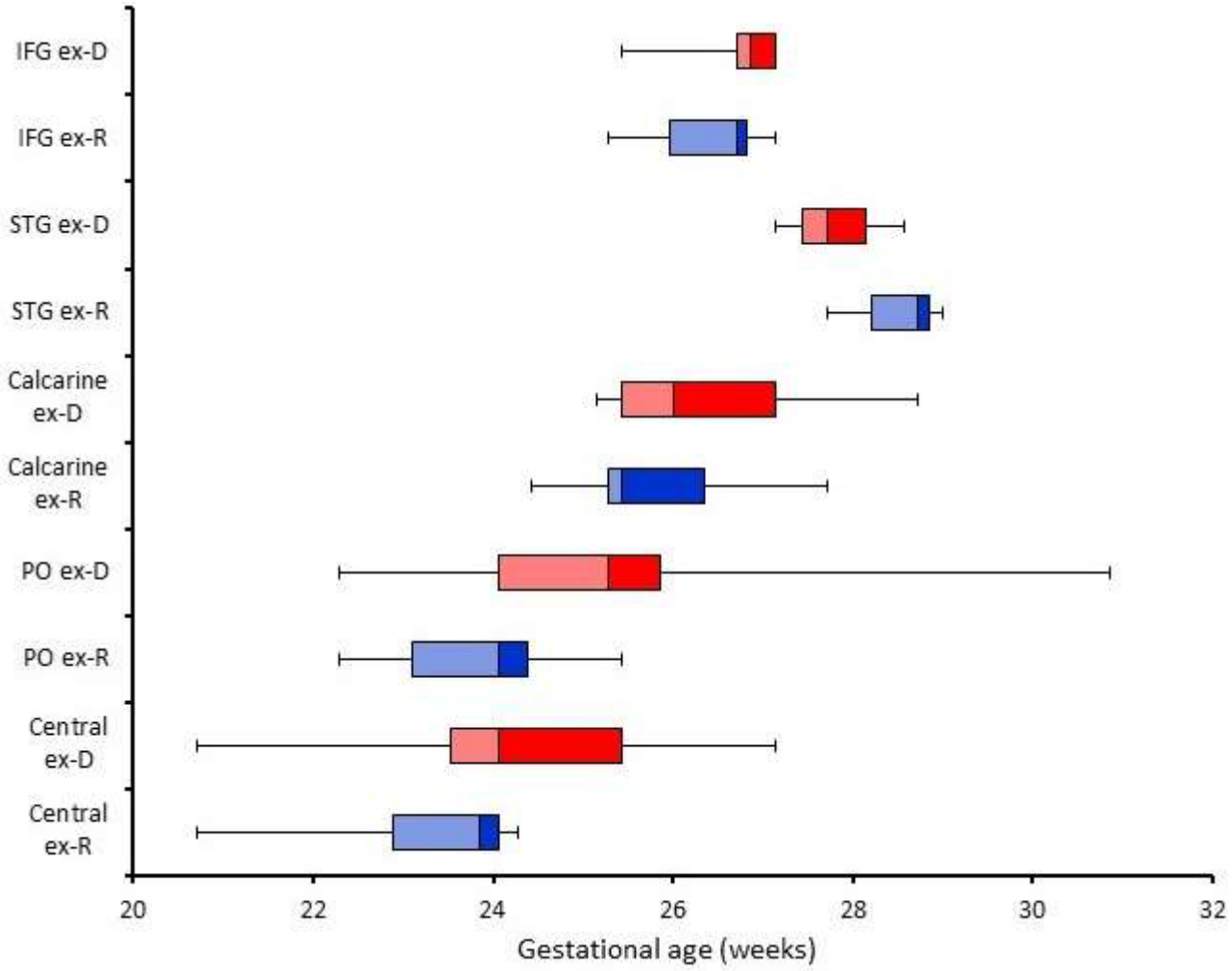


Figure 69. Gestational age of early sulcation in ex-recipient and ex-donor fetuses.

The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses with a cortical development score of 1. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. (Central: central sulcus, PO: parieto-occipital fissure, Cal: calcarine, STG: superior temporal gyrus, IFG: inferior frontal gyrus).

3.4.4.3 Correlation of maturation with GA

The strength of association between GA and development scores of 1 or above for all twin fetuses, as well as separated for ex-recipients and ex-donors, is presented for each cortical landmark in Table 24. Cortical development scores generally correlated well with GA for both ex-recipients and ex-donors although Spearman's r tended to be slightly lower in ex-donors.

	POF	Central	IFG	STG	Calcarine	Sylvian
All fetuses	0.76**	0.89**	0.81**	0.87**	0.78**	0.88**
Ex-recipients	0.87**	0.90**	0.84**	0.85**	0.86**	0.92**
Ex-donors	0.69**	0.88**	0.78**	0.88**	0.72**	0.87**

Table 24. Strength of association between cortical development score above 1 and GA in twin fetuses. Spearman's r is reported for each measure. ** p<0.0001 (Central: central sulcus, POF: parieto-occipital fissure, Cal: calcarine, STG: superior temporal gyrus, IFG: inferior frontal gyrus).

3.4.4.4 Frontal lobe (Inferior frontal gyrus)

The inferior frontal gyrus was present in 30 fetuses (16 ex-recipients and 14 ex-donors) (figure 70). Cortical development score increased with gestational age for all fetuses (score Spearman's r = 0.81, p<0.0001) and was similar in ex-recipients (score Spearman's r = 0.84, p<0.0001) and ex-donors (score Spearman's r = 0.78, p<0.0001). Development scores were not significantly different between ex-recipients and ex-donors within twin pairs (p=0.3; 1-sided). No scores of 6 were recorded.

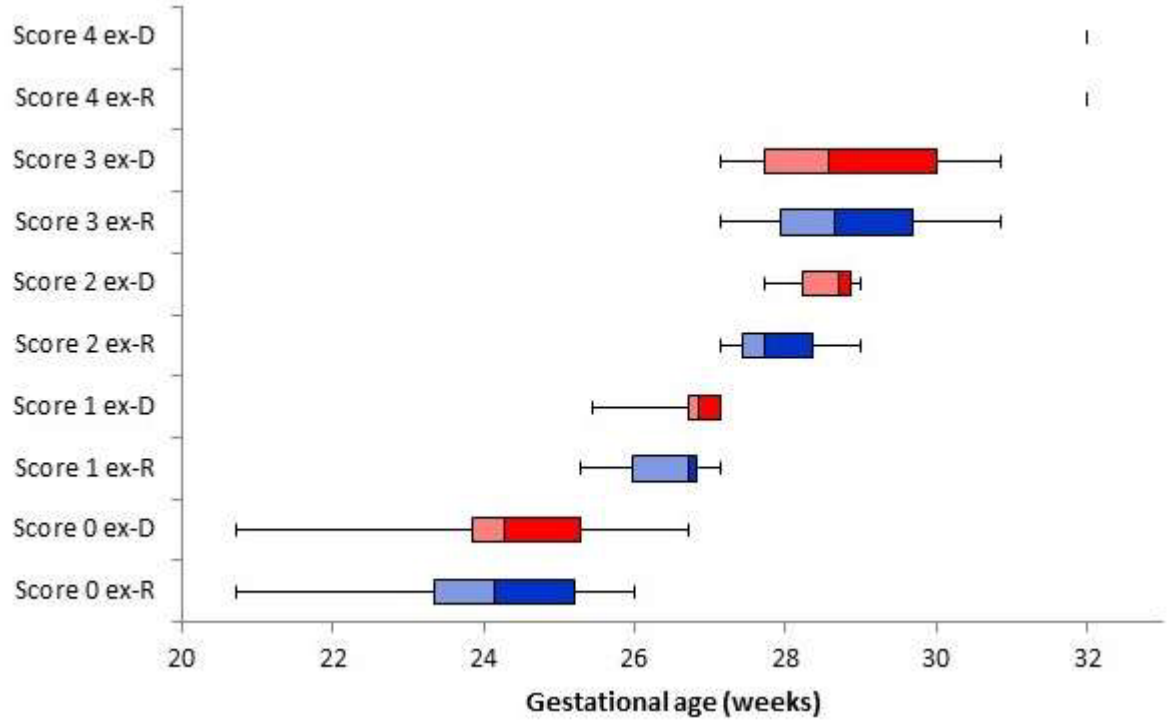


Figure 70. Cortical development scores for the inferior frontal gyrus in ex-recipient and ex-donor co-twins.
The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. Development scores were not significantly different within twin pairs (p = 0.3).

3.4.4.5 Frontoparietal border (central sulcus)

The central sulcus was present in 44 fetuses (25 ex-recipients and 19 ex-donors) (figure 71). Cortical development score increased with gestational age for all fetuses (score Spearman's r = 0.89, p<0.0001) and this was similar in ex-recipients (score Spearman's r = 0.90, p<0.0001) and ex-donors (score Spearman's r = 0.88, p<0.0001). Development scores were significantly higher in ex-recipients compared to ex-donors within twin pairs (p<0.001; 1-sided).

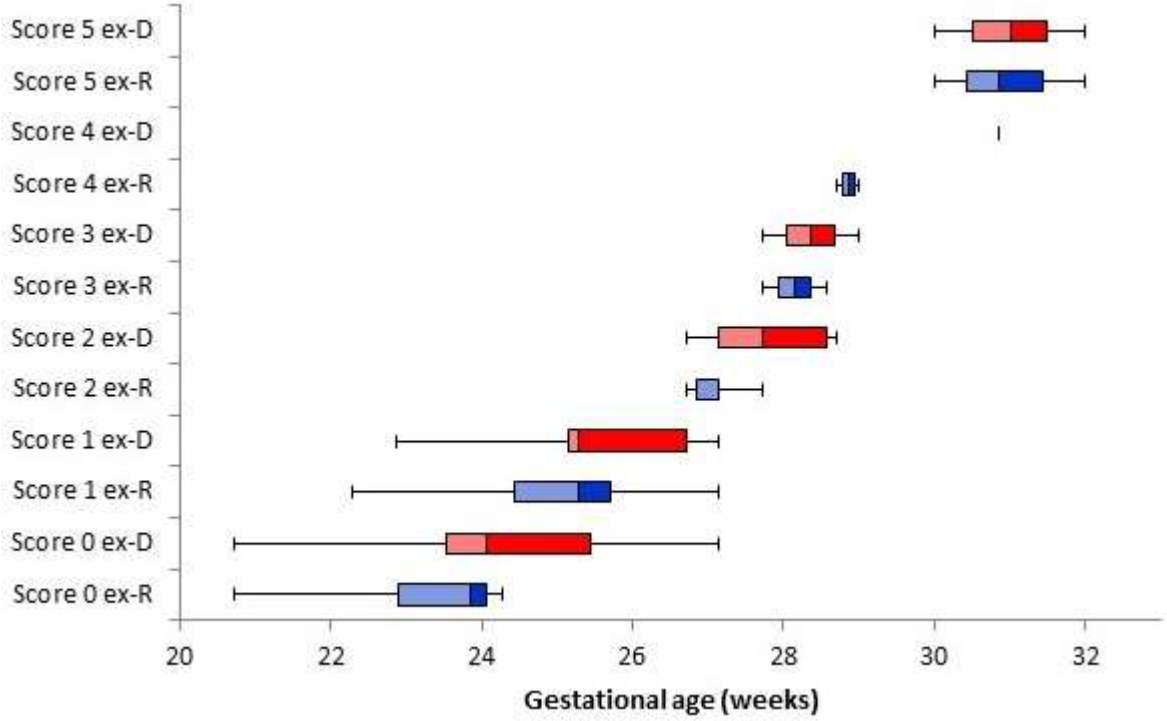


Figure 71. Cortical development scores for the central sulcus in ex-recipient and ex-donor co-twins.
The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. Development scores were significantly higher for ex-recipients compared to ex-donor co-twins (p<0.001).

3.4.3.2.6 Parietal lobe (Parieto-occipital fissure)

The parieto-occipital fissure was present in 56 fetuses (29 ex-recipients and 27 ex-donors) (figure 72). Cortical development score increased with gestational age for all fetuses (score Spearman's r = 0.76, p<0.0001) and this was similar in ex-recipients (score Spearman's r = 0.87, p<0.0001) and ex-donors (score Spearman's r = 0.69, p<0.0001). Developmental scores were significantly higher in ex-recipients compared to ex-donors within twin pairs (p<0.01; 1-sided).

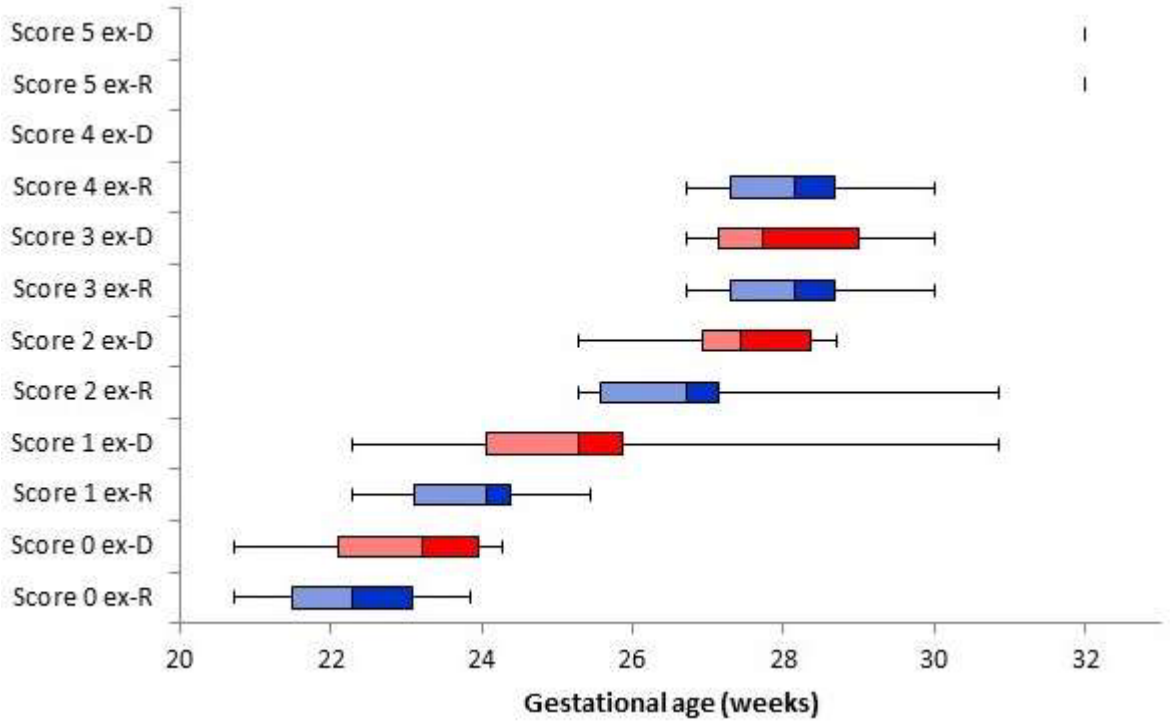


Figure 72. Cortical development scores for the parieto-occipital fissure in ex-recipient and ex-donor co-twins. The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. Development scores were significantly higher for ex-recipients compared to ex-donor co-twins (p<0.01).

3.4.3.2.4 Frontal/parietal/temporal border (Sylvian fissure)

The Sylvian fissure was present in all fetuses and measurable in 29 pairs. Figure 73 shows the insular-opercular ratio for ex-recipient and ex-donor fetuses. The insular-opercular ratio increased with gestational age when all twin fetuses where assessed (score Spearman's r = 0.88, p<0.0001). This association was similar for ex-recipients (score Spearman's r = 0.92, p<0.0001) and for ex-donors (score Spearman's r = 0.87, p<0.0001). Sylvian fissure ratio was significantly higher for ex-recipients compared to ex-donors within twin pairs (p<0.01).

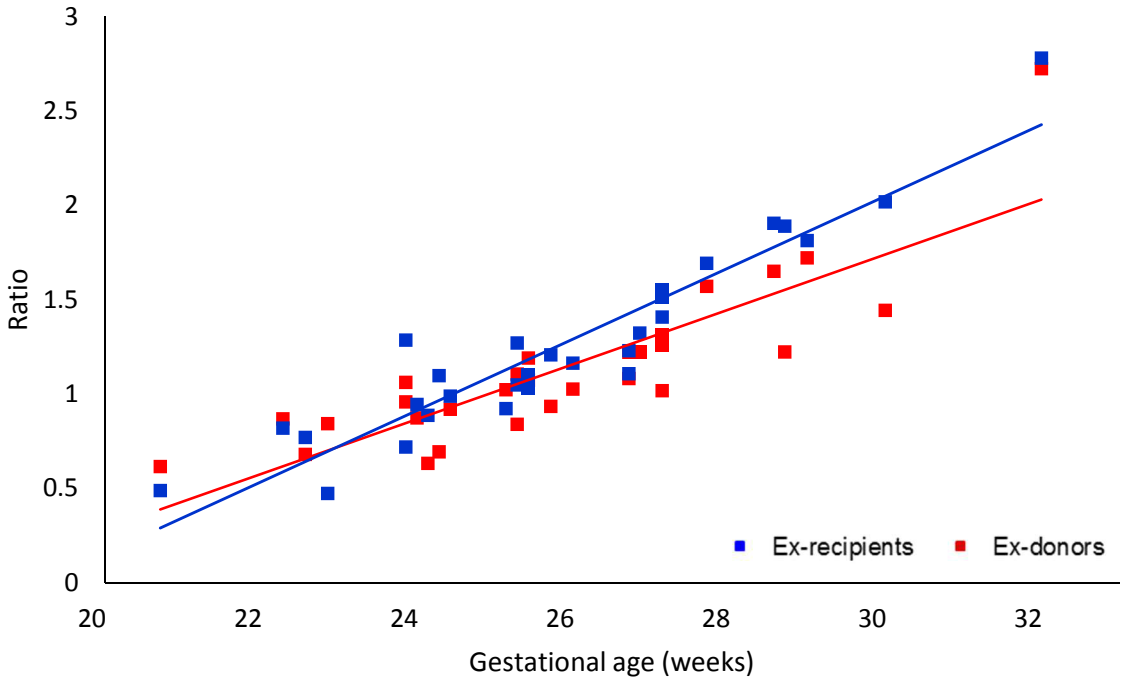


Figure 73. Sylvian fissure insular-opercular ratio in ex-recipient and ex-donor co-twins.
The insular-opercular ratio increased with gestational age for all fetuses. The ratio was significantly higher for ex-recipients compared to ex-donor co-twins (p<0.01).

Figure 74 shows the insular distance measurements for ex-recipients and ex-donors. There was a strong association between advancing gestational age and greater insular distance when assessed for all twin fetuses (Spearman's r=0.90, p<0.0001). This association was seen in ex-recipients (Spearman's r=0.92, p<0.0001) and ex-donors (Spearman's r=0.88, p<0.0001) when evaluated separately. Ex-recipient insular distance was significantly higher compared to ex-donors within twin pairs (p<0.05).

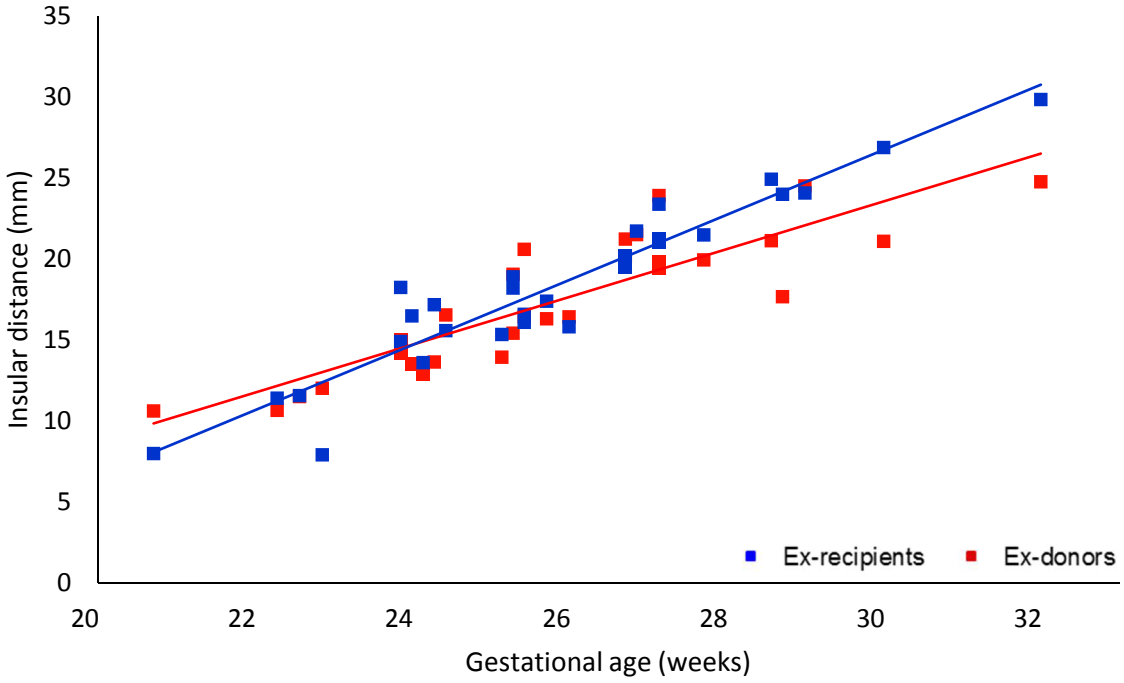


Figure 74. Sylvian fissure insular distance in ex-recipient and ex-donor co-twins.

The insular distance (the maximum distance from frontal to temporal boundaries along the insula-temporal cortex) increased with gestational age for all fetuses. The distance was significantly higher in ex-recipients compared to ex-donor co-twins (p<0.05).

Figure 75 shows the opercular distance measurements for ex-recipients and ex-donors. There was a moderate association between advancing gestational age and decreasing opercular distance when assessed for all twin fetuses (Spearman's r=-0.33, p<0.01). This pattern was seen in ex-recipients (Spearman's r=-0.56, p<0.001) however there was no significant association in ex-donors (Spearman's r=-0.14, p = 0.2). The opercular distance was significantly lower in ex-recipients compared to ex-donor co-twins (p<0.05).

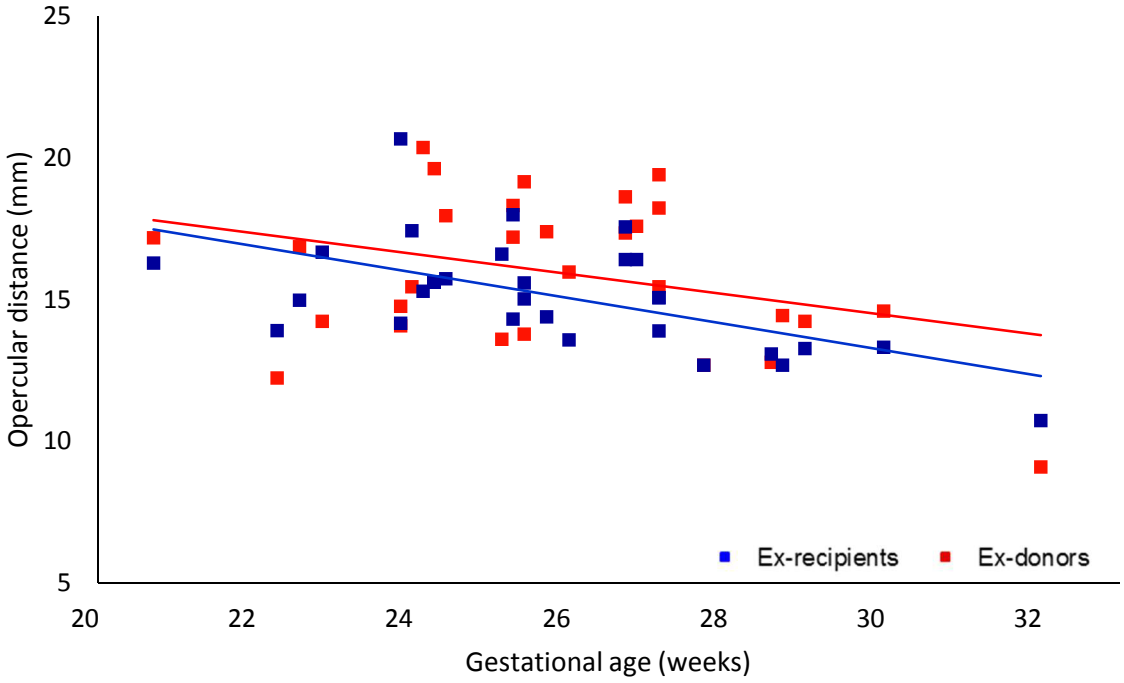


Figure 75. Sylvian fissure opercular distance in ex-recipient and ex-donor co-twins.

The opercular distance (the minimum distance from frontal to temporal borders of the operculum) decreased with gestational age in ex-recipients but not in ex-donor co-twins. The distance was significantly lower in ex-recipients compared to ex-donor co-twins (p<0.05).

Grouped linear regression analysis of Sylvian fissure development with gestational age between ex-recipients and ex-donors revealed that both Sylvian fissure ratios and insular distance showed a slower increase with gestational age in ex-donors compared to ex-recipients (t=2.1, p<0.05 and t=2.7, p<0.01 respectively). There was no significant slope difference between groups for opercular distance (p=0.7).

3.4.3.2.5 Temporal lobe (Superior temporal gyrus)

The superior temporal gyrus was present in 14 fetuses (8 ex-recipients and 6 ex-donors) (figure 76). Cortical development score increased with gestational age for all twin fetuses assessed together (Spearman's r = 0.87, p<0.0001) and this was similar in ex-recipients (Spearman's r = 0.85, p<0.0001) and ex-donors (Spearman's r = 0.88, p<0.0001). Developmental scores were not significantly different between ex-recipients and ex-donors within twin pairs (p=0.3; 1-sided).

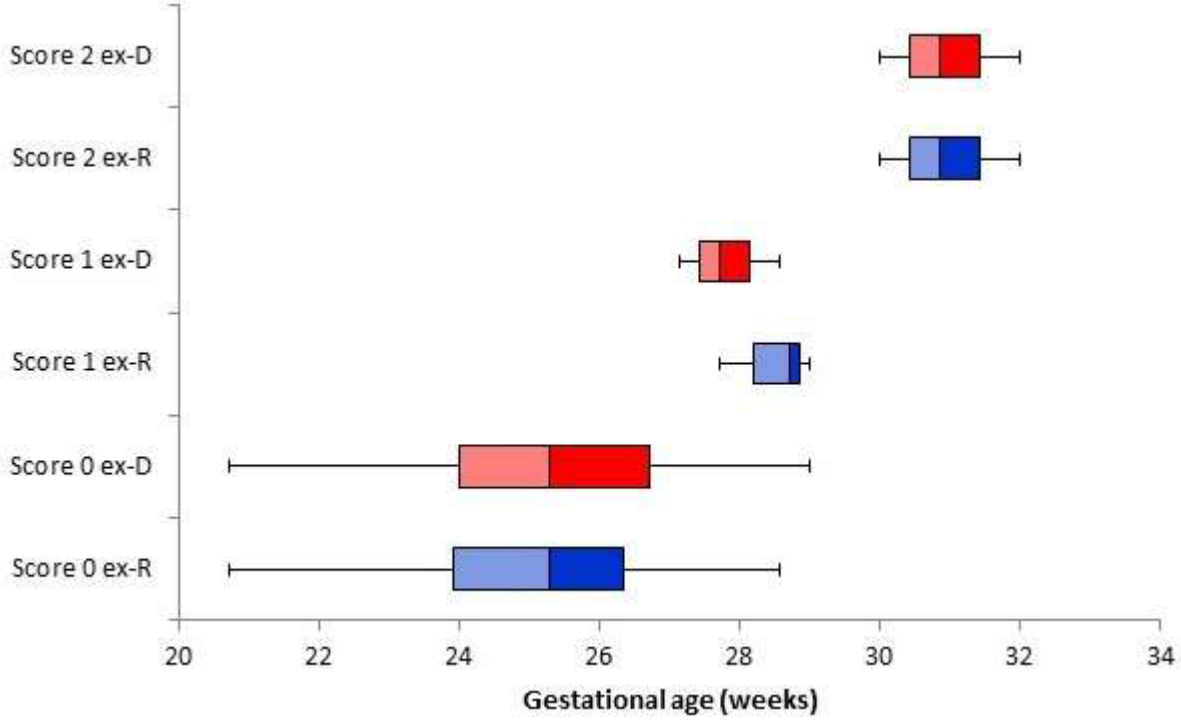


Figure 76. Cortical development scores for the superior temporal gyrus in ex-recipient and ex-donor co-twins. The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. Development scores were not significantly different for ex-recipients compared to ex-donor co-twins (p=0.3).

3.4.3.2.6 Occipital lobe (Calcarine sulcus)

The calcarine sulcus was present in 43 fetuses (22 ex-recipients and 21 ex-donors) (figure 77). Cortical development score increased with gestational age for all twin fetuses when assessed together (Spearman's r = 0.78, p<0.0001) and this was similar in ex-recipients (Spearman's r = 0.86, p<0.0001) and ex-donors (Spearman's r = 0.72, p<0.0001). Developmental scores were generally greater for ex-recipients compared to ex-donors within twin pairs but this did not reach statistical significance (p=0.1; 1-sided).

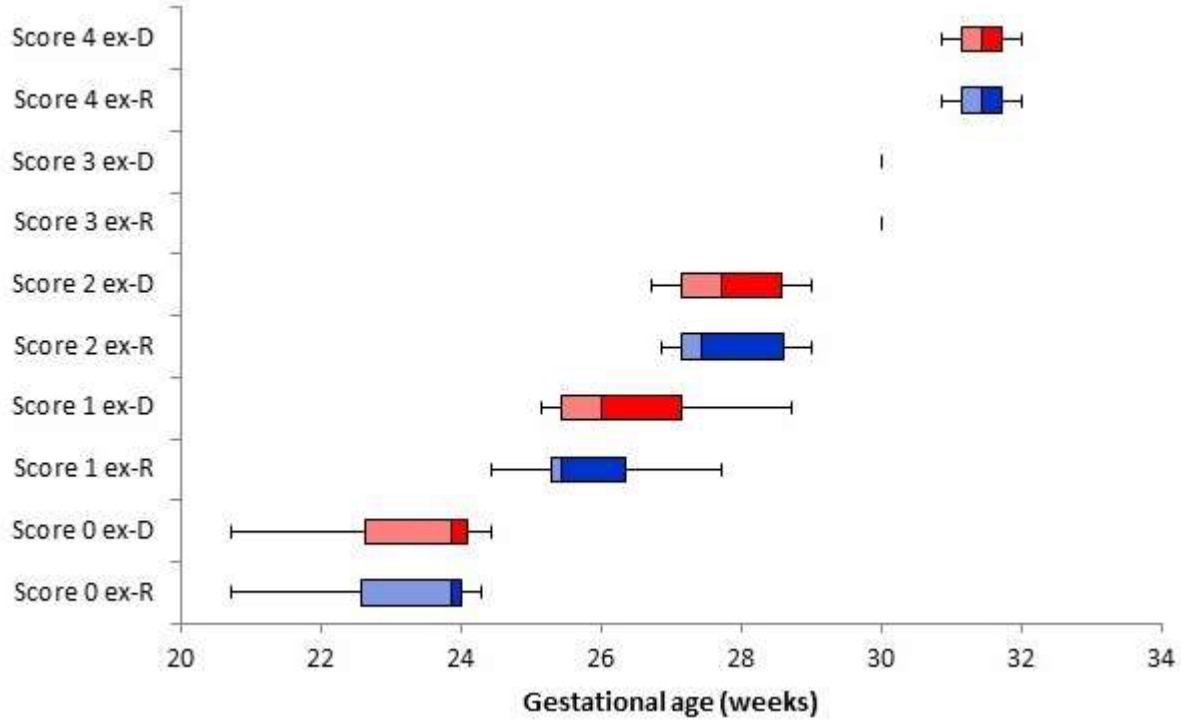


Figure 77. Cortical development scores for the calcarine sulcus in ex-recipient and ex-donor co-twins.
The data represent all ex-recipient (ex-R) and ex-donor (ex-D) fetuses. Ex-recipient data is shown in blue and ex-donor data in red. The blocked bars represent the 25-75% interval and the whiskers are minimum and maximum values. Development scores were not significantly different for ex-recipients compared to ex-donor co-twins (p=0.1).

3.4.5 Discussion

This chapter has assessed the appearance and development of specific cortical landmarks representative of each brain lobe in ex-recipient and ex-donor twin pairs treated for TTTS. The parieto-occipital fissure, inferior frontal gyrus and superior temporal gyrus were visualised at a later gestational age in the TTTS cohort relative to singleton controls. The parieto-occipital fissure was first visualised at a later gestational age in ex-donors compared to ex-recipients. Delayed maturation of the parieto-occipital fissure and central sulcus was demonstrated in ex-donors relative to their ex-recipient co-twins between 20 and 32 weeks, with aberrant development of the insula region.

The process of sulcation could be characterised in the twin cohort by application of the revised cortical development scheme. This was evidenced by the high correlation observed between development scores and gestational age. The Sylvian fissure was already present in all fetuses studied as expected. The parieto-occipital fissure began to appear at 24-25 weeks GA, followed by the central sulcus at 25 weeks and the calcarine fissure at around 25-26 weeks GA. The superior temporal gyrus and the inferior frontal gyrus were seen to develop from weeks 27-28 onwards.

This order of sulcation in the twin cohort was broadly consistent with that observed in previous studies in singleton controls. The appearance of individual landmarks was delayed by between one to four weeks compared to departmental MRI data in singletons as well as published studies (Fogliarini et al. 2005; Garel et al. 2001; Habas et al. 2012; Levine & Barnes 1999). There is some variation across singleton studies in both GA and sulcation order which may be accounted for by variability in anatomic criteria used, cohort size and the inclusion of multiple gestations and fetuses with CNS abnormalities.

Traditional anatomic studies have observed a delay in cortical maturation of approximately two weeks for twins relative to singletons (Chi & Dooling 1977). Only two previous studies describing fetal cortical maturation assessed by MRI have included a small subset of twins. Levine & Barnes (Levine & Barnes 1999) studied six sets of twins but did not specify chorionicity. Furthermore some fetuses had cerebral anomalies including ventriculomegaly in which cortical maturation has been shown to be delayed relative to normal singletons (Kyriakopoulou et al. 2014; Limperopoulos et al. 2013). They did not report a comparison with equivalent singleton data but report a variation in the lag of appearance of significant landmarks of zero to three weeks relative to anatomic specimens, which was similar to the lag observed in singletons. Dubois and colleagues (Dubois et al. 2008) measured sulcation index in 12 sets of twins (3 MCDAs and 9 DCDAs) and demonstrated delayed

but harmonious maturation relative to singleton controls, in line with the findings of this studyand those in anatomic specimens.

One possibility is that the delay in maturation observed between twins compared to singletons reflects a systematic bias in dating rather than a less mature brain for gestational age. Singleton charts are accurate to within 8 days (Butt et al. 2014), however growth discrepancy is common in twins (Salomon et al. 2005). Ultrasound based gestational age estimates in twins in the first trimester appear to straddle singleton normative data (Dias et al. 2010) however it is common, and recommended, practice to date by the larger twin to avoid missing growth restriction in the smaller twin (Morin & Lim 2011). These factors combined could theoretically result in a systematic overestimate in gestational age of up to two weeks that may account for the overall delay in cortical maturation seen in twins compared to singletons.

The early visualisation of the parieto-occipital fissure was delayed in ex-donors compared to ex-recipients. The Sylvian fissure, parieto-occipital fissure and central sulcus were less mature in ex-donors relative to their ex-recipient co-twins between 20 and 32 weeks. These differences were significant for within twin pair comparisons. This suggests that the pathology of TTTS is such that, even following successful treatment without apparent brain injury, genetically identical twins have already been influenced by imbalance in their shared in utero environment and embarked on divergent paths of cerebral development.

Singleton fetuses with CNS abnormalities have been shown to have delays in global cerebral development relative to normal controls (Levine & Barnes 1999). Slagle and colleagues (Slagle et al. 1989) demonstrated that in utero brain injury was associated with reduced cortical development. Neonates, imaged within the first few days of life, with intraventricular haemorrhage and ventricular dilatation, parenchymal haemorrhage, or cystic periventricular leukomalacia had local delays in convolutional maturation. Alterations in local nutrients and associated decreases in cellular proliferation have been hypothesised to delay sulcal development. However the fetuses in the twin cohort in this study all had clinically normal brains at the time of imaging and therefore local structural abnormalities cannot account for our observed within pair differences.

Assessment of maturation in fetuses with both early and late onset growth restriction has revealed disruption in development relative to healthy singletons (Dubois et al. 2008; Egaña-Ugrinovic et al. 2013; Egaña-Ugrinovic et al. 2014;). Cortical sulcation appears increased relative to surface area and may relate to reduced cortical thickness. The reduction in cortical thickness appeared to correlate with poorer subsequent neurobehavioural outcome (Dubois et al. 2008; Egaña-Ugrinovic

et al. 2014). These disruptions were particularly marked around the insular and cingulate regions (Egaña-Ugrinovic et al. 2013; Egaña-Ugrinovic et al. 2014). Scott and colleagues (2013) modelled surface curvature in 16 fetuses with ventriculomegaly compared to controls and reported reduced folding of the parietal occipital fissure. This limited finding may reflect the early and narrow gestational age range assessed in the study.

The lack of significant differences in the current study for later appearing sulci may relate to the small sample size for these landmarks. The sample size reflects the GA distribution of the cohort which was not broad enough to capture development of later appearing sulci. Therefore one cannot conclude whether differences observed reflect limited regional delay in appearance specific to the parietal occipital region or simply failure to capture global delay. However it is noteworthy that the studies in growth restricted fetuses (Egaña-Ugrinovic et al. 2013; Egaña-Ugrinovic et al. 2014) and those with ventriculomegaly (Scott et al. 2013) also report a concentration of disruption in the parieto-occipital and insular regions. Future studies should include imaging at later gestation to evaluate whether within pair differences in cortical maturation reflect regional or global effects.

In the cohort in this study ex-donors had greater opercular distances, and reduced insular distances relative to their ex-recipient co-twins leading to an overall reduction in Sylvian ratio. The Sylvian fissure is considered recognisable by around 14 weeks as a smooth depression (Toi et al., 2004). Opercularisation then proceeds as the temporal and parietal lobes form and enlarge to enclose the insula in a precisely defined manner until it is completely embedded by the operculum at 40 weeks GA (Chen et al., 1996). The role of the insula cortex is diverse (Nieuwenhuys 2012) encompassing emotion (Craig 2009), motor control (Fink et al. 1997), speech (Ackermann & Riecker 2010) as well as higher cognition including self-awareness (Tsakiris et al. 2007). The observed intertwin difference in development of the Sylvian fissure may reflect the early onset and longer time course of maturation in utero. Underdevelopment of the insula may relate to effects of underperfusion in donor twins during a crucial developmental time period prior to treatment. The quantitative rather than categorical assessment of Sylvian fissure development may have also increased the sensitivity for detection of aberrations.

Several hypotheses have been proposed regarding the process of normal gyrification in the fetal brain (Geschwind & Rakic 2013). Whilst it is clear that much is genetically pre-programmed, the factors that control expression of gene patterns as well as the mechanical sequence of events are poorly understood (Clowry et al. 2010). The extent to which sulcation is an active process of convolutional growth (Welker 1990), a passive consequence of cortical growth (Toro & Burnod 2005) or the result of differential tension in underlying white matter fibres (Hilgetag & Barbas 2006;

Van Essen 1997) is debated. Imaging studies suggest that tissue volume expands under gyral crests adjacent to sites of sulcation (Rajagopalan et al. 2011) and that it is growth of white matter axons that leads to tangential gyral expansion (Nie et al. 2012). Aberrations in cortical folding may therefore actually reflect primary dysfunction in cortical connectivity. This would account for a broad range of associations with later neurocognitive impairment. The potential influence of epigenetic or prenatal environmental factors is unclear (Dubois et al. 2008). However the response of the developing brain to injury is very different to that of the adult brain (Staudt 2010). Chronic hypoxia or early ischaemic injury as a result of haemodynamic flow imbalance between co-twins may play a role in stimulating reorganisation of cortex and corticospinal tracts in early fetal life.

3.4.6 Conclusion

In this chapter the revised cortical scoring system has been used to capture cortical maturation between 22 and 32 weeks GA in twins treated for TTTS. Cerebral development is delayed relative to singleton controls. The ex-donor fetal cerebral cortex appears less mature relative to its genetically identical co-twin. Whether this reflects global or regional delays in development is unclear. Early blood flow imbalance and discordant activation of the renin-angiotensin system in utero may have an effect on developing white matter tracts leading to aberrant cortical folding.

3.5 [1]HMRS assessment of cerebral metabolic ratios

3.5.1 Background

HMRS is an evolving tool for studying *in vivo* brain metabolism, although motion remains a major technical challenge. A range of biochemical changes that occur during fetal brain development have been quantified in normal singleton cohorts (Kok et al. 2002; Girard, Gouny, et al. 2006; Kreis et al. 2002) and in selected abnormal cohorts (Sanz-Cortes et al. 2015; Story et al. 2011; Story et al. 2013). No studies have investigated the feasibility of studying in vivo brain metabolism in twin gestations. In this chapter an optimised approach has been used to evaluate the metabolic differences between ex-recipient and ex-donor twins treated for TTTS. Spectra were obtained at short to long echo times to allow for detection and quantification of different metabolites.

3.5.2 Aims

To quantify differences in brain metabolite ratios between ex-recipient and ex-donor twins treated for TTTS at echo times of 42msec, 136msec and 270msec.

3.5.3 Methods

3.5.3.1 Acquisition of spectra

MRS data were acquired using the monovoxel Point Resolved Spectroscopy sequence (PRESS). A T2-weighted ssTSE was obtained prior to the ^{1}HMRS sequence to establish fetal position and guide placement of the region of interest. A 20x20x20mm voxel was placed centrally over the fetal brain sampling both white and grey matter. Data were acquired at three different echo times (42ms, 136ms, 270ms) to facilitate detection and quantification of a variety of metabolites. Thirty-two spectra were obtained at each echo time. Acquisition was performed using water suppression due to the large size of the water peak that may distort baseline and obstruct metabolite quantification. A further T2-weighted ssTSE scan was acquired following HMRS data acquisition to check that no significant change in fetal position had occurred.

3.5.3.2 Post-processing of spectra

Analysis of ^{1}HMRS spectra and quantification of metabolites was performed offline using jMRUI (www.mrui.uab.es/mrui/mrui_homePage.shtml, version 2.2). Spectrum quality was judged

according to the signal-to-noise ratio (height of metabolite peaks compared to background noise) and spectral resolution (peak width and differentiation between metabolites). Aberrant spectra indicative of fetal motion (as shown in figures 36 and 37 section 2.3) were removed. The remaining spectra were summed into a final spectrum. Datasets in which greater than 50% of spectra were affected by some form of motion corruption were deemed unsuitable for analysis. The water peak at 4.7 ppm was removed after noise reduction. Expected metabolite peaks were identified at each echo time based on their position within the spectrum. Metabolite ratios were calculated at each echo time as shown below in table 25. The presence of lactate was recorded at echo time 136ms.

Echo Time (ms)	Metabolite ratios
42	MI:Cho
	MI:Cr
	Cho:Cr
136	NAA:Cho
	NAA:Cr
	Cho:Cr
270	NAA:Cho
	NAA:Cr
	Cho:Cr

Table 25. Metabolite ratios quantified at each echo time.
The presence of lactate was also recorded at echo time 136ms. (MI: Myo-inositol; Cho: Choline; Cr: Creatine; NAA: N-acetylaspartate)

3.5.3.3 Statistical analysis

Fisher's exact test was used to evaluate differences between ex-recipients and ex-donors in the frequency of detection of lactate.

3.5.4 Results

3.5.4.1 Spectral acquisition at echo time 136ms

3.5.3.1.1 Cohort

Spectra were obtained for both fetuses in 13 twin pairs and in one additional ex-donor fetus. Data were suitable for analysis in 54% of ex-recipients and 50% of ex-donors. Only three pairs of twins had spectra suitable for interpretation in both twins. Median GA was similar for ex-donor (27.1 weeks; range 24-32) and ex-recipient fetuses (25.7; range 24-32) ($p<0.05$). One ex-donor fetus had evidence of grade IV IVH on MR imaging.

3.5.3.1.2 Metabolite ratios in fetuses at echo time 136ms

NAA, choline and creatine were identified in all spectra (figure 78).

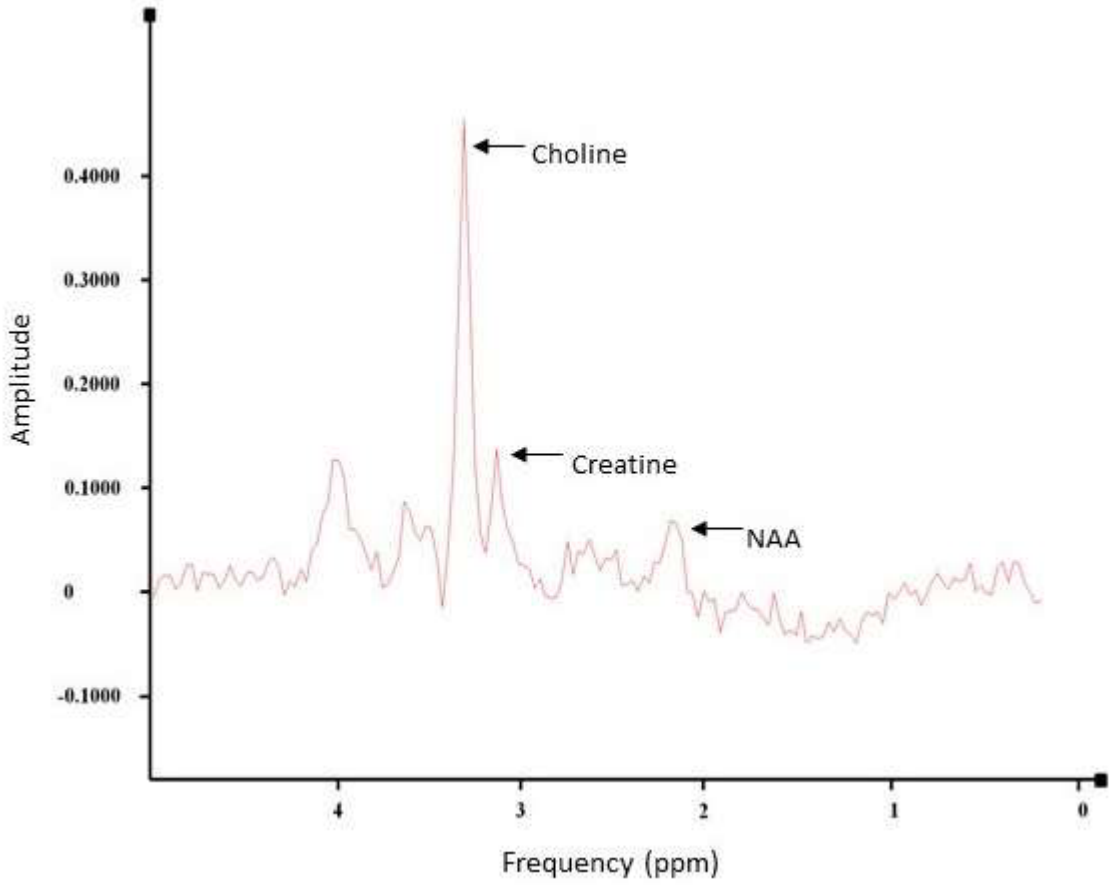


Figure 78. Example of a typical spectrum acquired at echo time 136ms.

Black arrows identify choline, creatine and NAA peaks. (NAA: N-acetylaspartate)

In ex-recipient fetuses no significant correlation was found between increasing GA and either NAA:Cho (Spearman's r = 0.67, p = 0.07), NAA:Cr (Spearman's r = 0.36, p = 0.22) or Cho:Cr (Spearman's r = -0.46, p = 0.13 respectively).

In ex-donor fetuses no significant correlation was found between increasing GA and either NAA:Cho or NAA:Cr (Spearman's r = 0.18, p = 0.36; Spearman's r = -0.54, p = 0.10 respectively). Cho:Cr significantly decreased with increasing GA (Spearman's r = -0.71, p <0.05).

There was no difference in either slope or intercept between ex-recipients and ex-donors for NAA:Cho (p = 0.4, p = 0.6) or NAA:Cr (p = 0.1, p = 0.8) and GA. There was no statistically significant difference for the slope of the relationship between the Cho:Cr ratio and GA between ex-recipients and ex-donors (p = 0.5). The intercept was lower in ex-donors but not significantly so (p = 0.09).

The distribution of metabolite ratios for NAA:Cho and NAA:Cr in ex-recipient and ex-donor fetuses is shown as box and whisker plots in figure 79.

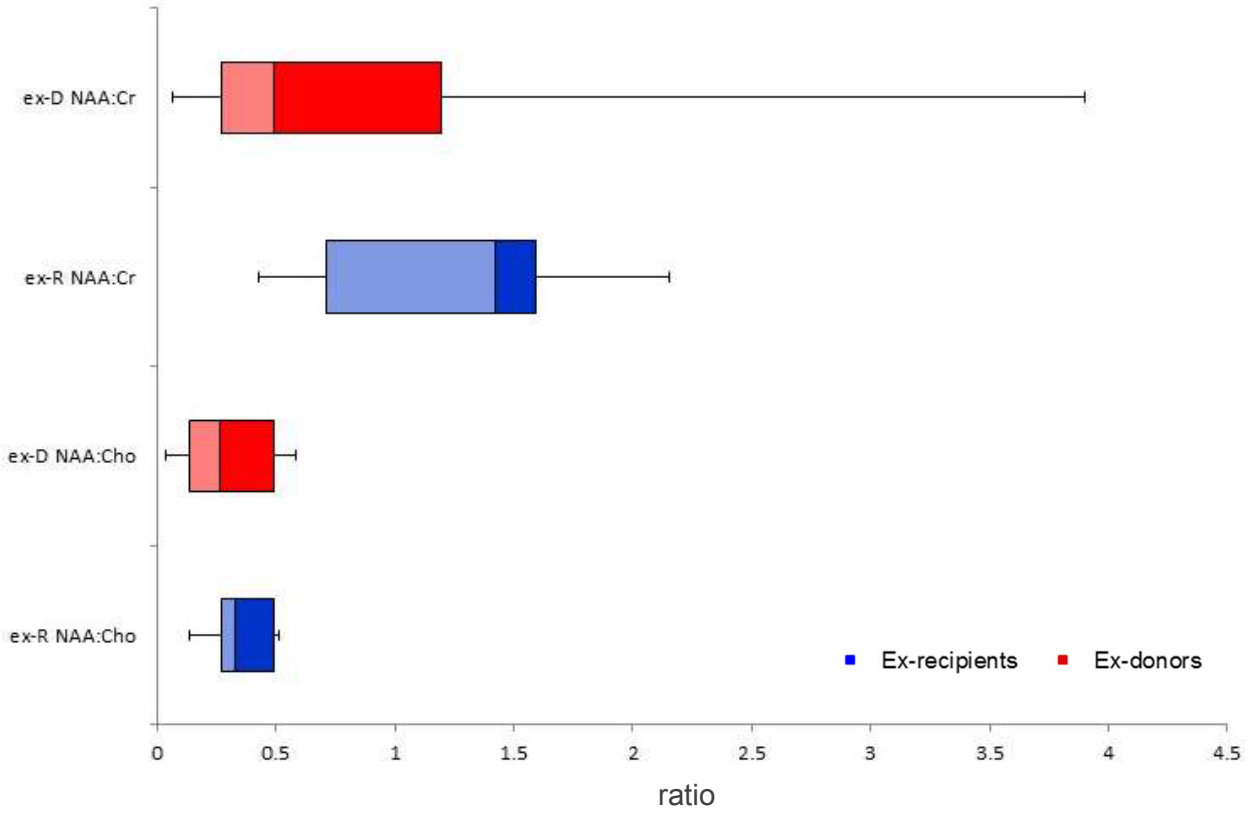


Figure 79. NAA:Cr and NAA:Cho at echo time 136ms for ex-recipient and ex-donor fetuses.
There was no significant difference in NAA:Cr or NAA:Cho ratios between ex-recipient and ex-donor fetuses. (NAA: N-acetylaspartate; Cr: Creatine; Cho: Choline)

The distribution of metabolite ratios for Cho:Cr in ex-recipient and ex-donor fetuses is shown in figure 80 as a scatter chart plotted against GA as there was a significant negative correlation between Cho:Cr and GA.

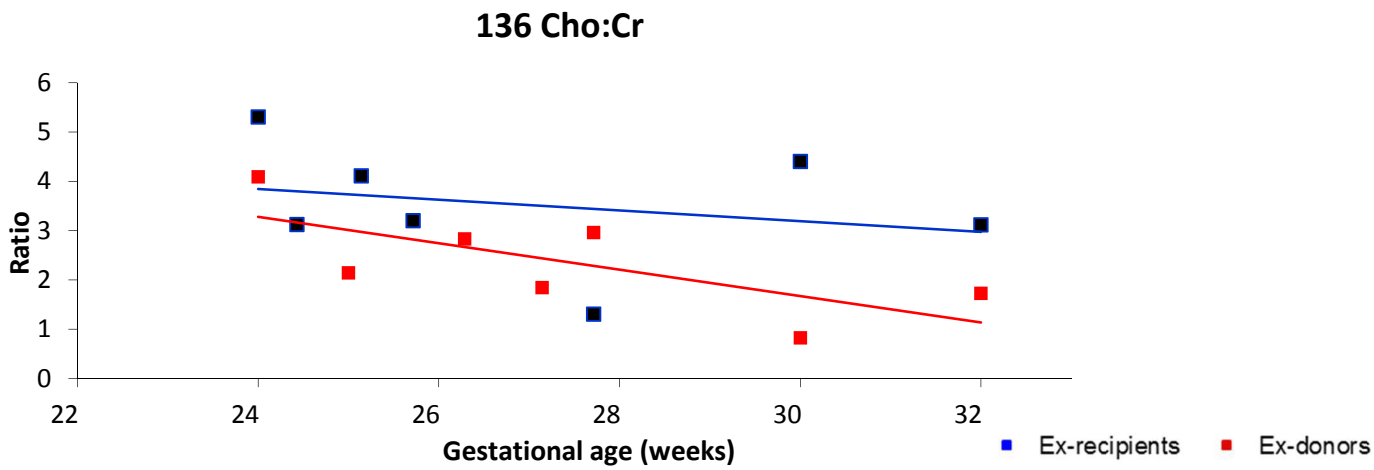


Figure 80. Relationship between GA and Cho:Cr at echo time 136ms for ex-recipient and ex-donor fetuses.
Cho:Cr decreased significantly with gestational age in ex-donors ($p<0.05$) but not in ex-recipients. (Cr: Creatine; Cho: Choline)

Lactate was present in two of the seven ex-recipient fetuses and in four out of seven ex-donor fetuses. This difference was not significantly different ($p = 0.35$). One of the ex-donor fetuses in whom lactate was present had grade IV IVH. Another ex-donor fetus with lactate detected had severe FGR and although there was no evidence of overt brain injury, the appearance of the brain was indistinct and could not be described as normal. The remaining fetuses had structurally normal brains. An example of a spectrum with a lactate peak present is shown in figure 81.

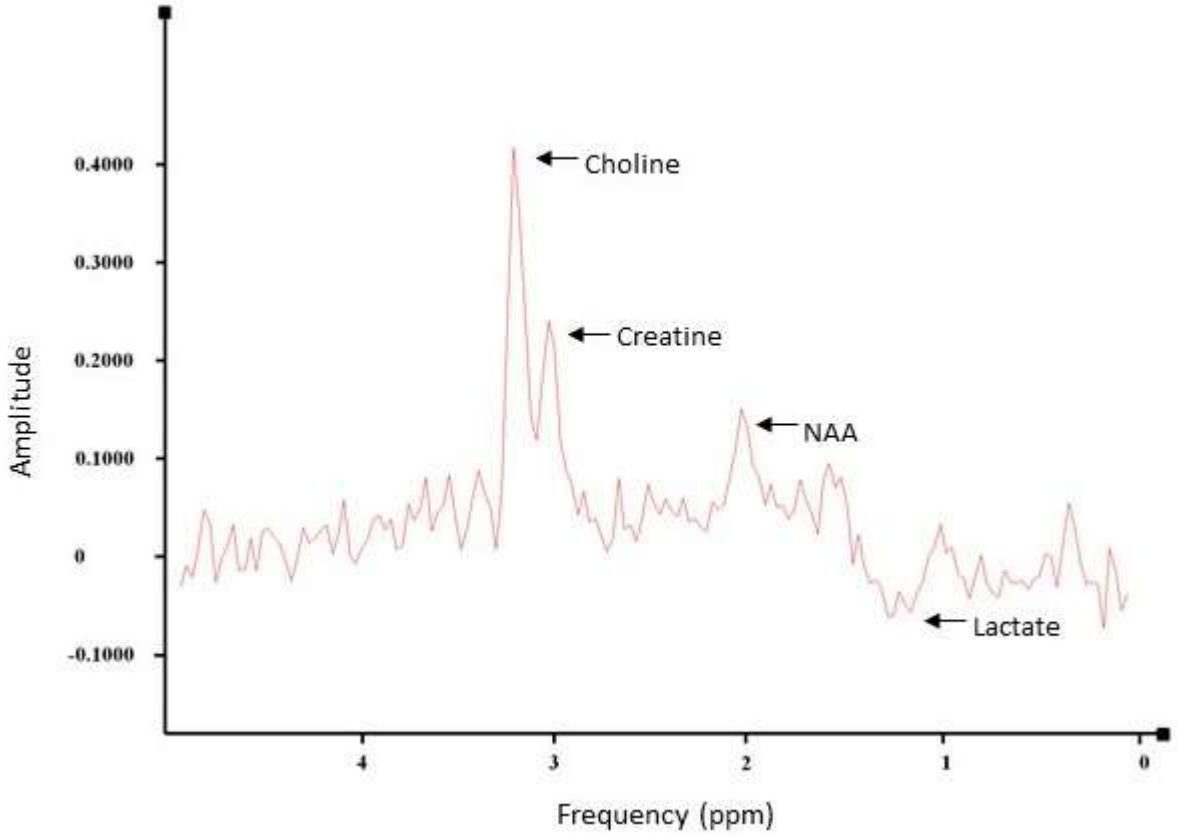


Figure 81. Example of lactate present in a spectrum acquired at echo time 136ms.

Metabolite ratio peaks are indicated. Lactate is present as a bifid inverted peak at 1.2ppm. (NAA: N-acetylaspartate)

Table 26 describes the case histories and outcomes for those fetuses with detectable lactate at echo time 136ms.

Case no	ex-R / ex-D	GA at laser	GA at MRI	MRI findings	Outcome
36	ex-R	22 1/7	25 1/7	normal	Neonatal death
30	ex-R	20 4/7	27 5/7	normal	Live birth; normal NDA at 1 year
6	ex-D	21	25	abnormal	Fetal death
28	ex-D	24	27 1/7	normal	Live birth
19	ex-D	19 4/7	24	normal	Live birth
5	ex-D	22	26 2/7	Grade IV IVH	Live birth; Neurodevelopmental delay at 1 year

Table 26. Summary characteristics and outcomes for fetuses with cerebral lactate detected.

Lactate was detected in six of 14 fetuses with interpretable spectra at echo time 136ms.

3.5.3.2 Spectral acquisition at echo time 42ms

3.5.3.2.1 Cohort

Spectra were obtained in 9 twin pairs and in one ex-donor and one ex-recipient fetus from different pairs. 60% of spectra were considered suitable for analysis in each group. Median GA was similar for ex-donor (26.6 weeks; range 24-30) and ex-recipient fetuses (25.4; range 24-30) ($p<0.05$). Two ex-donor fetuses had evidence of grade IV IVH on MR imaging.

3.5.3.2.2 Metabolite ratios in fetuses at echo time 42ms

MI, choline and creatine were identified in all spectra. An example of a typical spectrum is shown in Figure 82.

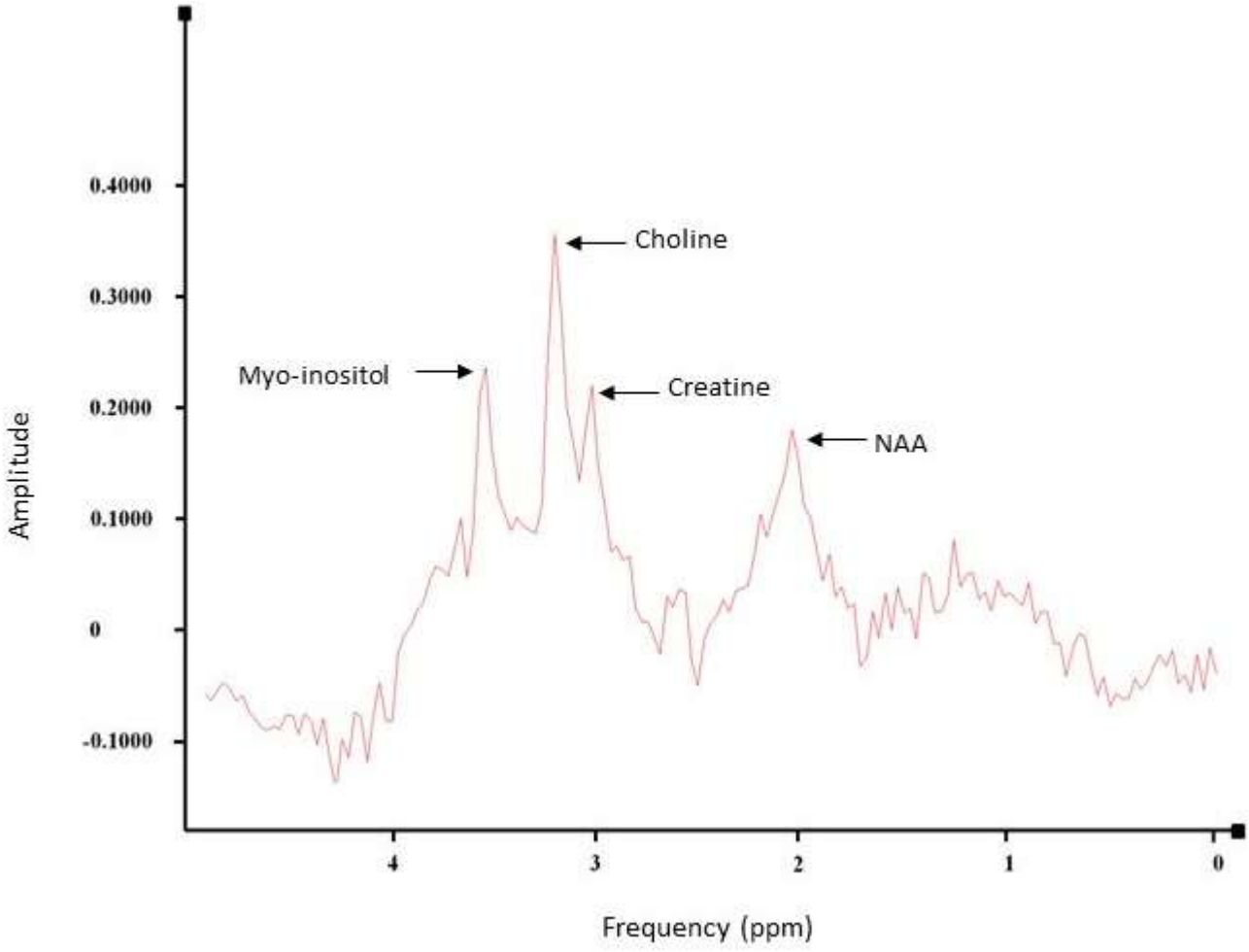


Figure 82. Example of a typical spectrum acquired at echo time 42ms.

Black arrows indicate MI, choline, creatine and NAA peaks. (NAA: N-acetylaspartate)

In ex-recipient fetuses MI:Cho, MI:Cr and Cho:Cr ratios did not significantly vary with increasing GA (Spearman's r = -0.03, p = 0.5; Spearman's r = 0.2, p = 0.4; Spearman's r = -0.03, p = 0.5).

In ex-donor fetuses MI:Cho, MI:Cr and Cho:Cr did not vary significantly with increasing GA (Spearman's r = -0.66, p = 0.07; Spearman's r = -0.26, p = 0.28; Spearman's r = -0.03, p = 0.46 respectively).

There was no difference in either slope or intercept between ex-recipients and ex-donors for MI:Cho (p = 0.2, p = 0.4), MI:Cr (p = 0.5, p = 0.6) or Cho:Cr (p = 0.9, p = 0.6) and GA.

The distribution of metabolite ratios for ex-recipient and ex-donor fetuses is shown in figure 83.

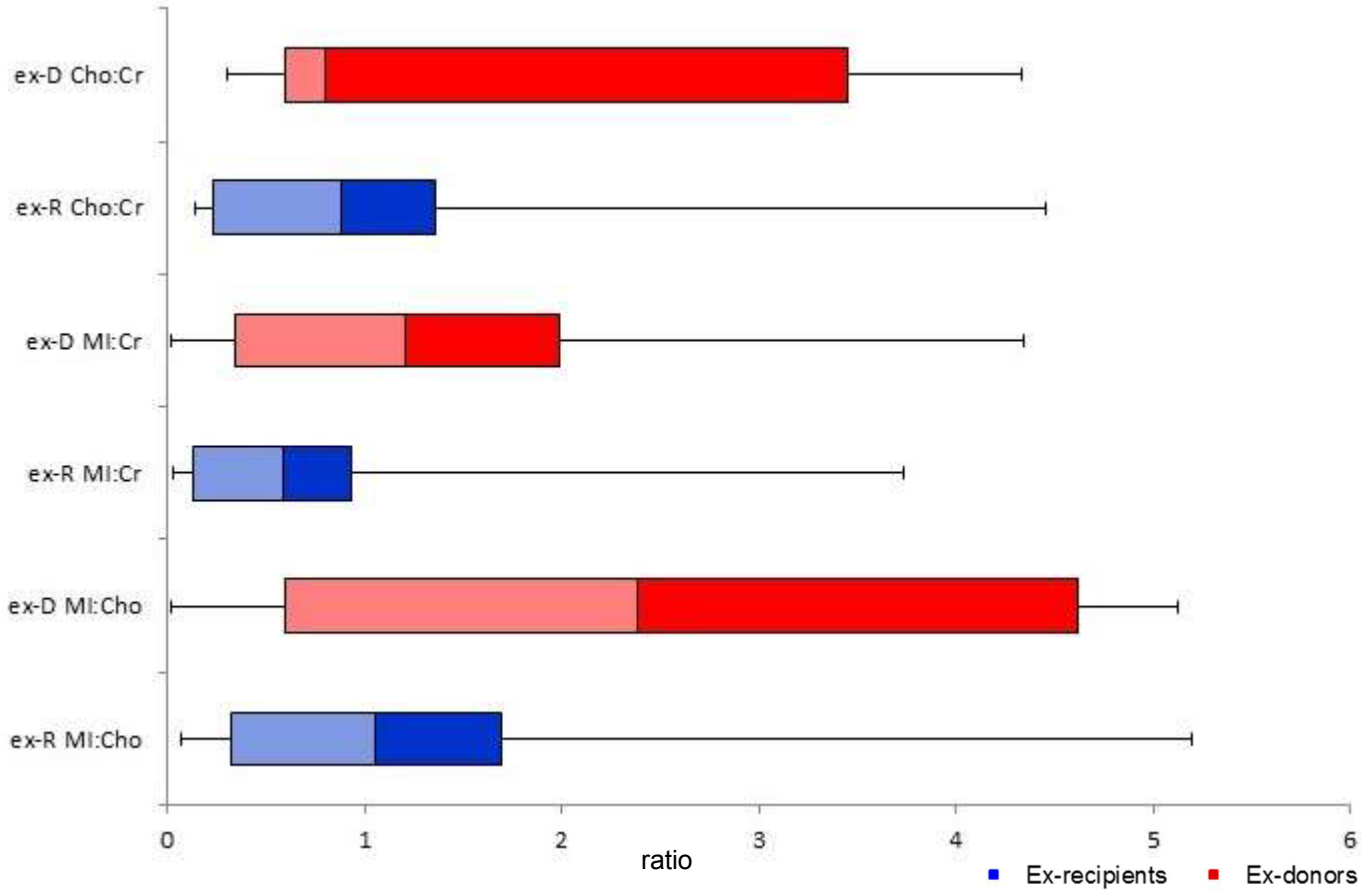


Figure 83. Metabolite ratios at echo time 42ms in ex-recipient and ex-donor fetuses.

There was no significant relationship with gestational age. (Cho: Choline; Cr: Creatine; MI: Myo-inositol)

3.5.3.3 Spectral acquisition at echo time 270ms

3.5.3.3.1 Cohort

Spectra were obtained in 7 twin pairs as well as 2 ex-recipient and 2 ex-donor fetuses from different pairs. As only 22% of spectra obtained were considered suitable for analysis, further analysis for statistical purposes was not undertaken.

3.5.3.3.2 Metabolite ratios in fetuses at echo time 270ms

NAA, choline and creatine were identified in interpretable spectra. An example spectrum is shown in Figure 84.

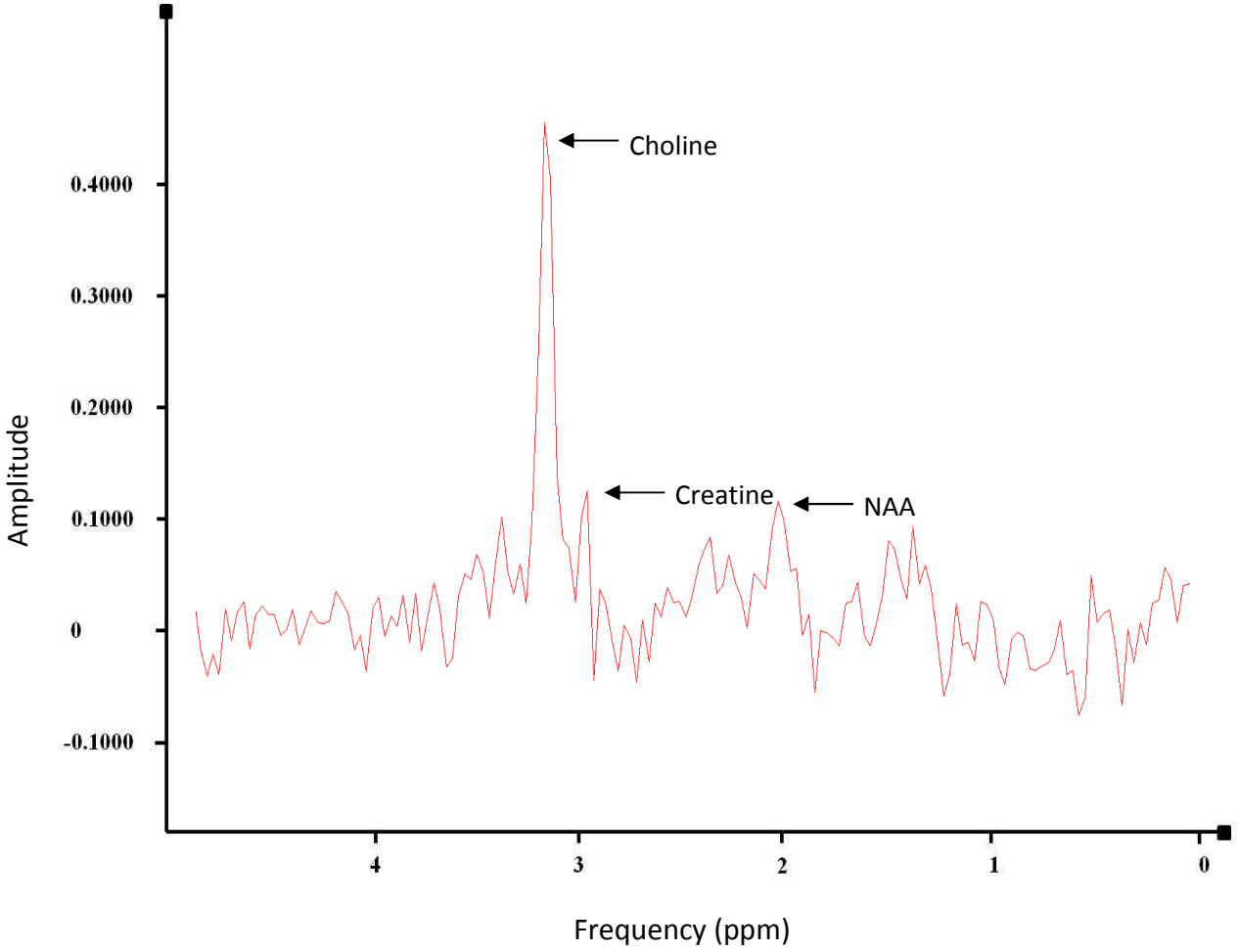


Figure 84. Example of a typical spectrum at echo time 270ms.

Black arrows indicate choline, creatine and NAA peaks. (NAA: N-acetylaspartate)

3.5.3.4. Reproducibility

Complete analysis of ten datasets (half at echo time 136ms and half at echo time 42ms) was repeated on a separate occasion to evaluate intra-rater variability. This re-analysis included all stages of post-processing from the raw data. Intra-class correlation coefficient was 0.91 ($p < 0.001$) suggesting good agreement.

3.5.5 Discussion

Acquisition of ^{1}HMRS data has demonstrated the presence of lactate in almost half of fetuses studied at echo time 136ms. There was a significant decrease in Cho:Cr ratios with advancing GA at echo time 136ms in ex-donors. Analysis was limited by small overall sample size and a high proportion of motion corrupted spectra that were not suitable for analysis.

The relatively long acquisition time in fetal spectroscopy is an extra burden on an already long imaging process in twin pregnancy. Spectroscopy sequences were performed for each fetus after T1 and T2 weighted images necessary for clinical examination of brain structure were obtained. As a result many participants terminated the imaging examination before acquisition of complete ^{1}HMRS data and interpretation of the data analysis was limited by the small sample size.

In addition a high proportion of spectra subsequently acquired were excluded at the post-processing analysis stage. At echo time 136ms and 42ms, 50% to 60% of spectra were considered suitable for analysis but at echo time 270ms only 22% were suitable. Of note data acquisition at echo time 270ms tended to be performed last. Reproducibility was high and there was no difference between ex-donor and ex-recipient rates of interpretable spectra. The majority of spectra that were unsuitable for analysis were excluded due to the lack of water peak suggestive of excessive motion. Motion may have been present in either the fetus, the mother or both. As the ^{1}HMRS sequence was always performed at the end of the imaging examination, participants were more restless by this stage. Spectra were also excluded due to contamination by large lipid molecules which distort or mask the resonances of molecules of interest. Lipid contamination may occur due to motion causing the voxel to become inadvertently placed over a region outside of the fetal brain.

Previous studies in singletons report rates of acquisition of interpretable spectra of 60 to 70% (Sanz-Cortes et al. 2015; Berger-Kulemann et al. 2013; Story et al. 2013; Story et al. 2011). Neither fetal presentation nor gestational age appear to influence the quality of spectra acquired (Berger-

Kulemann et al. 2013). There are no existing spectroscopy studies in twin pregnancies for comparison. Studies of frequency of fetal motion in twin pregnancies compared to singletons are not in agreement (Mulder et al. 2012; Sadovsky et al. 1986). The lower acquisition rate in the current study may reflect increased fetal motion in twin pregnancies as well as increased maternal motion due to longer total study time.

Analysis of the relationship between advancing GA and metabolite ratios revealed a trend towards decreasing Cho:Cr ratio with GA at echo time 136ms in ex-recipient fetuses and a significant decrease in Cho:Cr in ex-donors. This is consistent with results from studies in normal singletons (Berger-Kulemann et al. 2013; Story et al. 2011; Kok et al. 2002) and in growth restricted fetuses (Story et al. 2011). It is not clear whether the decreasing Cho:Cr ratio reflects a reduction in choline levels with advancing gestation or an increase in creatine. Absolute choline levels have been observed to decrease with gestational age in utero (Girard, Gouny, et al. 2006) as well as remain stable (Kok et al. 2002). Although an increase in absolute creatine levels with advancing gestational age has been detected at short echo times (Girard, Gouny, et al. 2006), the creatine peak generally appears relatively stable across gestation in normal singletons (Kok et al. 2002; Girard, Gouny, et al. 2006; Heerschap et al. 2003). It is therefore been suggested that decreasing Cho:Cr ratios reflect a reduction in choline levels due to acceleration in myelination and cell membrane formation as brain maturation occurs (Girard, Fogliarini, et al. 2006).

Of interest is the observation that the decreasing Cho:Cr ratio with GA only reached statistical significance in ex-donor fetuses. Furthermore Cho:Cr ratios appeared lower overall in ex-donor fetuses compared to co-twins, although this did not reach statistical significance. If a decrease in Cho:Cr with GA does indeed reflect reduction in choline due to accelerated myelination, our observation suggests more advanced white matter maturity in ex-donors compared to ex-recipients. This would be surprising given that the assessment of maturation according to cortical folding in the previous chapter suggested that ex-donor brains were less mature than their ex-recipient co-twins. An alternative explanation could be that lower Cho:Cr in ex-donors reflect a relative increase in creatine levels rather than a decrease in choline. Creatine is much more concentrated in glial cells than in neurons (Urenjak et al. 1993). As glial cells have an important role in response to inflammation and injury, it may be that chronic hypoxia in the ex-donor fetus results in more creatine rich glial cell proliferation compared to ex-recipients. Within pair comparisons of absolute metabolite levels in a larger sample population may clarify this.

Lactate was detected at echo time 136ms in 43% of fetuses in our study and twice as frequently in ex-donors compared to ex-recipients. Although there was no statistically significant difference

between lactate detection in ex-recipients compared to ex-donors, this may reflect the small sample size. The significance of the presence of lactate in the fetal brain is controversial. In the adult brain the presence of lactate is an abnormal finding suggestive of malignancy, infection and ischaemia amongst others (Shih et al. 2004). Animal models suggest that cerebral lactate is increased during hypoxia in the developing brain (Moxon-Lester et al. 2007) (van Cappellen et al. 1999). The presence of increased lactate associates with severity of hypoxic brain damage (Amess et al. 1999). In asphyxiated neonates the presence of brain lactate has been shown to be associated with neurodevelopmental delay (Barkovich et al. 1999; Amess et al. 1999).

However cerebral lactate has been detected in both preterm AGA and term newborns with normal brain appearances and these levels have been shown to decrease with advancing age (Xu et al. 2011; Cheong et al. 2006). In fetal ^{1}HMRS studies cerebral lactate has been demonstrated in apparently normal fetuses without adverse perinatal outcome (Story et al. 2011). Berger-Kulemann et al 2013 (Berger-Kulemann et al. 2013) studied a cohort of 75 fetuses that included a broad range of pathologies, as well as normal cases, and found no correlation between the presence of cerebral lactate and fetal pathology. Furthermore cerebral lactate was not associated with adverse neurodevelopmental outcome at one year.

Animal models suggest that the presence of lactate may indicate an adaptive response of the developing brain to hypoxia. Studies in newborn growth restricted piglets show that cerebral lactate reflects increased glycolytic activity as opposed to impaired mitochondrial function and may be neuroprotective (Moxon-Lester et al. 2007). In addition the immature brain appears to have the ability to metabolise lactate (Hernández et al. 1980; Vannucci & Vannucci 2000). Lactate released by astrocytes may be used as an oxidative substrate in aerobic metabolism by cortical neurons under specific conditions (Pellerin et al. 1998). This role of lactate as a supplementary fuel source in the developing brain has been widely debated (Korf 2006).

Whilst studies are limited, lactate does appear to be more frequently detected in growth restricted preterm neonates and fetuses compared to appropriately grown controls (Story et al. 2011; Leth et al. 1995). No difference in Lac:Cho ratios was shown between SGA and AGA fetuses in one fetal spectroscopy study but sample size was small and lactate resonances generally low (Roelants-van Rijn, van der Grond, et al. 2004). Cerebral lactate has been observed in 20% of fetuses with congenital heart disease (Limperopoulos et al. 2011) and two thirds of fetuses with hydrocephalus (Roelants-van Rijn, Groenendaal, et al. 2004). However the proportion of fetuses in this chapter with detectable lactate is much higher than rates observed in either apparently normal controls or those with pathology. Cerebral structural abnormality cannot account for this as no overt abnormality was

seen in the majority of fetuses with an identifiable lactate resonance. It may be that lactate presence reflects a compensatory response to chronic hypoxia induced impaired mitochondrial activity. This will certainly have occurred as a result of flow disturbances pre laser treatment and may be ongoing but subclinical following treatment.

3.5.6 Conclusion

The current study has shown that whilst ^{1}HMRS is particularly challenging in the TTTS cohort, the acquisition of good quality spectra is feasible particularly at echo times of 136ms and 42ms. Cerebral lactate was detected in almost half of the fetuses studied and may reflect an adaptive response to chronic hypoxia.

3.6 Neurodevelopmental Assessment

3.6.1 Background

The reported incidence of neurodevelopmental impairment in survivors of TTTS treated by fetoscopic laser therapy varies between 7% to 20% (Graeve et al. 2012; Gray et al. 2011; Lenclen et al. 2009; Ortibus et al. 2009; Rustico et al. 2012; Salomon et al. 2010; Sutcliffe et al. 2001; van Klink et al. 2011). Multiple follow up studies have failed to find a significant association between recipient or donor survivors and risk of neurodevelopmental delay (Maschke et al. 2011; Senat et al. 2004; van Klink et al. 2011). However the increased mortality in ex-donor twins (Chmait et al. 2011; Cincotta et al. 2009; Rossi & D'Addario 2009a) may have confounded this observed lack of association. In this chapter preliminary results of neurodevelopmental assessment in the cohort of surviving twins following treatment for TTTS are presented.

3.6.2 Aims

To assess the neurodevelopmental outcome of twin survivors of TTTS at 1 and 2 years of age and correlate these developmental scores with size of within pair differences in MR volumetric and maturation measures.

3.6.3 Methods

3.6.3.1 Developmental assessment

All parents that enrolled in the research program following successful treatment for TTTS were invited for a developmental assessment of their surviving children at one and two years of age. Assessment at one year of age (corrected for prematurity) was performed predominantly using the Griffiths Mental Development Scales (GMDS 0-2) by a clinical psychologist (BH) or paediatrician (AC or MM). Assessments at two years of age (corrected for prematurity) were performed predominantly using the Bayley Scales of Infant and Toddler Development-III (BSID-III). Developmental delay is diagnosed if scores are 1SD below the mean. Parent completed Ages & Stages Questionnaires (ASQ) were posted to parents that we were unable to contact or who had declined attendance for assessment.

3.6.3.2 Statistics

Comparison between cohort characteristics was by unpaired t-test or Mann-Whitney U test as appropriate. Comparison between incidence of developmental delay in ex-recipient and ex-donor children was by Fisher's Exact test. Developmental score comparisons within twin pairs were performed using paired t-test. Correlation between developmental scores and within pair measurement differences was assessed using Spearman's r correlation coefficient and Bonferroni correction applied for multiple comparisons. A 2-tailed $p < 0.05$ was considered significant unless stated otherwise.

3.6.4 Results

3.6.4.1 Cohort

A total of 58 children that had been live born following treatment for TTTS and enrolled in the fetal research program were invited for a developmental assessment when they reached their first year of age. A complete assessment was performed in 14 children at one year of age representing a 24% follow up rate at a mean chronological age of 15 (± 1.8) months and a range of 12-18 months (Table 27).

Neurodevelopmental assessment at 1 year of age		
Total children assessed	14/58	
Male	11	
Assessment age (months)	14.5	(12-18)
GA at delivery (months)	33.1	(27.7-36.6)
STB volume difference (%)	22.13	(28.3)
Sylvian ratio difference	0.31	(0.22)

Table 27. Assessment at 1 year of age (corrected for prematurity) cohort characteristics.
Age measures are presented as medians and (range). Sylvian fissure ratio and percentage STB volume within twin pair differences are shown as mean and (SD). (GA: Gestational age; STB: Supratentorial brain tissue)

Eleven children were assessed on the GDMS 0-2 and three children were assessed on the BSID-III. Eight were ex-recipients and six were ex-donors. All of the ex-donors were assessed with their ex-recipient co-twins. Of the remaining two ex-recipients that completed an assessment, one was a single survivor following selective feticide of the ex-donor co-twin (case 1). The other ex-recipient had a living ex-donor co-twin that was unable to attend as he remained an inpatient following pre-term delivery (case 9). In total 44 infants did not complete an assessment as shown in the flow chart below (figure 85), due to the following reasons: unable to complete due to time constraints (2), neonatal death (4), declined due to illness (3), refusal to attend for another reason (25), unable to contact parents (6), assessment missed (4).

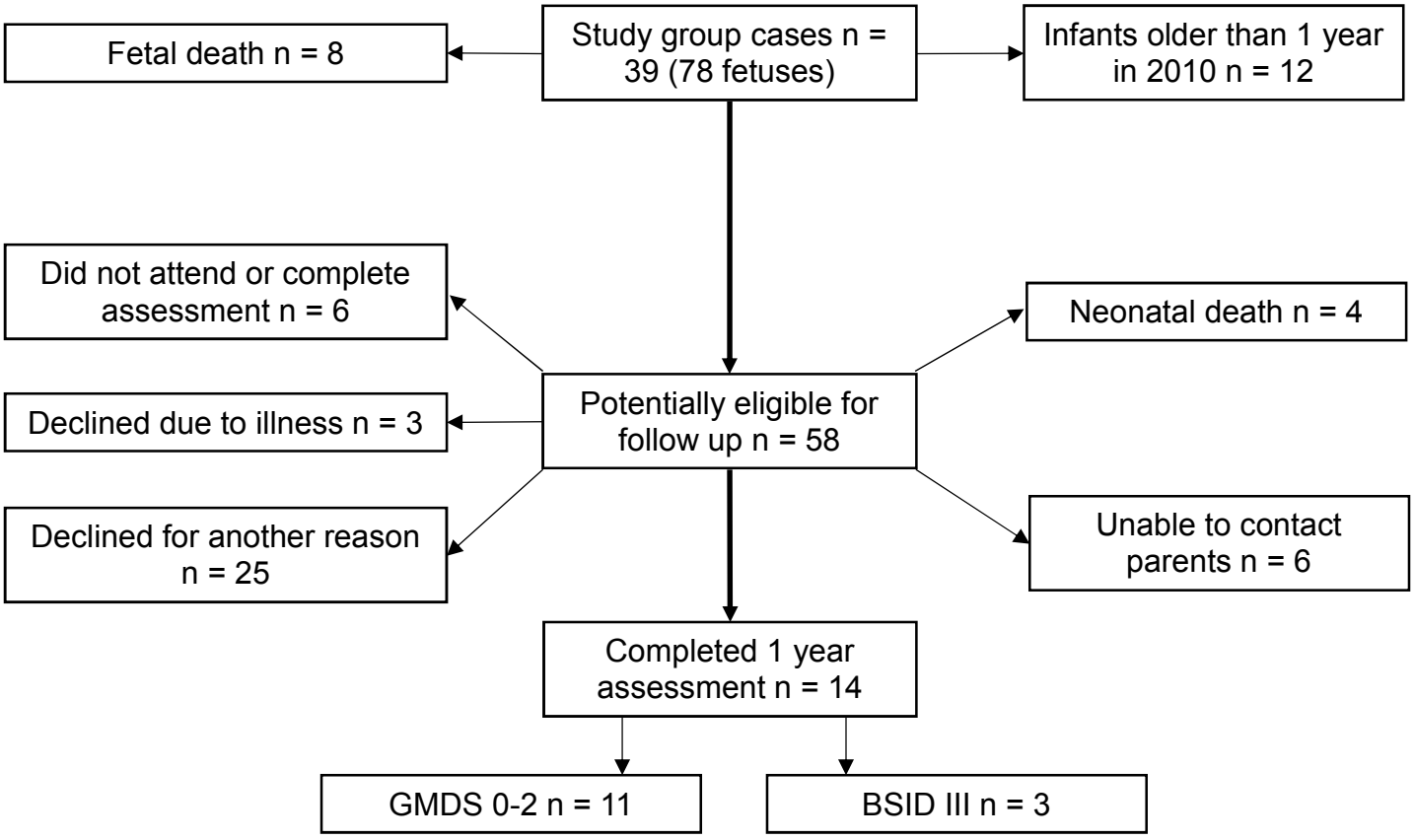


Figure 85. Participation in Neurodevelopmental Assessment at 1 year.

Fourteen of 58 potentially eligible children completed an assessment at one year of age.

A complete assessment was performed in 11 (of 43 eligible) children at 2 years of age representing a 25.6% follow up rate at a mean chronological age of 27.7 (± 3) months and a range of 24 to 33 months (Table 28).

32 infants did not complete an assessment due to the following reasons as shown in the flow chart below (figure 86) : declined due to illness (1), refusal to attend for another reason (20), unable to contact parents (9), assessment missed (2).

Neurodevelopmental assessment at 2 years of age		
Total children assessed	11/43	
Male	8	
Assessment age (months)	27	(24-33)
GA at delivery (weeks)	33.1	(29.4-36.6)
STB volume difference (%)	17.8	(14.5)
Sylvian ratio difference	0.43	(0.15)

Table 28. Assessment at 2 years of age (corrected for prematurity) cohort characteristics.

Age measures are presented as medians and (range). Sylvian fissure ratio and percentage STB volume within twin pair differences are shown as mean and (SD). (GA: Gestational age; STB: Supratentorial brain tissue)

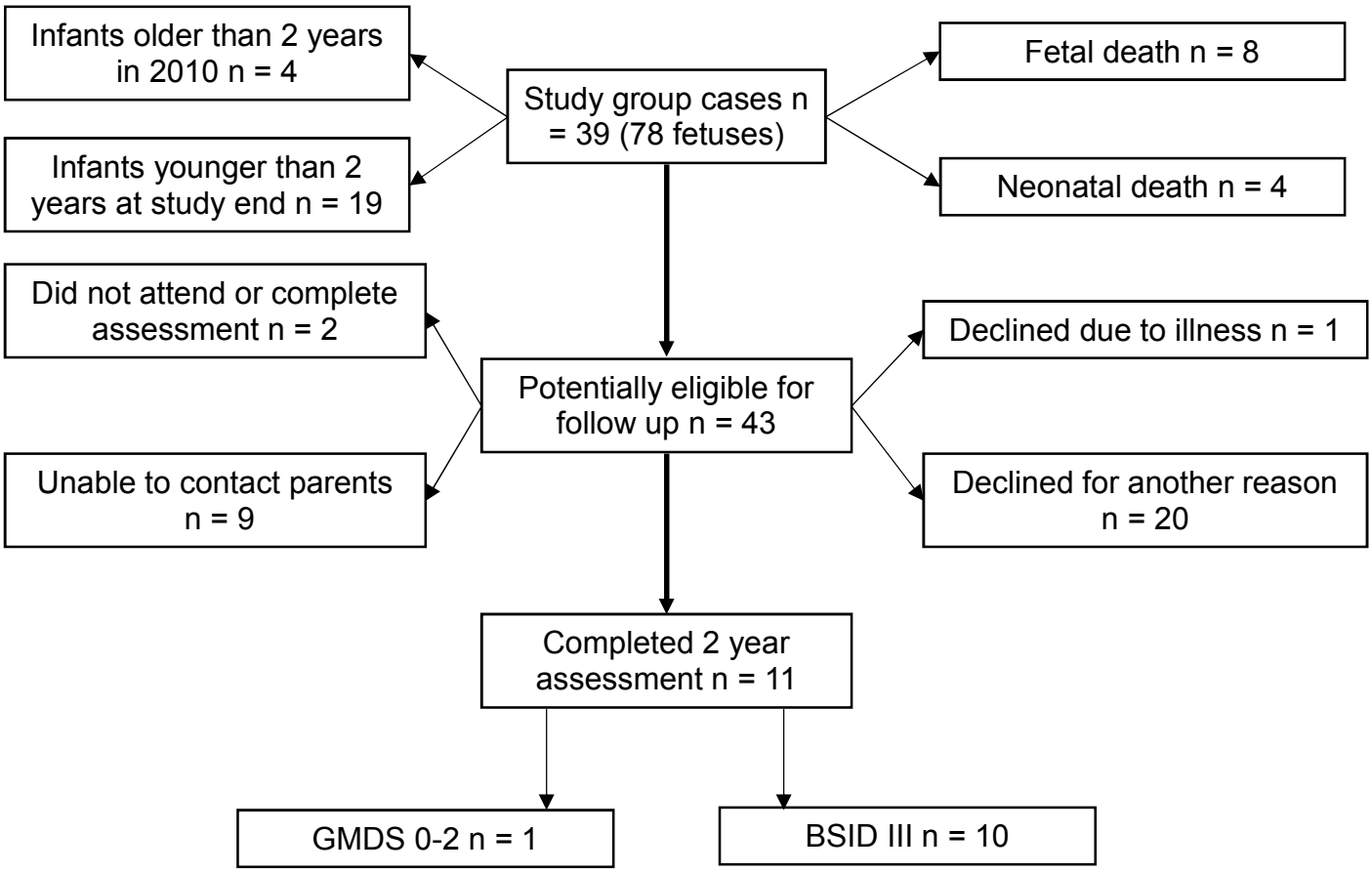


Figure 86. Participation in Neurodevelopmental Assessment at 2 years.

Fourteen of 58 potentially eligible children completed an assessment at two years of age.

Ages & Stages questionnaires were posted to the parents of eligible children that did not attend at two years and replies for two children (case 38 - one pair at 48 months chronological age) were received.

Three children had assessments at both one and two years (case 9 and 15). A total of 24 children, comprising 10 pairs of twin survivors and four ex-recipient children, therefore underwent complete assessment at some time period.

There was no significant difference in gestational age at delivery between those that did attend for assessment and those that did not at either year 1 ($p = 0.6$) or at year 2 ($p = 0.8$). The percentage within twin pair STB tissue volume difference was not significantly different in those that did attend for assessment compared to those that did not at either year 1 ($p = 0.14$) and year 2 ($p = 0.5$). The within twin pair Sylvian fissure ratio difference was significantly greater in those that did attend compared to those that did not attend at both year 1 ($p < 0.05$) and year 2 ($p < 0.01$).

3.6.4.2 One year assessment scores

According to the results of the GMDS 0-2 for 11 children and BSID-III for 3 children, eight (57.1%) children assessed had a global development score within the normal range. Five of the children that had a global development score below the normal range had been ex-donor fetuses. Only three children (21.4%) had normal scores across all of the domains assessed. These three had all been ex-recipient fetuses.

In the Locomotor (GDMS 0-2) or Gross Motor (BSID-III) domain seven children (50%) showed normal development (five ex-recipients). Of those seven with significant delay four were ex-donors. Two children had severe impairment consistent with possible cerebral palsy.

In the Eye-Hand Co-ordination (GDMS 0-2) and Fine Motor (BSID-III) domain eight children (57.1%) showed normal development (six ex-recipients) while six children showed significant delay (four were ex-donors).

In the Language and Hearing (GDMS 0-2) and composite language (BSID-III) domain, nine children (64.2%) had normal language development (six ex-recipients) while five children showed significant delay. Three of the children with significant delay were ex-donors.

In the Performance (GDMS 0-2) and Cognitive (BSID-III) domain 10 children (71.4%) had normal development (seven ex-recipients) and four showed significant delay, three of whom were ex-donors.

In the Personal-Social (GDMS 0-2) all children had development scores in the normal range.

The assessor commented on poor attention skills and difficulty concentrating during the assessment in three children (27.3%, two ex-donors).

3.6.4.3 Two year assessment

According to the results of the BSID-III for 10 children and the GMDS 0-2 for 1 child, seven (63.6%) children assessed had a global development score within the normal range. Three of the children that had a global development score below the normal range had been ex-recipient fetuses. Only three children (27.3%) had normal scores across all of the domains assessed (1 ex-recipient and 2 ex-donors).

Three children (27.3%) had significant delay on the Gross Motor scale. Two of these children were ex-recipients and one was an ex-donor. The ex-donor child had severe gross motor delay consistent with cerebral palsy. In the Fine Motor scales one child (9%) that was an ex-recipient showed delayed development.

On the Language scale, only four children (36.4%) had scores in the normal range (two ex-recipients and two ex-donors). One child (9%) had delayed receptive communication skills (an ex-recipient), three children (two ex-donors and one ex-recipient) had delayed expressive communication skills (27.3%) and three ex-recipients had delays in both (27.3%). Four children had severe delay that required referral to speech and language therapy.

On the Cognitive scale nine children showed development within the normal range (81.8%). Both of those children with abnormal cognitive development were ex-recipients and had significant delay in language development. One of them also had significant delay in fine motor development. One child (ex-donor) with a cognitive score at the lower end of the normal range had significant composite language delay.

One ex-recipient child that showed delay in cognitive, fine motor and language domains performed so poorly that scores were unable to be calculated and referral to specialist behavioural services recommended.

3.6.4.4 Children assessed at both year 1 and year 2

Three children were assessed at year 1 and year 2 of age. The results of the developmental assessments are summarised in Table 29.

Case no	Development at year 1	Development at year 2
15 ex-R	normal	***language delay***
15 ex-D	***language / gross motor delay***	***language / gross motor delay***
9	***gross motor delay***	***language / cognitive delay***

Table 29. Comparison between developmental outcomes in three children that were assessed at both 1 and 2 years.

Case numbers refer to pregnancies described in Table 9 section 2.2.3

Cases 15 were co-twins from the same pregnancy delivered due to pre-term labour at 33/40. The ex-recipient twin scores were below the mean but within the normal range for all domains when assessed at year 1. The ex-donor twin had gross motor function consistent with cerebral palsy. Case 9 was an ex-recipient twin whose co-twin was unable to attend as he remained an inpatient following pre-term delivery at 29 weeks and 3 days. Gross motor performance was within normal limits by year 2 assessment with significant cognitive delay and severe language delay.

3.6.4.5 ASQ results

Two children (from one twin pair, case 38) completed Ages & Stages questionnaire. Both children had scores on the Problem Solving and Personal-Social scales within the normal range and scores on the Communication and Fine Motor scales in the borderline range. The ex-recipient child had a Gross Motor score in the borderline range whereas the ex-donor scored within the normal range.

3.6.4.6 Influence of ex-recipient or ex-donor status

Overall in our follow up group of 24 children we found an overall rate of significant developmental delay (as assessed by a global development score below the normal range in either the BSID-III or GDMS 0-2) of 20.8%. This represents two ex-recipient children (14.3%) and three ex-donor children (30%) but the difference was not statistically significant (p = 0.2)

Within pair comparison for 10 twins (20 children) that underwent complete assessment did not reveal a significant difference between ex-recipient and ex-donor twins in the Cognitive domain (p = 0.28), Gross Motor domain (p = 0.49) or Hearing and Language domain (p = 0.33). Ex-donor twins showed a trend towards lower scores in the Fine Motor domain but this did not reach statistical significance (p = 0.06).

3.6.4.7 Correlation with MR measurements

Given the small numbers at year 1 and year 2 assessments, a provisional analysis was undertaken of the combined data. The scores for each sub-scale in the GMDS and BSID-III were transformed into months delay from the mean for ex-recipient and ex-donor children and correlated with within pair differences in STB tissue and Sylvian fissure ratio (table 30). One child was excluded as performance had been so poor in the assessment that scores could not be calculated. In the three cases that underwent assessment at year 1 and year 2 (see table 29), only the year 2 results were included.

Intertwin difference	**Cognition / Performance**		**Fine motor / Co-ordination**		**Gross motor / Locomotor**		**Hearing & Language / Communication**	
	ex-R	ex-D	ex-R	ex-D	ex-R	ex-D	ex-R	ex-D
Supratentorial brain volume	0.33	0.40	-0.48	-0.65	0.01	0.77*	0.51	0.73^{+}
Sylvian fissure ratio	0.08	0.07	-0.62^{+}	-0.85*	0.29	0.61	0.68*	0.89**

Table 30. Relationship between MR measurements and developmental outcome scores.

Increasing intertwin Sylvian fissure ratio difference correlated positively with increasing delay in language scores and negatively with increasing delay in fine motor scores in ex-donors. Increasing intertwin STB tissue volume correlated positively with increasing delay in gross motor scores in ex-donors. Correlation was assessed using the Spearman's r. * $p<0.05$ ** $p<0.01$ $^{+}$ $p = 0.06$

Magnitude of the within pair difference in Sylvian fissure ratio showed a positive correlation with increasing delay in language scores in ex-recipients (Spearman's $r = 0.68$, $p<0.05$) and ex-donors (Spearman's $r = 0.89$, $p < 0.01$). Sylvian fissure ratio difference showed a negative correlation with increasing delay in fine motor skills in ex-donors (Spearman's $r = -0.85$, $p < 0.05$) and a similar trend in ex-recipients that did not reach statistical significance ($p = 0.06$).

Magnitude of the within pair difference in STB tissue volume showed a positive correlation with increasing delay in gross motor scores in ex-donors (Spearman's $r = 0.77$, $p < 0.05$). Supratentorial brain tissue volume difference was positively correlated with increasing delay in language scores in ex-donors but did not reach statistical significance ($p = 0.06$). However these correlations did not withstand Bonferroni correction for multiple comparisons.

3.6.5 Discussion

The overall rate of delay in global developmental score in the cohort was 20.8%. Whilst the majority of children assessed had a normal global developmental outcome, only 16.7% of children had scores within the normal range in all developmental domains. In this preliminary analysis the deficits detected were mostly within the motor domain at one year (64%) and in the language domain at two years (63.6%). Two children had motor impairment consistent with cerebral palsy and another was unable to perform the majority of tasks and referred to specialist behavioural services. Over half of children with language delay at 2 years required referral to speech and language therapy. Cognitive development however was within normal ranges for 75% of the cohort. No significant differences were found between global or domain specific impairment between ex-recipient and ex-donor twins. Larger within pair discrepancy in Sylvian fissure ratio correlated with degree of language impairment in both ex-recipients and ex-donors but negatively correlated with fine motor delay in ex-donors. Large within pair discrepancy in STB tissue volume correlated with gross motor impairment in ex-donors. These results are limited by small sample size and require verification with larger numbers.

The observed incidence in this study of 21% significant neurodevelopmental impairment is at the higher end, but consistent with reports in the literature. Outcome studies report rates of 7-20% (Graeve et al. 2012; Gray et al. 2011; Lenclen et al. 2009; Ortibus et al. 2009; Rustico et al. 2012; Salomon et al. 2010; Sutcliffe et al. 2001) that vary depending on the definition of impairment. When restricted to a diagnosis of cerebral palsy or bilateral deafness, severe impairment is generally less than 10% (Graeve et al. 2012; Rustico et al. 2012; Salomon et al. 2010; van Klink et al. 2011) which

would be consistent with our results (8.3%). The definition of impairment in the current study included scores lower than 1SD below the mean in GMDS 0-2 or BSID-III. Ortibus and colleagues (Ortibus et al. 2009) assessed neurodevelopmental outcome using the BSID and report a 10% incidence of developmental impairment, however they used a more stringent cut-off of 2SD below the mean. Similarly Gray and colleagues (Gray et al. 2011) report a lower incidence of impairment of 12.4% but only included cognitive impairment that scored less than 2SD below the mean. Language or motor delay was not included unless it constituted cerebral palsy. Graeve and colleagues (Graeve et al. 2012) assessed 190 children at a mean age of 6 years. They report a combined rate of major and minor impairment (which includes language or motor delay scores of more than 1SD below the mean) of 20.5% in line with the findings reported here.

Although consistent with existing reports in the literature, it is possible that the incidence of neurological impairment reported in this chapter represents an overestimate due to the low follow up rate. This low rate is partly due to the number of children not yet eligible for assessment at the time of analysis but also due to poor parental uptake in accepting follow up invitations or completing questionnaires. It is therefore possible that this follow up cohort is biased as parents with concerns regarding their children's progress may be more likely to participate in follow up.

Lower gestational age at birth in our follow up cohort may also contribute to the high incidence of neurodevelopmental impairment. The median gestational age at delivery in this study was 33.1 weeks and therefore almost half would have been delivered at 32 weeks or less. Lower gestational age at birth is associated with increased risk of neurological impairment (Graef et al. 2006; Lenclen et al. 2009; Maschke et al. 2011; Ortibus et al. 2009) with delivery at less than 32 weeks a significant risk factor for subsequent neurological impairment (Graeve et al. 2012; Lopriore et al. 2009). One study found no difference in neurological morbidity at 4 year follow up between twin survivors treated for TTTS and twin controls matched for prematurity (Kowitt et al. 2012). The rate of preterm delivery in the current study is similar to other large outcome studies (Gray et al. 2011; Rustico et al. 2012) although some report have reported later median gestation at delivery (Graeve et al. 2012; Ortibus et al. 2009). Importantly in the current study, gestational age at delivery was similar between those children that did attend for assessment and those that did not, so does not result in bias in the follow up group.

Several follow up studies have failed to find a significant association between recipient or donor survivors and risk of neurodevelopmental impairment (Graeve et al. 2012; Maschke et al. 2011; Senat et al. 2004; van Klink et al. 2011). None of these studies have looked at within twin pair comparisons however. In addition most are restricted to incidence of major neurological morbidity

and do not assess milder functional impairment that may be different between co-twins. In our follow up cohort the percentage of ex-donors with global developmental impairment was twice as high as ex-recipients although this difference was not statistically significant. Further analysis in a larger cohort is warranted.

One caveat concerns the phenomenon of 'perinatal switching'. Antenatal ultrasound labelling does not correspond to birth order in approximately one third of twin pregnancies (D'Antonio et al. 2013). Failure to find association between recipient or donor status and risk of neurodevelopmental impairment in previous studies may relate to confounding by incorrect identification of twin status at follow up. In our study actual birthweight was used as an additional check for identification of ex-donor / ex-recipient status at delivery. In addition parents were asked to confirm when they attended for neurodevelopmental assessment which twin had been the donor or recipient in utero. However despite these attempts to remove ambiguity there may still have been some cases where the incorrect ex-donor / ex-recipient status is assigned at follow up.

Both of the children with major motor impairment consistent with cerebral palsy were ex-donors. Both had had normal fetal MR imaging. In one case delivery was at 33 weeks for growth restriction. This child has significant speech and language delay requiring speech and language therapy input and remains on supplemental nutrition via gastrostomy tube at night. In the other case delivery was at 30 weeks and 4 days. Growth velocities of the twins were symmetrical and stable following laser treatment and spontaneous vaginal delivery occurred 2 weeks after preterm rupture of membranes of the ex-recipient twin. The mother did not receive steroids. This child also had major cognitive impairment although speech and language were within the normal range.

The analysis of the correlation between within pair measures of discrepancy in brain tissue volume and Sylvian fissure ratio with developmental delay was limited by the small sample size. Within pair discrepancy in Sylvian fissure development appeared to correlate with more domains of development delay than discrepancy in STB tissue. In particular discordant Sylvian fissure ratio correlated with degree of language impairment in both ex-recipients and ex-donors but appeared to negatively correlate with fine motor delay. The Sylvian fissure represents a convergence point for the frontal, parietal and temporal lobes and houses the insular cortex. As such it has a role in a vast range of functions including motor control, higher cognitive function, emotion and sensation as well as language (Jones et al. 2010; Nieuwenhuys 2012; Stephani et al. 2011). In addition the temporal lobe forms the auditory cortex along the inferior border of the fissure and it is therefore not surprising we have found that impaired development of this sulcus correlates with language delay.

3.6.6 Conclusion

In conclusion, a significant incidence of minor and major neurodevelopmental impairment in the cohort of twins treated for TTTS has been reported here. Preliminary analysis demonstrates evidence of a relationship between fetal MR derived measures of brain maturation and growth and degree of developmental impairment. Neurodevelopmental follow up data continues to be acquired and further analysis is merited.

4. Main Discussion

TTTS develops in approximately 10% of monochorionic pregnancies (Simpson 2013) and the aetiology remains incompletely understood (Fisk et al. 2009). Advances in treatment have improved perinatal mortality rates. However longer-term outcome remains a significant issue, even with apparently successful in utero laser therapy. Most research has focused on treatment strategies, outcome prediction and risk stratification. TTTS provides a unique opportunity to study the effect of discordant in utero environment on fetal brain development in genetically identical twins. Only one study has commented on fetal brain development in TTTS and this was limited to 2D biometric measures on non-motion corrected MR images (Tarui et al. 2012). Imbalance in placental blood flow prior to laser therapy results in hypoperfusion of the donor fetus with upregulation of the renin-angiotensin system (Mahieu-Caputo et al. 2005). Fetal brain volume is reduced in congenital heart disease, particularly in association with reduced ventricular output (Limperopoulos et al. 2011) but preserved in fetal growth restriction (Damodaram et al. 2012) relative to singleton controls. Downregulation of the RAS appears to be neuroprotective in animal (Dong et al. 2011; Villapol et al. 2015) and human models (Rouch et al. 2015). Therefore it is hypothesised that ex-donor fetuses will have smaller as well as less mature brains than their ex-recipient co-twins. The main aims and findings of this thesis are summarised in Table 31.

Aims	Methods	Main findings
To establish intertwin differences in multiple intracranial structures in fetal twin pairs treated for TTTS	Volumetric reconstruction and segmentation of fetal intracranial structures	Ex-donor fetuses had smaller STB tissue than ex-recipient co-twins. However cerebellar and cortical grey matter growth was similar within pairs when corrected for brain size.
To establish how fetal brain growth relates to overall fetal body growth in twins treated for TTTS.	Manual segmentation of fetal body	Ex-donors show larger brain tissue volumes with reduced ECF volumes when corrected for overall fetal body volume relative to ex-recipient co-twins.
To establish whether differences in fetal cortical maturation take place within twin pairs.	Cortical scoring system	Cortical landmark development was delayed in ex-donors compared to their co-twins. This was evident particularly in the frontal parietal region, specifically at the central sulcus, parietal occipital fissure and Sylvian fissure.
To evaluate feasibility of ^{1}HMRS in complex twin pregnancy and evaluate the differences in brain metabolite ratios between ex-recipient and ex-donor fetuses.	^{1}HMRS	Spectral acquisition rates were lower than in published literature for singletons but the technique is feasible. Cho:Cr ratios decreased with gestational age in ex-donor fetuses. A lactate peak was observed in 43% of fetuses.
To assess the neurodevelopmental outcome of laser treated TTTS survivors at 1 and 2 years of age and correlate with intertwin discordance in fetal brain MR measurements.	Neurodevelopmental assessment	Delay in global developmental score was found in 21% of children at preliminary follow up. At 2 years 63.6% had delay in the language domain. Intertwin discordance in Sylvian fissure maturation correlated with delayed scores in specific developmental domains.

Table 31. Main study aims and findings.

In this study volumetric analysis has been performed on reconstructed images from 60 structurally normal MR scans of fetuses from 21 to 32 weeks GA following treatment for TTTS. Results have been compared to data from singleton controls and shown that ex-donors have smaller STB tissue, cortical grey matter, cerebellar and extracerebral fluid volumes. When corrected for overall fetal body volume, STB tissue volume is relatively larger in ex-donors compared to their recipient co-twins whilst extracerebral fluid volume remains relatively reduced. This brain sparing appears to have a global effect as volumetric analysis of cortical grey matter and cerebellar volumes showed growth in similar proportion to overall brain tissue volume within twin pairs.

There are no comparable studies assessing intracranial volumetry in the fetal twin population. One 2D MR study (Tarui et al. 2012) has found smaller BPDs in donor fetuses compared to recipients with smaller bitemporal diameter corrected TCDs. Biparietal diameter was not corrected for overall weight and treatment for TTTS was not commented upon. It has been shown in this thesis that cerebellar volume correlates strongly with TCD and is similar between co-twins when corrected for overall brain tissue volume. This disagreement may reflect the smaller sample size (n=18) and younger gestational age (median 21 weeks) in the study by Tarui et al (2012). In the current study smaller corrected cerebellar and cortical volumes were observed in the twin cohort compared to singleton controls. This finding has been observed in healthy MCDA neonates compared to singleton neonates (Knickmeyer et al. 2011) and so one cannot conclude whether the findings of this thesis reflect the influence of TTTS or monochorionicity more generally. Segmentation of the whole fetus revealed significantly smaller volumes in ex-donors compared to their ex-recipient co-twins. Analysis corrected brain tissue volume corrected by whole fetal volume demonstrated evidence of brain sparing in ex-donors. This has been shown in one volumetry study in fetal growth restriction (Damodaram et al. 2012), and of greater magnitude than we have found in our ex-donor cohort. One other group has reported smaller corrected brain volumetry in fetal growth restriction (Egaña-Ugrinovic et al. 2013) however significant limitations in that study make their findings hard to interpret. In our study ex-donor fetuses had significantly smaller brain volumes than their genetically identical co-twins. Whilst there is evidence of some compensatory growth through the mechanism of brain sparing, ex-donor fetuses in our cohort remained unable to achieve their full genetic brain growth potential. Hypoperfusion and aberrant endocrine function may account for donor fetuses' failure to achieve their full genetic brain growth potential and also impact brain development.

Having demonstrated significant intertwin differences in fetal growth of intracranial structures, the next section of this study aimed to assess variability in the temporospatial schedule of cortical

development. To quantify in utero cortical maturation we selected individual gyri and sulci representative of each brain lobe within the gestational age limits of our cohort and assigned a cortical folding score to each. Regional differences in cortical maturity were observed between ex-recipient and ex-donor fetuses within twin pairs treated for TTTS. In addition it has been shown that the gestational age at which early development of gyri and sulci is visible was delayed in the twin TTTS cohort by up to three weeks relative to singleton controls. This is consistent with limited existing studies comparing maturation between singletons and twins (Chi & Dooling 1977; Dubois et al. 2008). Delay in cortical folding in ex-donors relative to their co-twins was most prominent around the parietal region, in particular at the central sulcus, parietal-occipital fissure and Sylvian fissure. These landmarks appear earliest in the temporospatial course of sulcation. It is not clear whether this finding represents true regional delay or failure to capture global delay due to the young gestational age of our cohort. Studies in cortical development in singletons with growth restriction or ventriculomegaly have also identified the insular and parietal regions as specifically susceptible to aberrant development (Egaña-Ugrinovic et al. 2014; Scott et al. 2013). The Sylvian fissure is the earliest identifiable landmark in cortical maturation, developing at around 14 weeks (Toi et al. 2004). Therefore this region is may be particularly vulnerable to the impact of progressing TTTS pathology in the second trimester compared to landmarks which form later in gestation. The complex configuration of the Sylvian fissure prevents assessment by the scoring system and assessment of the ratio of the insular to opercular distance was found to be significantly lower in ex-donors compared to co-twins. Hypotheses regarding the control of cortical folding emphasise the driving role of white matter development (Sur & Rubenstein 2005; Toro & Burnod 2005; Van Essen 1997). High renin and angiotensin levels as well as cerebral hypoperfusion in TTTS donors early in the second trimester may produce abnormalities in white matter connectivity that impair subsequent development of the cortical surface and predispose to later neurodevelopmental deficits.

The next component of the study assessed the metabolic profile of the developing brain in fetuses treated for TTTS. Acquisition rates of spectra suitable for interpretation were about 10% lower in our cohort compared to published studies in singletons. There are no published twin spectroscopy studies for comparison and the lower acquisition rate may reflect increased fetal and maternal motion. Although feasible in twin pregnancy as has been demonstrated here, sample size was small and makes conclusive interpretation difficult. In the current study Cho:Cr ratio significantly decreased with GA in ex-donor fetuses and appeared lower overall compared to ex-recipients. Choline appears to decrease with GA in normal fetuses as molecules are incorporated into myelin lipids reflecting white matter maturation (Girard, Gouny, et al. 2006). Given the relatively more immature cortical folding of the ex-donor fetal brain observed in chapter 3.4, it would be surprising to conclude that relatively lower Cho:Cr ratios in ex-donors reflect increased white matter

maturation. Instead it may be that the lower ratio reflects higher creatine levels in ex-donors. Creatine is found in high concentration in glial cells (Urenjak et al. 1993) and levels appear stable in normal fetuses (Girard, Gouny, et al. 2006). Creatine levels may be abnormally high in ex-donor fetuses as a result of accelerated pro-inflammatory signalling and glial cell activation in response to RAS upregulation (Guilherme et al. 2009). Lactate was detected in 43% of fetuses in our cohort. This is higher than has been reported in either normal fetal populations (Story et al. 2011; Berger-Kulemann et al. 2013) or those with pathology including congenital heart disease and FGR (Story et al. 2011; Limperopoulos et al. 2011). The significance of cerebral fetal lactate is unclear but it may function as a supplementary fuel under conditions of oxidative stress (Pellerin et al. 1998) and even be neuroprotective (Moxon-Lester et al. 2007). Further validation of the findings in chapter 3.5 with a larger sample size is necessary but the feasibility of ^{1}HMRS in this population has been demonstrated as well as within pair differences in metabolite ratios detected. These results may reflect underlying inflammatory responses relating to RAS activation in TTTS that could impact white and grey matter maturity.

Finally, neurodevelopmental outcome in a proportion of children enrolled in this study were presented. Almost 21% of children treated in utero for TTTS had neurodevelopmental impairment. Deficits detected were predominantly within the motor domain at year 1 and in the language domain at year 2. Results from this preliminary cohort are in agreement with the current literature reporting an approximately 10% incidence of severe impairment (van Klink et al. 2011). In chapter 3.6 more detailed data on developmental delay within specific domains was reported. The magnitude of intertwin fetal discordance in Sylvian fissure development correlates with poorer language development scores in childhood. Intertwin discordance in brain maturity rather than growth may be a better marker of longer term outcome and merits further investigation.

In conclusion, fetal phenotypic brain differences have been demonstrated between genetically identical twins arising in the discordant uterine environment that the TTTS model provides. Significant differences exist between co-twin fetuses treated for TTTS in brain structure as well as development. The ex-donor fetus is characterized by brain sparing that does not reach full genetic growth potential and has a less mature cortical gyrification pattern. The magnitude of these differences may appear to correlate with later neurodevelopmental impairment. Further investigation of the impact of TTTS on white matter function and connectivity may and the role of the brain renin-angiotensin system may improve our ability to predict longer term outcome.

5. Future Work

5.1 Volumetric segmentation of white matter, basal ganglia and thalami

This study has shown that whilst size differences in supratentorial tissue volume exist within twin pairs, the cortical grey matter and cerebellum develop in proportion to overall STB volume. It has not been determined whether this is the case for cortical white matter or other intracranial structures such as the basal ganglia, thalami and brainstem. The segmentation algorithm described in chapter 2.3 delineates white matter however further manual segmentation is required for accurate volumetric measurement and is currently underway. White matter development may be specifically disrupted by activation of the renin-angiotensin system and therefore is an important component to quantify. Future studies could also investigate white matter connectivity differences between ex-recipient and ex-donor twins through fetal diffusion tensor imaging.

5.2 Placental influence

The placenta is the substrate for the pathophysiology that develops in TTTS. Indeed a proportion of twin pregnancies complicated by TTTS also demonstrate sFGR likely secondary to placental insufficiency. There is almost certainly overlap between the pathophysiological processes occurring in TTTS and FGR. Up to 40% of donor fetuses meet criteria for FGR (Vanderbilt et al. 2014; Chmait et al. 2011). Whilst the total fetal body volume was segmengted in a subset of cases with suitable imaging, the placenta was not examined. Future work could evaluate total placental MRI volumetry and include post delivery placental injection studies. These findings could be correlated to discordance in cerebral growth and cortical development. Other markers of placental function and flow including fetal Doppler waveforms could be included to help stratify the risk of fetal brain discordance. Important comparison groups to isolate the role of the placenta versus RAS activation would be healthy MCDA twin controls as well as those with sFGR.

5.3 Lateralisation

The most mature cortical landmark was selected for scoring in assessment of cortical folding. Reliable left / right orientation of the brain in otherwise normal appearing brains after post processing is complex. There is little reason to expect lateralization differences in cerebral development within twin pairs and therefore it was not considered that this would provide significant additional value to

the project within the time constraints. It could be undertaken for completeness to demonstrate symmetrical folding patterns within twin pairs.

5.4 Neurodevelopmental follow up

Neurodevelopmental follow in the cohort of twins treated for TTTS in this study is on-going. Larger numbers are required to validate the finding of an association between intertwin discordance in brain tissue volume and cortical maturation with neurodevelopmental delay scores. Larger numbers will also allow the impact of recipient or donor status on more subtle measures of neurodevelopmental impairment to be explored. This has not been examined in large outcome studies to date.

Appendix 1: Fetoscopic laser therapy

The majority of cases were performed under local anaesthesia. In four cases epidural anaesthesia was used.

A 10F trocar was inserted into the uterine cavity percutaneously under ultrasound guidance. A curved viewing fetoscope was then inserted and the placental vasculature mapped and intertwin anastomoses identified. An operating fetoscope was then introduced with a laser fibre inserted down a side port. A pulse laser was then used to coagulate the AV and VV anastomoses along the vascular equator using the selective technique. Amnio-exchange was performed in cases where amniotic fluid discolouration impaired visualization of the surface of the placenta. Placental vasculature was remapped following the procedure to ensure complete anastomotic ablation. The surgery was performed by two experienced operators (SK and RW).

Women were given prophylactic antibiotic cover with intravenous Cefuroxime 750mg. In addition a 400mg Cyclogest pessary was prescribed OD for four weeks and Indomethacin 100mg PR for 3-5 days.

Fetal heart beats were confirmed post procedure and women were admitted for overnight observation and follow up at 24 hours. Further follow up was offered at two weekly intervals.

Appendix 2: Information Sheet for Patients

You will be given a copy of this information sheet

Study Title:

Quantification of fetal brain development and growth using magnetic resonance imaging

Invitation to take part

We are inviting you to take part in a research study, which uses a technique called magnetic resonance 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 normal fetus (baby), magnetic resonance is usually better for detecting and assessing both normal structures and abnormalities. This is why your obstetrician may have referred you for an MR scan. We are interested in developing the MR technique further to improve our abilities to detect abnormalities and to try and understand more about their impact on your child's later development.
We therefore plan to image your baby once or twice prior to delivery. Where possible we also seek your permission to scan your baby after delivery and in the event of a premature delivery at term equivalent age. We would also like to assess your child's development over the first few years.

The technique 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.

Why have I been chosen?

We seek permission for these studies from:

1. Women with complicated pregnancies,
2. Women where antenatal ultrasound has shown a possible problem with the fetus,
3. Women with normal pregnancies and normal fetuses.

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?

If you have been referred for an MR scan by your doctor this will be performed as usual. 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. 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 MR spectroscopy information from your baby's brain and liver and your placenta. We need information on these organs to increase our understanding of their role when a baby is not growing well. 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.

What do I have to do?

For your scans we will ask you to lie as still as possible within a magnetic scanner while the machine produces images.

What is the procedure that is being tested?

The procedures being tested are magnetic resonance imaging and spectroscopy. We will be optimising the scanner's abilities to obtain the most detailed information possible about the unborn baby. These developments will allow us, for instance, to accurately measure areas of a baby's brain at different gestations and to identify an area of injury soon after it has occurred.

What are the side effects of taking part?

The 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 basic 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?

MRI 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 MRI. All the findings on your scan will be discussed with yourself and with your obstetrician. This will allow your obstetrician to advise on the management of your pregnancy with all the available information.

What happens when the research study stops?

The present research has been continuing for over 20 years and the results have been published regularly. We intend to continue this.

What if something goes wrong?

In the unlikely event of you suffering any adverse effects as a consequence of your participation in the study you will be compensated through the Imperial College School of Medicines "No Fault Compensation Scheme".

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 your gestation, ultrasound details of your baby. This information would be given to us by your obstetrician or GP and would be handled confidentially. Any imaging data that is shared with colleagues in collaborating units will be anonymised.

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.

Who has reviewed the study?

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

Contact for Further Information

Mary Rutherford

Professor in Perinatal Imaging
MRC Clinical Sciences Centre
Imaging Sciences Department
Hammersmith Hospital
Du Cane Road,
London W12 OHS
Phone: 020 8383 3298
m.rutherford@imperial.ac.uk

Appendix 3: Information Sheet for Patients

You will be given a copy of this information sheet

Study Title:

Fetal Brain Growth and Development in Twins: An MR study.

Invitation to take part

We are inviting you to take part in a research study, which uses a technique called magnetic resonance 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 (MRI) is a method of obtaining pictures of the inside of the body. While ultrasound is excellent for showing the anatomy of the normal fetus, MRI is usually better for detecting and assessing both normal structures and abnormalities. This is why your obstetrician may have referred you for an MR scan. We are interested in developing the MR technique further to improve our abilities to detect abnormalities and to try and understand more about their impact on your child's later development. We are therefore asking your permission to image your baby prior to delivery and where possible after delivery. We would also like to assess your child's development during the first few years after birth.

The technique uses a large magnet and radiowaves. 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.

Why have I been chosen?

You have been chosen because your pregnancy has been affected by an uncommon complication and required specialist obstetric care. Because such complications are uncommon we are trying to understand more about any impact that such problems may have on early and later childhood brain development.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you are 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.

What will happen to me if I 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. We will give you an appointment to attend for an MRI scan at your convenience.

Initial MRI scan: During the MRI scan you will be lying slightly tilted to one side on cushions inside the MRI scanner. We will make you as comfortable as possible. Your head will be level with the opening of the scanner. You will be wearing headphones so that we can talk to you during the scan. We can also play music through the headphones so please feel free to bring a CD. You may still hear a knocking noise during the procedure which is the sound that the magnet makes. Your partner or a friend or relative may accompany you to the scanning room. You will be given an alarm bell to sound at any time if you are upset or worried during the examination. If the procedure does not suit you for any reason, it can be stopped at any time. During the examination we will collect imaging data on your baby's brain. We may also collect MR spectroscopy information from your baby's brain. The duration of the scan will depend on how much the baby is moving. Singleton babies take up to an hour to scan and twin babies may take up to 90 minutes. You will have a break outside of the scanner if the anticipated scan time is longer than 60 minutes. If you become uncomfortable at any time we can stop the scan. It is possible to continue scanning after you have had a break or a change in position. After your scan we will review the images with you where possible and provide a report for your notes. A formal report will be made available to your referring obstetrician. You may ask for a copy of your images if you wish. You may arrange to come back at a later date to review the images if you prefer.

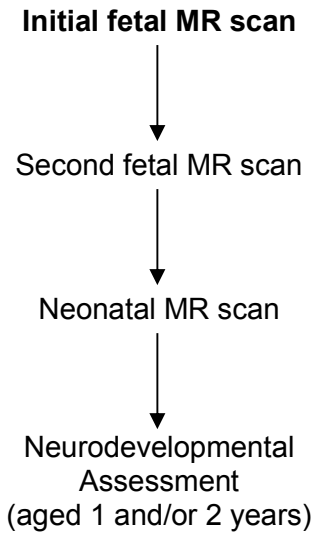
Further Assessments: Depending on the findings of your initial MRI scan, further assessments to provide additional clinical information for you and your clinician may be informative and would be discussed with you and arranged as appropriate. We seek consent to use data from these assessments in our study. Where clinical follow up has not been arranged, we seek consent to contact you later in pregnancy and after birth to arrange further assessments as described below and shown in the flow chart at the end of this section. We would like you to participate in as much as you feel able to. You may choose to decline a particular assessment at any stage. You may ask not to be contacted in the future at any stage.

Second MRI scan later in pregnancy: We would like to perform a further MRI scan later in pregnancy to allow us to measure development over time. This would be similar to the first one. We will ask you if you would like to arrange this after your initial MRI.

MRI after birth: We would like to perform an MRI scan of your baby's brain up to 6 weeks after delivery to continue measuring development over time. This takes approximately 50 minutes and would be performed during a natural nap time or under sedation if you prefer. We will contact you after delivery to ask if you would like to arrange this. If you are having a neonatal MR scan for research purposes, a further information sheet would be provided and you would be asked to sign a separate consent form.

Developmental assessments: We would like to be able to follow your child's development after birth and offer an overall developmental assessment of your child at one and/or two years of age. This would provide us with very useful information when quantifying our MR scans. Developmental assessments involve your child spending about an hour with a clinical psychologist at Hammersmith Hospital who uses games and toys to measure how your child's development compares to the average for that age. We will contact you closer to the time to ask if you would like to arrange this and discuss practical arrangements.

Overview of Study Assessments



After your initial fetal MR scan, we would like you to participate in as much or as little of the subsequent further assessments as you feel able to. You may decline a particular assessment at any stage. We will ask you at each stage if you are happy to be contacted about future assessments.

What do I have to do?

For your scans we will ask you to lie as still as possible within a magnetic scanner while the machine produces images.

What is the procedure that is being tested?

The procedures being tested are magnetic resonance imaging and spectroscopy. We will be optimising the scanner's abilities to obtain the most detailed information possible about the unborn baby. These developments will allow us, for instance, to accurately measure areas of a baby's brain at different gestations and to identify an area of injury soon after it has occurred.

What are the side effects of taking part?

The 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 we are imaging your baby, 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. 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 basic examination is not believed to have hazards associated with it when operated within National Radiological Protection Board Guidelines (which we do). We appreciate the use of your time and resources in participating in the study. To minimise inconvenience we aim to offer appointments at a time suitable for you and will reimburse the reasonable cost of travel to appointments for research purposes to and from the department. If you wish to claim costs, please let us know in advance so that we can provide appropriate advice regarding mode of transport and use of receipts to ensure prompt reimbursement.

What are the possible benefits of taking part?

Your initial 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 after birth by MRI and developmental assessment. You may find information that is produced from these assessments reassuring or it may help plan any treatment or extra therapy that your child may require.

What if new information becomes available?

Any test carries the possibility of an unexpected finding. Where MR or neurodevelopmental assessments have been requested for clinical purposes, all findings will be discussed with yourself and your clinician who will advise you on further appropriate management. Where unexpected information of potential clinical importance is found during a further assessment for research purposes, we will discuss the matter with you and, if you give your permission, refer the matter to your GP or any other appropriate doctor. Most often the finding will be a normal variation and not clinically significant. In addition whilst we anticipate that your tests will show normal results, this cannot be considered as a guarantee that your baby will have normal development throughout childhood. Some children with significant problems with their development have normal appearances on their MRI scans even when performed later in childhood.

What happens when the research study stops?

When the research study ends we will analyse our data and publish the results in the medical literature. If the study were to stop unexpectedly we would contact you to let you know as there would be no further follow up. There would be no change to your clinical care.

What if something goes wrong?

Imperial College London holds insurance policies which 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 a 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 the Investigator (Marisa Taylor-Clarke, contact details below). The normal National Health Service complaint 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.

Will my taking part in this study be kept confidential?

The information obtained from your study is covered by the Data Protection Act. Any information stored on NHS and Imperial College computers 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 your gestation, ultrasound details of your baby. This information would be given to us by your obstetrician and would be handled confidentially. Any image information sent out from our unit to colleagues in a collaborating unit will be anonymised. We will give you a copy of your consent form to keep in your handheld notes as well as our contact details and ethical approval number that will inform any healthcare professionals involved in your care that you have participated in our study.

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. Part of this study will form the basis of a PhD research project.

Who is organising and funding the research?

The research is organised and funded by Tiny Tickers, The Wiseman Trust, Medical Research Council, Imperial College School of Medicine and charities such as Action for Medical Research.

Who has reviewed the study?

The study has been reviewed by NRES Committee London – Riverside 11/LO/1744

Appendix 4: Consent Form 1st December 2011 V4 11/LO/1744

NAME:_____

Quantification of brain development and growth in twin pregnancies using MRI

(please tick each statement if it applies to you)

I have read the Information Sheet for this study 1st December 2011 V4 ☐

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 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 participation in this study will be recorded in my maternity notes. ☐

I agree that image data may be used in future research with appropriate ethical approval. ☐

I understand that my/my child's personal data may 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. ☐

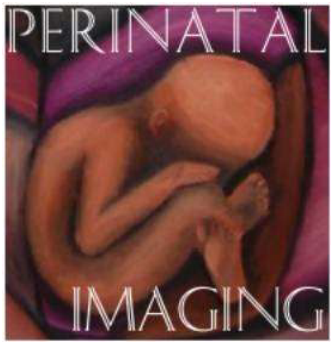
Signed.....Date.....

(NAME IN BLOCK CAPITALS).....

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

(NAME IN BLOCK CAPITALS).....

Appendix 5: Parental Questionnaire



Fetal Imaging Programme
Parental Questionnaire

Dear Parents,

We are trying to improve our clinical and research service and would really value your views. It would be helpful if you could answer the following questions for us. The completed questionnaire will be anonymous unless you wish to give your name. We have given you a stamped addressed envelope to make it easier to let us have your replies.

Yours faithfully



Mary Rutherford
Professor in Perinatal Imaging

m.rutherford@imperial.ac.uk

Please put a circle around your chosen answer i.e. ◯ NO or tick the box

1. **Which hospital were you referred from?**

   Queen Charlottes Other hospital

2. **Why were you referred for an MRI scan? Because there was a problem with my**

   Baby's brain baby's body my pregnancy other

   None of the above I am a volunteer for research ☐

3. **How did you receive your appointment?**

   Hand/In person Phone Mail

4. **Were you able to confirm the appointment easily or contact us for more details?**

   YES NO

5. **Did you receive an information sheet about our research?**

   YES NO

6. **Did you have enough time to read through it?**

   YES NO

7. **Did you understand it?**

   YES NO

8. **Would you have preferred it to be in a different language from English?**

   YES NO

9. **If you answered "Yes" which language? _____**

10. **Did you have the opportunity to ask any questions about the information sheet?**

    YES NO

11. **Were you clear which aspects of your examination were research and which were clinical?**

    YES NO

12. **Were the practical details about the scan and safety issues explained to you?**

YES NO

13. Did you receive (on appointment letter and information sheet) the internet based video link showing further details of MR scan?

YES NO

14. Did you view it?

YES NO

15. Did you find it helpful?

YES NO

16. What further information would you have liked to receive prior to the scan?

YES NO

17. Was there anything about the scan itself that worried you?

18. In what ways could the scan have been better for you

19. Were you shown the images after the scan?

YES NO

If you answered NO did you want to see the images?

YES NO

20. Did you understand what you were told about the images?

YES NO

21. Could the discussion have been improved in anyway?

YES NO

22. Did you discuss the scan findings <u>again</u> with one of your doctors?

YES NO

23. Did you feel the scan helped you understand the problems affecting your

baby or your pregnancy?

YES NO

24. Did you feel the scan was a help in making decisions about your pregnancy?

YES NO

25. As part of the research we have asked your permission to contact you again. Were you happy with this?

YES NO

26. We may have asked your permission for further scans of your baby. Did you agree?

YES NO

If you did not agree for further scans could we ask your reasons for saying no?

27. We may have asked whether we could follow your baby's development. Did you agree?

YES NO

If you did not agree for further scans could we ask your reasons for saying no?

28. Finally on a scale of 1-5, where 1 is not useful at all and 5 is very useful, do you feel that having the scan was a useful thing to have done?
(Please put a circle around the number.)

1 2 3 4 5

Thank you very much indeed for filling this in. We would be very grateful if you could put the questionnaire into the pre addressed and stamped envelope
Also we would welcome,

ANY EXTRA COMMENTS, IDEAS OR SUGGESTIONS:

Appendix 6: 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:	○	○	○	____

E101240200

Ages & Stages Questionnaires®, Third Edition (ASQ-3™), Squires & Bricker
© 2009 Paul H. Brookes Publishing Co. All rights reserved.

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*

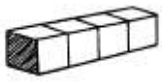
FINE MOTOR

	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.)* 	○	○	○	___
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

E101240500

Ages & Stages Questionnaires®, Third Edition (ASQ-3™), Squires & Bricker
© 2009 Paul H. Brookes Publishing Co. All rights reserved.

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

E101240700

Ages & Stages Questionnaires®, Third Edition (ASQ-3™), Squires & Bricker
© 2009 Paul H. Brookes Publishing Co. All rights reserved.

Appendix 7: Singleton Survivor Data

Single fetal demise is a significant risk in monochorionic pregnancies (Hack, Derks, et al. 2008; Shek et al. 2014) particularly following treatment for TTTS (Rossi & D'Addario 2008; Roberts et al. 2014). Estimates of single fetal demise following laser for TTTS vary between 17% and 36% (Middeldorp et al. 2007; Huber et al. 2006; Cavicchioni et al. 2006; Cincotta et al. 2009; Chmait et al. 2011; Rustico et al. 2012). Selective feticide is a potential treatment option in TTTS that may result in a singleton survivor but is also frequently performed for a variety of other indications. Short term prognosis for survivors of iatrogenic co-twin demise (selective feticide or post laser) appears better than for survivors of spontaneous co-twin demise (Jelin et al. 2008; O'Donoghue et al. 2009) although one recent study has reported similar rates of brain injury (Griffiths et al. 2015). The impact of iatrogenic co-twin demise on brain development and longer term outcome for the surviving twin is unclear (Hillman et al. 2010; Hillman et al. 2011).

Imaging data from singleton survivors of laser therapy for TTTS were excluded from the main study. Imaging data from a cohort of singleton survivors following selective feticide for TTTS and other pathologies, as well as singleton survivors of spontaneous fetal demise were also available. This appendix presents a preliminary analysis of brain volumetry data from this heterogeneous group of singleton survivors in comparison with singleton controls as well as our laser treated TTTS dual survivor cohort. The aims and results of the preliminary study are presented with the main areas for future analysis.

7.1 Aims

To compare fetal supratentorial brain tissue and lateral ventricular volumes between singleton controls and singleton survivors of iatrogenic co-twin death.

To compare fetal supratentorial brain tissues volumes between singleton survivors of spontaneous co-twin death and iatrogenic co-twin death.

To compare fetal supratentorial brain tissue volumes between singleton survivors of iatrogenic co-twin death in TTTS (selective feticide or post laser) and double survivors of laser treated TTTS.

7.2 Methods

Fifty five MCDA pregnancies were recruited between September 2008 and September 2012 following referral for fetal brain MR imaging after single death of a co-twin, as is recommended clinical practice. Exclusion criteria were brain anomalies on MRI and images unsuitable for 3D volume reconstruction.

MR images were acquired, reconstructed, reoriented and resampled as described in section 2.3. Semi-automatic volumetric segmentation of intracranial structures included the STB tissue and lateral ventricles.

7.2.1 Statistics

Demographics were reported as medians with IQR. ANOVA was used for comparison. Spearman's correlation coefficient was used for the evaluation of the relationship between gestational age and brain volumetry measures. A p value of < 0.05 was considered significant. All tests were 2 tailed unless otherwise stated. Grouped linear regression with covariance analysis was used to compare age corrected STB tissue and lateral ventricle volumes between groups.

7.3 Results

7.3.1 Cohort

Both the singleton control group and the dual TTTS survivor cohort were described in chapter 3.3. Imaging data was available for 55 singleton survivors of co-twin death. Forty one fetuses were survivors of iatrogenic co-twin death and 14 were surviving fetuses of spontaneous co-twin death. Figure 87 shows the number of cases excluded and the subgroups of survivors of iatrogenic co-twin death. Reasons for exclusion of cases included brain abnormalities on imaging and MR data unsuitable for volume reconstruction. Brain abnormalities were large pseudocysts in two cases, isolated moderate ventriculomegaly in one case and three cases of severe haemorrhage.

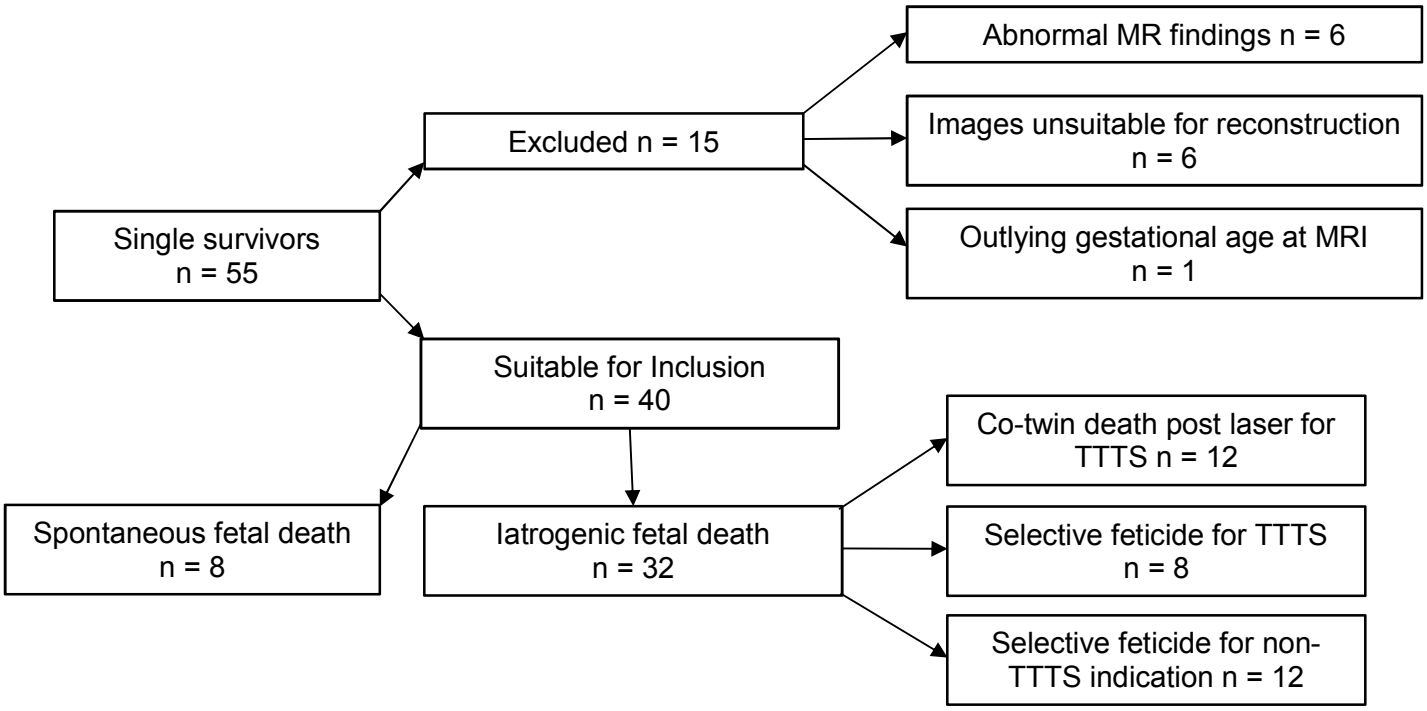


Figure 87. Flowchart of cases included and grouping according to mechanism of fetal death

A total of forty survivors of single fetal death were available for analysis.

7.3.2 Singleton survivors of iatrogenic co-twin death

Maternal and pregnancy characteristics for the singleton survivors (iatrogenic), double survivors of laser treated TTTS and singleton controls are shown in table 32.

	Singleton survivor	Double survivor	Singleton control
Maternal age (weeks)	30.0 (27.8 – 33.2)	33.7 (28.6 – 36.9)	35.1 (29.9 – 38.7)
GA at MRI (weeks)	24.7 (22.5 – 26.91) [a, b]	25.6 (24.2 – 27.1)	26.3 (23.8 – 28.6)
Male (%)	50	71	52

Table 32. Demographic data for singleton survivors, double TTTS survivors and singleton controls.

Medians and IQR are provided. [a] GA at MRI comparison with double survivor group $p = 0.08$. [b] GA at MRI comparison with singleton control group $p < 0.05$.

Median maternal age at MRI was 5.1 years lower in the singleton survivor group compared to the singleton control group and 3.7 years lower compared to the double survivor group. Median GA at MRI was 1.6 weeks lower in the singleton survivor group compared to the singleton control group ($p<0.05$) and 0.9 weeks lower compared to the double survivor group ($p = 0.08$). (ANOVA $p < 0.05$).

The median GA at which iatrogenic fetal death occurred in the singleton survivor group was 19.8 weeks (range 16.1 to 25.9).

7.3.2.1 Supratentorial brain tissue volume

Supratentorial brain tissue volume in singleton survivors of iatrogenic co-twin death increased linearly with GA (Spearman's r = 0.94; p < 0.001) from 44.78 cm^3 at 22 weeks to 162.73 cm^3 at 32 weeks at a rate of 11.8 cm^3 (15.3%) per week. Goodness of fit was similar for both linear (r^2 = 0.91) and exponential function curves (r^2 = 0.91).

Age-corrected supratentorial brain tissue volumes were not significantly different to singleton controls (p=0.5) or ex-recipient co-twins from surviving twin pairs of laser treated TTTS (p = 0.7). However age-corrected supratentorial brain volumes were an average of 6.91 cm^3 (9.0%) larger than ex-donor co-twins from surviving twin pairs of laser treated TTTS (p < 0.01). Slope comparisons are shown in figure 88.

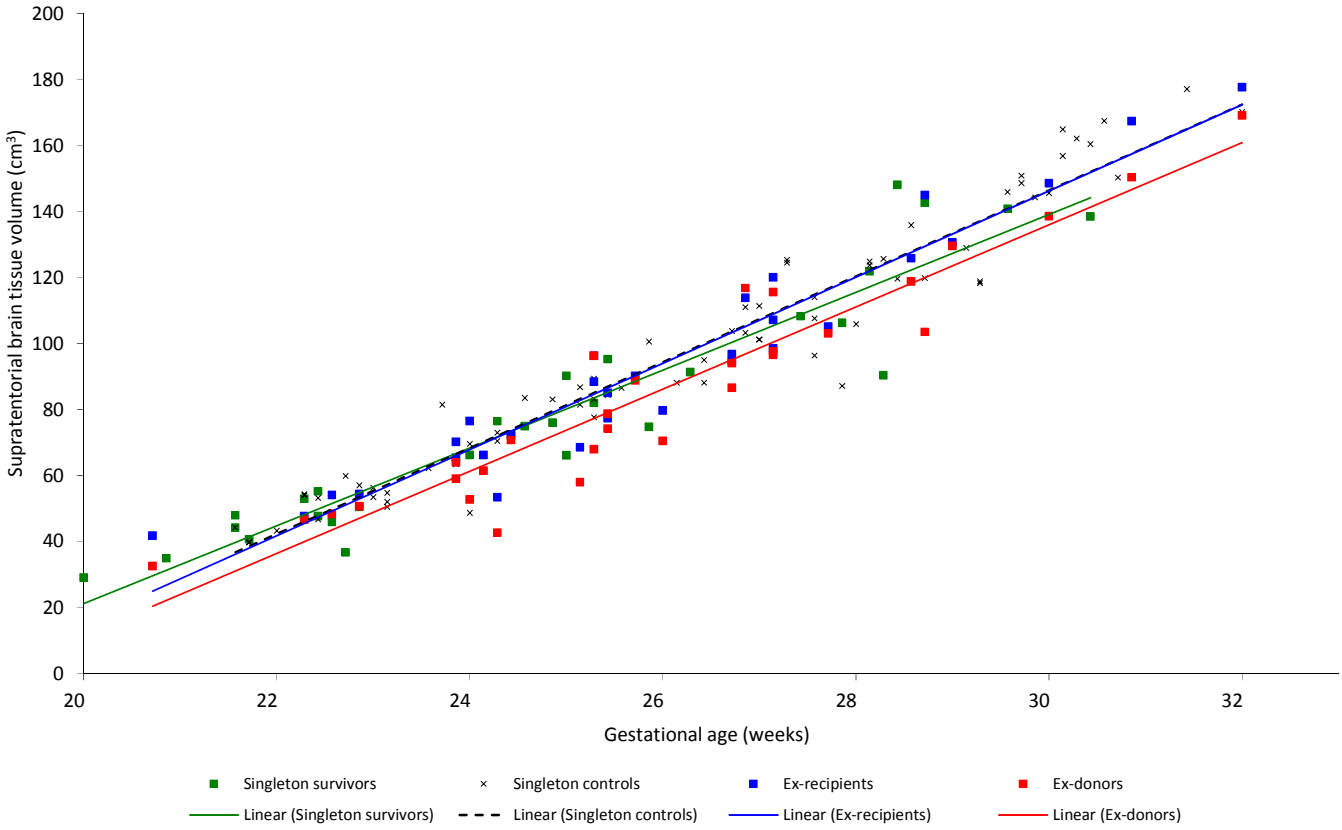


Figure 88. The relationship between GA and supratentorial brain tissue volume in singleton survivors of iatrogenic co-twin death, singleton controls and dual survivors of laser treated TTTS.

There was no significant difference between singleton survivors and singleton controls (p = 0.5). Ex-donors from dual surviving twin pairs have significantly smaller supratentorial brain tissue volumes (p < 0.01).

7.3.2.2 Lateral ventricle volume

Total lateral ventricular volume in singleton survivors of iatrogenic co-twin death increased moderately with GA (Spearman's r = 0.49; p < 0.01) from 22.87 cm^3 at 22 weeks to 51.32 cm^3 at 32 weeks at a rate of 2.8 cm^3 (9.3%) per week. Goodness of fit was similar for both linear (r^2 = 0.26) and exponential function curves (r^2 = 0.26).

Age-corrected lateral ventricle volumes were not significantly different between groups (p=0.6). Slope comparisons are shown in figure 89.

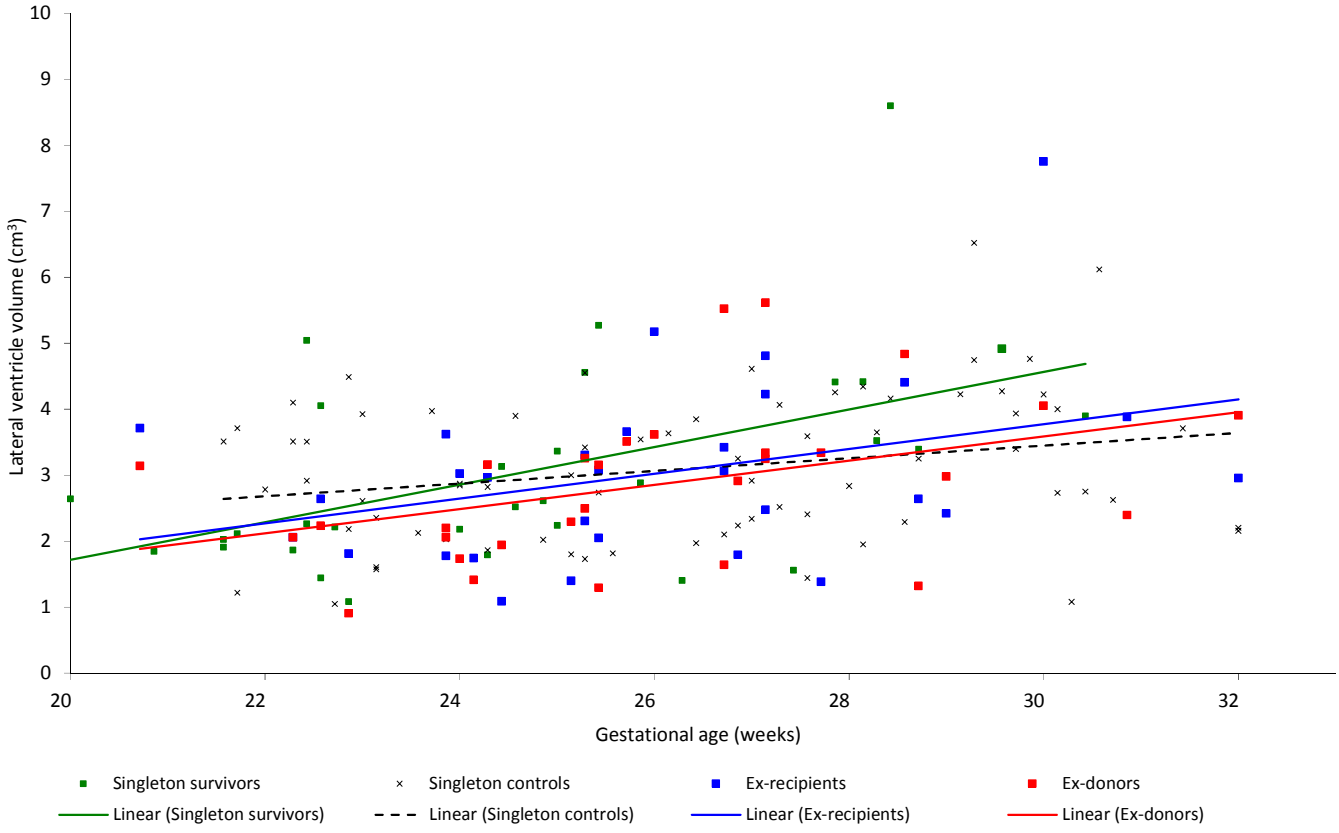


Figure 89. The relationship between GA and lateral ventricle volume in singleton survivors of iatrogenic co-twin death, singleton controls and dual survivors of laser treated TTTS.

There was no significant difference between groups (p = 0.6).

Correlation coefficients and estimated volumes are shown in table 33 alongside the data for the singleton control group (as presented in chapter 3.3) for ease of comparison.

Volume measured	STB		LV	
Singleton group	Survivors	Controls	Survivors	Controls
Spearman's r	0.94**	0.97**	0.49**	0.22*
Relative growth rate (% per week)	15.3	13.1	9.3	3.2
Average volume (cm^3)				
At 22 weeks	44.78	42.35	22.87	2.68
At 26 weeks	91.96	94.39	34.25	3.07
At 30 weeks	139.14	146.43	45.63	3.45

Table 33. Correlation coefficients, relative growth rates and estimated volumes in singleton survivors and controls.

** p <0.01

7.3.3 Singleton survivors of co-twin death subgroups

7.3.3.1 Iatrogenic '**post laser**' subgroup

Twelve cases of co-twin death following laser treatment for TTTS were analysed. All laser procedures were carried out for stage 3 disease. The same operators performed the procedure using the technique described in Appendix 1. Pregnancy characteristics and outcomes are shown in table 34 :

Case no	Maternal age (years)	GA at laser (weeks)	GA at fetal death (weeks)	Survivor status	GA at MRI (weeks)	Survivor outcome
1	34.4	20 5/7	23 5/7	recipient	24	IUD 33 6/7
2	27.1	18 2/7	18 3/7	recipient	23 6/7	LB 25 2/7
3	21.8	21 3/7	25 6/7	donor	26 1/7	IUD 29 1/7
4	33.3	17 4/7	20 2/7	recipient	27	LB 36 6/7
5	36.1	22 3/7	25 2/7	donor	26 2/7	LB 32 0/7
6	27.3	24 3/7	24 4/7	donor	25	LB 37 5/7
7	42.0	17 4/7	17 5/7	recipient	23	LB 37 0/7
8	28.9	20 4/7	20 5/7	recipient	26 4/7	LB 34 3/7
9	33.7	17 5/7	19 5/7	recipient	27 1/7	LB
10	32.3	16 5/7	16 6/7	recipient	24 2/7	LB 40 0/7

11	42.3	16 2/7	18 1/7	recipient	25 2/7	LB 39 0/7
12	29.4	19 1/7	19 2/7	recipient	23 6/7	LB 36 3/7

Table 34. Pregnancy characteristics and outcome (singleton death post laser).

Median GA at treatment in the post-laser death subgroup was 18.7 weeks (range 16.3 to 24.4). This was not significantly different to the GA at laser treatment in the double survivor group (unpaired t-test p = 0.31 2 sided). Median treatment to MRI interval in the post-laser death subgroup was 5.1 weeks (range 3.3 to 11.3 weeks). This was not significantly different to the same interval in the double survivor group (Mann-Whitney p = 0.70).

7.3.3.2 Iatrogenic '**RFA_TTTS**' subgroup

Selective feticide was carried out for TTTS in eight cases. Selective feticide was carried out by radiofrequency ablation in all cases according to standard protocol at the single referring unit.

Table 35 presents pregnancy characteristics and outcomes for the eight cases of selective feticide for TTTS. Seven cases were performed for stage 3 TTTS and one for stage 4 TTTS (case 18).

Case no	Maternal age (years)	GA at RFA (weeks)	Survivor status	GA at MRI (weeks)	Survivor outcome
13	19.3	16 1/7	recipient	20	LB 26 2/7 (NND)
14	31.0	22 2/7	recipient	24 6/7	LB
15	31.0	16 3/7	recipient	24 4/7	LB 38 0/7
16	28.4	24 4/7	recipient	27 3/7	LB 33 2/7
17	32.5	21 0/7	recipient	28 1/7	IUD 30 0/7
18	36.7	16 3/7	donor	22 5/7	IUD 33 1/7
19	24.5	17 4/7	recipient	22 3/7	LB 38 5/7
20	28.3	19 6/7	recipient	25 0/7	LB 37 0/7

Table 35. Pregnancy characteristics and outcome (selective feticide for TTTS).

7.3.3.3 Iatrogenic 'RFA_Other' subgroup

Selective feticide was carried out for non-TTTS pathology in a further twelve cases. Selective feticide was carried out by radiofrequency ablation in all cases according to standard protocol at the single referring unit.

Table 36 below presents pregnancy characteristics and outcomes for the twelve cases of selective feticide for pathology other than TTTS.

Case no	Maternal age (years)	GA at RFA (weeks)	Indication for RFA	GA at MRI (weeks)	Survivor outcome
21	38.2	16 2/7	TRAP	24 3/7	LB 36 0/7
22	32.7	22 1/7	sFGR	28 2/7	LB 38 0/7
23	29.8	21 3/7	TRAP	26 2/7	LB 31 1/7
24	26.7	17 5/7	sFGR	21 5/7	LB 32 0/7
25	24.9	16 2/7	Exomphalos	22 3/7	LB 27 5/7
26	30.2	21 3/7	DWM	25 2/7	LB 39 1/7
27	33.0	17 0/7	Anencephaly	22 2/7	LB 39 0/7
28	29.4	20 6/7	Spina bifida	24 2/7	LB 36 0/7
29	32.5	16 6/7	Anencephaly	25 0/7	LB 38 0/7
30	21.7	21 2/7	ACC	25 3/7	LB 40 4/7
31	28.6	23 3/7	DWM	25 6/7	LB 38 1/7
32	29.0	19 4/7	Anencephaly	25 5/7	LB 39 1/7

Table 36. Pregnancy characteristics and outcome (selective feticide for non-TTTS pathology).
Selective feticide was performed for a variety of indications as shown. The majority were CNS abnormalities. (ACC : Agenesis of the corpus callosum; DWM : Dandy Walker malformation; TRAP : Twin Reversed Arterial Perfusion)

7.3.3.4 'Spontaneous' subgroup

There were eight cases in the 'spontaneous' co-twin death subgroup. Pregnancy characteristics and co-twin outcome are shown in table 37.

Case no	Maternal age (years)	GA at IUD (weeks)	Probable cause	GA at MRI (weeks)	Survivor outcome
33	32.3	17 0/7	sFGR	23 3/7	Unknown
34	29.1	23 1/7	Unknown	26 0/7	LB 40 0/7
35	44.8	16 1/7	Unknown	24 3/7	LB 39 0/7
36	35.1	23 0/7	TTTS	26 6/7	LB 36 3/7
37	44.3	18 1/7	TTTS	23 1/7	LB
38	33.4	28	sFGR	31 6/7	LB 38 2/7
39	34.0	20 3/7	sFGR	23 3/7	LB 36 4/7
40	41.4	22 2/7	TTTS	26 5/7	LB 32 6/7

Table 37. Pregnancy characteristics and outcome (spontaneous single fetal death).

Table 38 shows the gestational age at which fetal death occurred, the GA at MRI and the interval between the two for each of the four singleton survivor subgroups. There was no significant difference in GA at which fetal demise occurred between groups (p = 0.4 ANOVA), in GA at MRI (p = 0.9) or in the interval between the two (p = 0.7).

	Post laser	RFA TTTS	RFA Other	Spontaneous
GA fetal death (weeks)	20.0 (18.3 – 24.1)	18.7 (16.4 – 21.6)	20.2 (16.9 – 21.4)	21.4 (17.9 – 23.0)
GA MRI (weeks)	23.4 (22.1 – 28.61)	24.7 (22.6 – 25.6)	25.1 (23.8 – 25.7)	25.2 (23.4 – 26.8)
Mean Interval (SD)	4.45 (2.71)	5.12 (2.0)	5.23 (1.79)	4.71 (1.84)

Table 38. GA at which fetal death occurred and GA at MRI in singleton survivor subgroups.
Medians and IQR are reported for gestational age data. Fetal death to MRI interval is reported as mean and SD.

7.3.3.5 Supratentorial brain tissue volume subgroup analysis

The difference between regression slopes overall from grouped linear regression analysis did not reach significance (p = 0.06). Individual slope comparisons revealed no significant slope difference between ‘RFA TTTS’ and ‘laser’ (p = 0.66). There was a significant difference in slope between ‘RFA

other' and 'post laser' ($p < 0.01$) and 'RFA other' and 'spontaneous' ($p<0.05$), with no significant difference between 'RFA other' and 'RFA TTTS' ($p = 0.08$). Covariance analysis showed no significant difference between groups overall ($p = 0.2$) however supratentorial brain tissue volume was significantly smaller in survivors of spontaneous co-twin death compared to survivors in each of the three iatrogenic subgroups ($p<0.05$). Figure 89 shows the relationship between the subgroups.

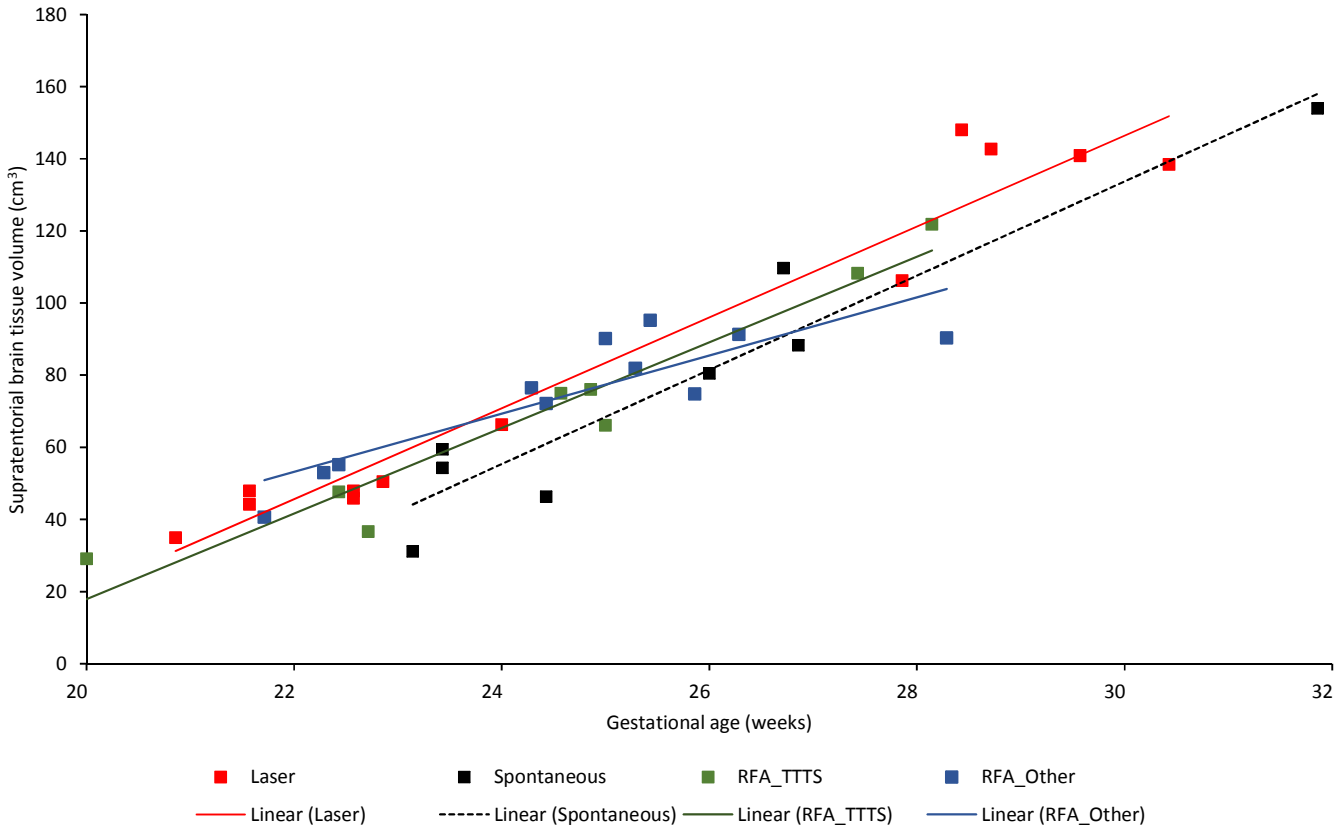


Figure 89. The relationship between GA and supratentorial brain tissue volume in singleton survivor subgroups.

Supratentorial brain tissue volumes were significantly smaller in survivors of spontaneous co-twin death compared to other subgroups ($p<0.05$).

Grouped regression analysis comparing singleton TTTS survivors (combined 'post laser' co-twin death and selective feticide 'RFA TTTS') against singleton controls and ex-recipient / ex-donor co-twins revealed a common slope ($p < 0.001$) and no significant slope difference ($p = 0.8$). Analysis of covariance was significantly different between groups ($p < 0.001$). Supratentorial brain tissue volume was significantly greater in TTTS singleton survivors compared to ex-donor co-twins ($p < 0.01$) and similar to both singleton controls ($p = 0.9$) and ex-recipient co-twins ($p = 0.9$). These comparisons are shown in figure 90.

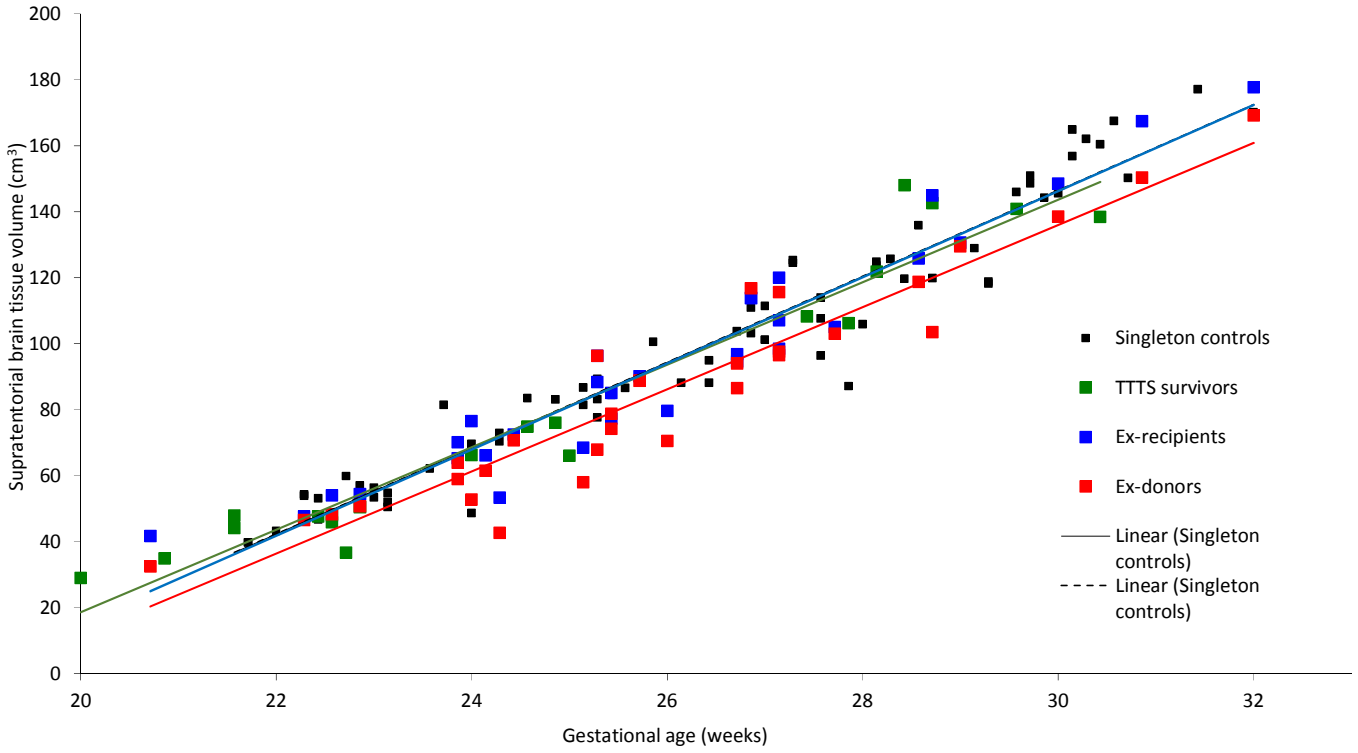


Figure 90. The relationship between GA and supratentorial brain tissue volume in TTTS singleton survivors, singleton controls and surviving TTTS twin pairs.

Supratentorial brain tissue volume was significantly lower in ex-donors from surviving twin pairs compared to all other groups ($p < 001$).

Within the TTTS survivor subgroup there were 16 ex-recipient singletons and 4 ex-donor singletons. Small sample size precludes further statistical analysis. Visual inspection of the data revealed four data points that lay below the trend line for ex-donor co-twin STB tissue volume. Two of these points were from ex-donor singletons and two from ex-recipient singletons.

7.3.4 Singleton survivor outcomes

Perinatal outcomes were available for 39 out of 40 pregnancies and are shown in table 39. Survival rate at 28 days was 87.2% (34/39).

Co-twin death	Post laser	RFA TTTS	RFA other	Spontaneous	Overall
N	12	8	12	7	39
Perinatal survival (%)	83.3	62.5	100	100	87.1

GA at delivery (weeks)	34.6 (32 – 37)	33.2 (30 – 38)	38 (34 – 39.1)	37.4 (36.5 – 38.8)	36.6 (33 – 38.1)
Birthweight (g)	2310 (1770 – 2735)	2218 (1288 – 2860)	2570 (2314 – 3195)	2650 (2480 – 2710)	2480 (2250 – 2940)

Table 39. Perinatal outcomes in singleton survivors.

Perinatal outcomes for the four singleton survivor subgroups are presented. Median and IQR are reported.

7.4 Discussion and prospective work

Singleton survivors of all iatrogenic mortality in MCDA pregnancies have similar fetal STB tissue and lateral ventricle volumes compared to singleton controls. Segmentation of the cortical grey matter and cerebellum is underway in the singleton survivor group to compare growth of these structures with singleton controls. Comparison between our double survivor TTTS cohort and singleton controls showed that brain tissue corrected cortical grey matter and cerebellar volumes were smaller in TTTS twins (chapter 3.3). Results of the ongoing analysis will determine whether this pattern is maintained in survivors of iatrogenic co-twin demise.

Our preliminary analysis suggests that there is no difference in brain growth between singleton survivors of co-twin death following laser treatment for TTTS and selective feticide for TTTS. Furthermore brain tissue volumes appear similar to ex-recipients of surviving twin pairs and significantly larger than ex-donors in surviving twin pairs. Recruitment of singleton survivors of iatrogenic co-twin death in TTTS is ongoing. Work is underway in segmentation of cortical grey matter and cerebellum to determine whether the volume of these structures is increased following co-twin death and comparable to singleton controls. Assessment of cortical folding in TTTS singleton survivors will determine whether the delay in cortical maturation observed in surviving co-twins following laser treatment persists following single twin death.

Neurodevelopmental assessment data has been collected in 46.4% of singleton survivors and is ongoing. Further analysis will focus in particular on differences in long term developmental outcome between singleton and double survivors in TTTS.

References

Ackermann, H. & Riecker, A., 2010. The contribution of the insula to speech production: a review of the clinical and functional imaging literature. *Brain Structure and Function*, 214(5-6), pp.419–33.

Acosta-Rojas, R. et al., 2007. Twin chorionicity and the risk of adverse perinatal outcome. *International Journal of Gynaecology and Obstetrics*, 96(2), pp.98–102.

Adegbite, A. et al., 2005. Prevalence of cranial scan abnormalities in preterm twins in relation to chorionicity and discordant birth weight. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 119(1), pp.47–55.

Akkermans, J. et al., 2014. Is the sequential laser technique for twin-to-twin transfusion syndrome truly superior to the standard selective technique? A meta-analysis. *Fetal Diagnosis and Therapy*, 37(4), pp.251–8.

Altman, D. & Chitty, L., 1997. New charts for ultrasound dating of pregnancy. *Ultrasound in Obstetrics and Gynecology*, 10(3), pp.174–91.

Amess, P. et al., 1999. Early brain proton magnetic resonance spectroscopy and neonatal neurology related to neurodevelopmental outcome at 1 year in term infants after presumed hypoxic-ischaemic brain injury. *Developmental Medicine and Child Neurology*, 41(7), pp.436–45.

Anderson, P., et al. 2010. Underestimation of developmental delay by the new Bayley-III scale. *Archives of Pediatrics and Adolescent Medicine.* 164(4), pp. 352-6.

Armstrong, E. et al., 1995. The Ontogeny of Human Gyrification. *Cerebral Cortex*, 5(1), pp.56–63.

Azpurua, H. et al., 2008. Metabolic assessment of the brain using proton magnetic resonance spectroscopy in a growth-restricted human fetus : case report. *American Journal of Perinatology*, 1(212), pp.305–309.

Baillieux, H. et al., 2008. Cerebellar neurocognition: insights into the bottom of the brain. *Clinical Neurology and Neurosurgery*, 110(8), pp.763–73.

Bajoria, R., Sullivan, M. & Fisk, N., 1999. Endothelin concentrations in monochorionic twins with severe twin – twin transfusion syndrome. *Human Reproduction*, 14(6), pp.1614–18.

Bajoria, R., Ward, S. & Chatterjee, R., 2003. Brain natriuretic peptide and endothelin-1 in the pathogenesis of polyhydramnios-oligohydramnios in monochorionic twins. *American Journal of Obstetrics and Gynecology*, 189(1), pp.189–194.

Bajoria, R., Ward, S. & Chatterjee, R., 2002. Natriuretic peptides in the pathogenesis of cardiac dysfunction in the recipient fetus of twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 186(1), pp.121–127.

Bajoria, R., Ward, S. & Sooranna, S., 2001. Atrial natriuretic peptide mediated polyuria: pathogenesis of polyhydramnios in the recipient twin of twin-twin transfusion syndrome. *Placenta*, 22(8-9), pp.716–24.

Bajoria, R., Ward, S. & Sooranna, S.R., 2004. Influence of vasopressin in the pathogenesis of oligohydramnios-polyhydramnios in monochorionic twins. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 113(1), pp.49–55.

Baker, P. et al., 1994. A three-year follow-up of children imaged in utero with echo-planar magnetic resonance. *American Journal of Obstetrics and Gynecology*, 170(1), pp.32–33.

Baker, P. et al., 1995. Measurement of fetal liver, brain and placental volumes with echo-planar magnetic resonance imaging. *British Journal of Obstetrics & Gynaecology*, 102(1), pp.35–39.

Banek, C. et al., 2003. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 188(4), pp.876–880.

Barkovich, A., 2010. Current concepts of polymicrogyria. *Neuroradiology*, 52(6), pp.479–487.

Barkovich, A. et al., 1999. Proton MR spectroscopy for the evaluation of brain injury in asphyxiated, term neonates. *American Journal of Neuroradiology*, 20(8), pp.1399–405.

Barrea, C. et al., 2006. Impact of selective laser ablation of placental anastomoses on the cardiovascular pathology of the recipient twin in severe twin-twin transfusion syndrome. *American Journal of Obsterics & Gynecology*, 195(5), pp.1388–95.

Baschat, A. et al., 2013. Outcome after fetoscopic selective laser ablation of placental anastomoses vs equatorial laser dichorionization for the treatment of twin-to-twin transfusion syndrome. *American Journal of Obsterics & Gynecology*, 209(3), pp.234.e1–8.

Baschat, A. et al., 2011. Twin-to-twin transfusion syndrome (TTTS). *Journal of Perinatal Medicine*, 39(2), pp.107–12.

Bastian, A. & Thach, W., 2002. *The cerebellum and its disorders. Structure and function of the cerebellum.* M. Manto & M. Pandolfo, eds., Cambridge, UK: Cambridge University Press.

Battin, M., Bevan, C. & Harding, J., 2008. Power and interpretation of a randomized study on the treatment of severe twin-to-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 198(5), pp.606–7.

Bayley, N., 1969. *Manual for the Bayley scales of infant development.* San Antonio, TX: The Psychological Corporation.

Bayley, N., 1993. *Bayley scales of infant development.* 2nd ed., San Antonio, TX: PsychCorp, Pearson Assessment Inc

Bayley, N., 2006. *Bayley scales of infant and toddler development.* 3rd ed., San Antonio, TX: Harcourt Assessment.

Becker, J. et al., 2006. Stage-dependent fetal umbilical blood flow changes induced by laser therapy and amniodrainage in twin-to-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 28(5), pp.674–80.

Behrman, R. et al., 1970. Distribution of the circulation in the normal and asphyxiated fetal primate. *American Journal of Obstetrics & Gynecology*, 108(6), pp.956–69.

Bell, C., Han, V. & Sawtell, N., 2008. Cerebellum-like structures and their implications for cerebellar function. *Annual Review of Neuroscience*, 31, pp.1–24.

Bendon, R., 1995. Twin transfusion: pathological studies of the monochorionic placenta in liveborn twins and of the perinatal autopsy in monochorionic twin pairs. *Pediatric Pathology and Laboratory Medicine*, 15(3), pp.363–76.

Berger, H., de Waard, F. & Molenaar, Y., 2000. A case of twin-to-twin transfusion in 1617. *The Lancet*, 356(9232), pp.847–848.

Berger-Kulemann, V. et al., 2013. MR spectroscopy of the fetal brain: is it possible without sedation? *American Journal of Neuroradiology*, 34(2), pp.424–31.

Berghella, V. & Kaufmann, M., 2001. Natural history of twin-twin transfusion syndrome. *The Journal of Reproductive Medicine*, 46(5), pp.480–4.

Bermúdez, C. et al., 2002. Placental types and twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 187(2), pp.489–494.

Bhakoo, K., Craig, T. & Pearce, D., 2006. N-acetylaspartate metabolism in neural cells. *Advances in Experimental Medicine and Biology*, 576, pp.27–47.

Blickstein, I., 2002. Cerebral palsy in multifoetal pregnancies. *Developmental Medicine and Child Neurology*, 44(5), pp.352–5.

Blickstein, I., 2006. Monochorionicity in perspective. *Ultrasound in Obstetrics and Gynecology*, 27(3), pp.235–8.

Bode, M., et al. 2014. Predictive validity of the Bayley, Third edition at 2 years for intelligience quotient at 4 years in preterm infants. *Journal of Developmental and Behavioral Pediatrics,* 35(9), pp.570-5.

Van Bogaert, P. et al., 1996. Congenital bilateral perisylvian syndrome in a monozygotic twin with intra-uterine death of the co-twin. *Developmental Medicine and Child Neurology*, 38(2), pp.166–70.

Bolduc, M.-E., Du Plessis, A., et al., 2012. Cerebellar malformations alter regional cerebral development. *Developmental Medicine and Child Neurology*, 53(12), pp.1128–34.

Bolduc, M.-E., du Plessis, A., et al., 2012. Regional cerebellar volumes predict functional outcome in children with cerebellar malformations. *Cerebellum*, 11(2), pp.531–42.

Bolduc, M.-E. et al., 2011. Spectrum of neurodevelopmental disabilities in children with cerebellar malformations. *Developmental Medicine and Child Neurology*, 53(5), pp.409–16.

Brighina, E. et al., 2009. Human fetal brain chemistry as detected by proton magnetic resonance spectroscopy. *Pediatric Neurology*, 40(5), pp.327–42.

Brody, B. et al., 1987. Sequence of central nervous system myelination in human infancy. An autopsy study of myelination. *Journal of Neuropathology and Experimental Neurology*, 46(3), pp.283–301.

Butt, K. et al., 2014. Determination of gestational age by ultrasound. *Journal of Obstetrics and gynaecology Canada*, 36(2), pp.171–81.

Campbell, W. et al., 1994. Use of the transverse cerebellar diameter/abdominal circumference ratio in pregnancies at risk for intrauterine growth retardation. *Journal of Clinical Ultrasound*, 22(8), pp.497–502.

Van Cappellen, M. et al., 1999. Hypoxia, the subsequent systemic metabolic acidosis, and their relationship with cerebral metabolite concentrations: An in vivo study in fetal lambs with proton magnetic resonance spectroscopy. *American Journal of Obsterics & Gynaecology*, 181(6), pp.1537–45.

Carnes, K. & Magin, R., 1996. Effects of in utero exposure to 4.7T MR imaging conditions on fetal growth and testicular development in the mouse. *Magnetic Resonance Imaging*, 14(3), pp.263–74.

Carr, S. et al., 2004. Antenatal necrotic injury in severe twin-to-twin transfusion syndrome. A case and review. *Fetal Diagnosis and Therapy*, 19(4), pp.370–2.

Carver, A. et al., 2011. Monochorionic diamniotic twin pregnancy: timing and duration of sonographic surveillance for detection of twin-twin transfusion syndrome. *Journal of Ultrasound Medicine*, 30(3), pp.297–301.

Casanova, M. et al., 2004. Reduced brain size and gyrification in the brains of dyslexic patients. *Journal of Child Neurology*, 19(4), pp.275–81.

Cavicchioni, O. et al., 2006. Intrauterine fetal demise following laser treatment in twin-to-twin transfusion syndrome. *British Journal of Obstetrics & Gynaecology*, 113(5), pp.590–4.

Cetin, I. et al., 2011. Lactate detection in the brain of growth-restricted fetuses with magnetic resonance spectroscopy. *American Journal of Obstetrics & Gynecology*, 205(4), pp.350.e1–7.

Chao, C.P., Zaleski, C.G. & Patton, A.C., 2006. Neonatal Hypoxic-ischemic encephalopathy : multimodality imaging findings. *Radiographics*, 32224, pp.159–173.

Charlemaine, C. et al., 2000. Fetal biometric parameters, twin type and birth weight difference. A longitudinal study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 93(1), pp.27–32.

Chaudhary, T. et al., 2013. Predictive and concurrent validity of standardized neurodevelopmental examinations by the Griffiths scales and Bayley scales of infant development II. *Klinische Padiatrie,* 225(1), pp.8-12.

Chen, S. et al., 2009. Ischemia-induced brain damage is enhanced in human renin and angiotensinogen double-transgenic mice. *American Journal of Physiology*, 297(5), pp.R1526–31.

Cheong, J. et al., 2006. Proton MR spectroscopy in neonates with perinatal cerebral hypoxic-ischemic injury: metabolite peak-area ratios, relaxation times, and absolute concentrations. *American journal of neuroradiology*, 27(7), pp.1546–54.

Chescheir, N. & Seeds, J., 1988. Polyhydramnios and oligohydramnios in twin gestations. *Obstetrics and Gynecology*, 71(6), pp.882–4.

Chew, S. et al., 2001. The effects of 1.5T magnetic resonance imaging on early murine in-vitro embryo development. *Journal of Magnetic Resonance Imaging*, 13(3), pp.417–20.

Chi, J. & Dooling, E., 1977. Gyral development of the human brain. *Annals of Neurology*, 1(1), pp.86–93.

Chmait, R. et al., 2008. Fetal growth after laser therapy for twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 199(1), pp.47.e1–6.

Chmait, R. et al., 2011. Stage-based outcomes of 682 consecutive cases of twin-twin transfusion syndrome treated with laser surgery: the USFetus experience. *American Journal of Obsterics & Gynecology*, 204(5), pp.393.e1–6.

Chmait, R., Kontopoulos, E. & Quintero, R., 2014. Sequential laser surgery for twin-twin transfusion syndrome. *American Journal of Perinatology*, 31(S1), pp.13–8.

Cincotta, R. et al., 2009. Selective fetoscopic laser ablation in 100 consecutive pregnancies with severe twin-twin transfusion syndrome. *The Australian & New Zealand Journal of Obstetrics & Gynaecology*, 49(1), pp.22–7.

Clements, H. et al., 2000. Infants exposed to MRI in utero have a normal paediatric assessment at 9 months of age. *The British Journal of Radiology*, 73(866), pp.190–4.

Clouchoux, C. et al., 2011. Normative fetal brain growth by quantitative in vivo magnetic resonance imaging. *American Journal of Obstetrics and Gynecology*, 206(2), pp.173.e1–8.

Clouchoux, C. et al., 2012. Quantitative in vivo MRI measurement of cortical development in the fetus. *Brain Structure and Function*, 217(1), pp.127–39.

Clowry, G., Molnár, Z. & Rakic, P., 2010. Renewed focus on the developing human neocortex. *Journal of Anatomy*, 217(4), pp.276–88.

Courchesne, E. et al., 2001. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology*, 57(2), pp.245–54.

Craig, A.D.B., 2009. How do you feel now? The anterior insula and human awareness. *Nature Reviews. Neuroscience*, 10(1), pp.59–70.

Crombleholme, T. et al., 2007. A prospective, randomized, multicenter trial of amnioreduction vs selective fetoscopic laser photocoagulation for the treatment of severe twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 197(4), pp.396.e1–9.

D'Antonio, F., Dias, T. & Thilaganathan, B., 2013. Does antenatal ultrasound labeling predict birth order in twin pregnancies? *Ultrasound in Obstetrics and Gynecology*, 41(September 2012), pp.274–277.

Damodaram, M. et al., 2012. Foetal volumetry using Magnetic Resonance Imaging in intrauterine growth restriction. *Early Human Development*, 88(S1), pp.S35–40.

Degani, S. et al., 2006. Instability of Doppler cerebral blood flow in monochorionic twins. *Journal of Ultrasound in Medicine*, 25(4), pp.449–54.

Delle Urban, L. et al., 2004. Prenatal ultrasound detection of bilateral focal polymicrogyria. *Prenatal Diagnosis*, 24(10), pp.808–11.

Denbow, M. et al., 1998. Colour Doppler energy insonation of placental vasculature in monochorionic twins: absent arterio-arterial anastomoses in association with twin-to-twin transfusion syndrome. *British Journal of Obstetrics & Gynaecology*, 105(7), pp.760–5.

Denbow, M. et al., 1998. Haematological indices at fetal blood samplng in monochorionic pregnancies complicated by feto-fetal transfusion syndrome. *Prenatal Diagnosis*, 18, pp.941–46.

Denbow, M. et al., 2000. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. *American Journal of Obstetrics and Gynecology*, 182(2), pp.417–26.

Denbow, M. et al., 2000. Twin fetuses : Intravascular microbubble US contrast agent administration - early experience. *Radiology*, 214, pp.724–8.

Denegre, J. et al., 1998. Cleavage planes in frog eggs are altered by strong magnetic fields. *Proceedings of the National Academy of Sciences of the United States of America*, 95(25), pp.14729–32.

Dhouib, A. et al., 2011. Correlation between pre- and postnatal cerebral magnetic resonance imaging. *Ultrasound in Obstetrics and Gynecology*, 38(2), pp.170–8.

Dias, T. et al., 2010. First-trimester ultrasound dating of twin pregnancy: are singleton charts reliable? *British Journal of Obstetrics & Gynaecology*, 117(8), pp.979–84.

Dias, T. et al., 2011. Second-trimester assessment of gestational age in twins: validation of singleton biometry charts. *Ultrasound in Obstetrics and Gynecology*, 37(1), pp.34–7.

Diehl, W. et al., 2001. Placental vascular anastomoses visualized during fetoscopic laser surgery in severe mid-trimester twin – twin transfusion syndrome. *Placenta*, 22(10), pp.876–81.

Diemert, A. et al., 2010. Laser therapy of twin-to-twin transfusion syndrome in triplet pregnancies. *Ultrasound in Obstetrics and Gynecology*, 35(1), pp.71–4.

Dilmen, G. et al., 1996. Transverse cerebellar diameter and transverse cerebellar diameter/ abdominal circumference index for assessing fetal growth. *Fetal Diagnosis and Therapy*, 11(1), pp.50–6.

Dong, Y.-F. et al., 2011. Attenuation of brain damage and cognitive impairment by direct renin inhibition in mice with chronic cerebral hypoperfusion. *Hypertension*, 58(4), pp.635–42.

Dorovini-Zis, K. & Dolman, C.L., 1977. Gestational development of brain. *Archives of Pathology & Laboratory Medicine*, 101(4), pp.192–5.

Drost, D., Riddle, W. & Clarke, G., 2002. Proton magnetic resonance spectroscopy in the brain: report of AAPM MR Task Group #9. *Medical Physics*, 29(9), pp.2177–97.

Dubois, J. et al., 2008. Primary cortical folding in the human newborn: an early marker of later functional development. *Brain*, 131(8), pp.2028–41.

Edwards, M., Saunders, R. & Shiota, K., 2006. Effects of heat on embryos and foetuses. *International Journal of Hyperthermia*, 19(3), pp.295–324.

Edwards, P., et al. 2002. Increasing response rates to postal questionnaires: a systematic review. *British Medical Journal,* 324(7347), pp. 1183-1192.

Egaña-Ugrinovic, G. et al., 2013. Differences in cortical development assessed by fetal MRI in late-onset intrauterine growth restriction. *American Journal of Obstetrics and Gynecology*, 209(2), pp.126.e1–8.

Egaña-Ugrinovic, G. et al., 2014. Fetal MRI insular cortical morphometry and its association with neurobehavior in late-onset small for gestational age fetuses. *Ultrasound in Obstetrics and Gynecology*, 44(3), pp.322–9.

Espinar, A. et al., 1997. Histological changes during development of the cerebellum in the chick embryo exposed to a static magnetic field. *Bioelectromagnetics*, 18(1), pp.36–46.

Van Essen, D.C., 1997. A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature*, 385(6614), pp.313–8.

Etzel, R. et al., 1997. Noise: A Hazard for the Fetus and Newborn. *Pediatrics*, 100(4), pp.724–7.

Fenton, B. et al., 2001. The fetus at term: in utero volume-selected proton MR spectroscopy with a breath-hold technique--a feasibility study. *Radiology*, 219(2), pp.563–6.

Ferrer, I. & Catalá, I., 1991. Unlayered polymicrogyria: structural and developmental aspects. *Anatomy and Embryology*, 184(5), pp.517–28.

Fink, G. et al., 1997. Multiple nonprimary motor areas in the human cortex. *Journal of Neurophysiology*, 77(4), pp.2164–74.

Fisher, S., Novak, J.E. & Agranoff, B.W., 2002. Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. *Journal of Neurochemistry*, 82(4), pp.736–54.

Fisk, N., Duncombe, G. & Sullivan, M., 2009. The basic and clinical science of twin-twin transfusion syndrome. *Placenta*, 30(5), pp.379–90.

Fogliarini, C. et al., 2005. Assessment of cortical maturation with prenatal MRI. Part I: Normal cortical maturation. *European Radiology*, 15(8), pp.1671–85.

Frick, N. et al., 2015. The reliability of fetal MRI in the assessment of brain malformations. *Fetal Diagnosis and Therapy*, 37(2), pp.93–101.

Galea, P. et al., 2008. The placenta contributes to activation of the renin angiotensin system in twin-twin transfusion syndrome. *Placenta*, 29(8), pp.734–42.

Garden, A. & Roberts, N., 1996. Fetal and fetal organ volume estimations with magnetic resonance imaging. *American Journal of Obstetrics and Gynecology*, 175(2), pp.442–8.

Gardiner, H. et al., 2014. Cardiac function in 10-year-old twins following different fetal therapies for twin-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 43(6), pp.652–7.

Gardiner, H.M., 2001. Early changes in vascular dynamics in relation to twin-twin transfusion syndrome. *Twin research : the official journal of the International Society for Twin Studies*, 4(5), pp.371–377.

Garel, C. et al., 2001. Fetal cerebral cortex: normal gestational landmarks identified using prenatal MR imaging. *American journal of neuroradiology*, 22(1), pp.184–9.

Garel, C. et al., 2003. Fetal MRI: normal gestational landmarks for cerebral biometry, gyration and myelination. *Child's Nervous System*, 19(7-8), pp.422–5.

Garel, C. & Alberti, C., 2006. Coronal measurement of the fetal lateral ventricles: comparison between ultrasonography and magnetic resonance imaging. *Ultrasound in Obstetrics & Gynecology*, 27(1), pp.23–7.

Geschwind, D. & Rakic, P., 2013. Cortical evolution: judge the brain by its cover. *Neuron*, 80(3), pp.633–47.

Gholipour, A. et al., 2011. Fetal brain volumetry through MRI volumetric reconstruction and segmentation. *International Journal of Computer Assisted Radiology and Surgery*, 6(3), pp.329–39.

Gholipour, A., Estroff, J. & Warfield, S., 2010. Robust super-resolution volume reconstruction from slice acquisitions: application to fetal brain MRI. *IEEE Transactions on Medical Imaging*, 29(10), pp.1739–58.

Gilmore, J. et al., 1996. Fetal brain development of twins assessed in utero by ultrasound: implications for schizophrenia. *Schizophrenia Research*, 19(2), pp.141–149.

Girard, N., Gouny, S., et al., 2006. Assessment of normal fetal brain maturation in utero by proton magnetic resonance spectroscopy. *Magnetic Resonance in Medicine*, 56(4), pp.768–75.

Girard, N. et al., 2001. Fetal brain MR imaging. *Magnetic Resonance Imaging Clinics of North America*, 9(1), pp.19–56, vii.

Girard, N. et al., 2003. MR imaging of acquired fetal brain disorders. *Child's Nervous System*, 19(7-8), pp.490–500.

Girard, N., Fogliarini, C., et al., 2006. MRS of normal and impaired fetal brain development. *European Journal of Radiology*, 57(2), pp.217–25.

Girard, N. & Chaumoitre, K., 2012. The brain in the belly: What and how of fetal neuroimaging? *Journal of Magnetic Resonance Imaging*, 36(4), pp.788–804.

Glenn, O., 2010. MR imaging of the fetal brain. *Pediatric Radiology*, 40(1), pp.68–81.

Glenn, O., 2009. Normal development of the fetal brain by MRI. *Seminars in Perinatology*, 33(4), pp.208–19.

Glinianaia, S. et al., 2002. Fetal or infant death in twin pregnancy: neurodevelopmental consequence for the survivor. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 86(1), pp.F9–15.

Glover, P. et al., 1995. An assessment of the intrauterine sound intensity level during obstetric echo-planar magnetic resonance imaging. *The British Journal of Radiology*, 68(814), pp.1090–4.

Goldstein, I. et al., 1987. Cerebellar measurements with ultrasonography in the evaluation of fetal growth and development. *American Journal of Obstetrics and Gynecology*, 156(5), pp.1065–9.

Goldstein, I. & Reece, E., 1995. Cerebellar growth in normal and growth-restricted fetuses of multiple gestations. *American journal of obstetrics and gynecology*, 173(4), pp.1343–8.

Goldstein, I., Reece, E. & Tamir, A., 2002. Cerebellar growth in normal fetuses of multiple gestations. *The Journal of Maternal-Fetal & Neonatal medicine*, 11(3), pp.188–91.

Gollenberg, A. et al., 2010. Concurrent validity of the parent-completed Ages and Stages Questionnaires, 2nd Ed. with the Bayley Scales of Infant Development II in a low-risk sample. *Child: Care, Health and Development*, 36(4), pp.485–90.

Gong, Q. et al., 1998. Fetal and fetal brain volume estimation in the third trimester of human pregnancy using gradient echo MR imaging. *Magnetic Resonance Imaging*, 16(3), pp.235–240.

Graef, C. et al., 2006. Long-term neurodevelopmental outcome of 167 children after intrauterine laser treatment for severe twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 194(2), pp.303–8.

Graeve, P. et al., 2012. Neurodevelopmental outcome at 6 years of age after intrauterine laser therapy for twin-twin transfusion syndrome. *Acta Paediatrica*, 101(12), pp.1200–5.

Grafe, M.R., 1993. Antenatal cerebral necrosis in monochorionic twins. *Pediatric Pathology*, 13(1), pp.15–9.

Gratacós, E. et al., 2002. Impact of laser coagulation in severe twin-twin transfusion syndrome on fetal Doppler indices and venous blood flow volume. *Ultrasound in Obstetrics and Gynecology*, 20(2), pp.125–30.

Gray, P. et al., 2011. Neurodevelopmental outcome and risk factors for disability for twin-twin transfusion syndrome treated with laser surgery. *American Journal of Obstetrics and Gynecology*, 204(2), pp.159.e1–6.

Griffiths, P. et al., 2015. Fetal brain injury in survivors of twin pregnancies complicated by demise of one twin as assessed by in utero MR imaging. *Prenatal Diagnosis*, 35(6), pp.583–91.

Griffiths, R. *The abilities of young children.* London: Child Development Research Centre. 1970.

Griffiths, R. & Huntley, M. *The Griffiths mental development scales - revised manual: from birth to 2 years.* High Wycombe. ARICD. 1996.

Grossman, R. et al., 2006. Quantitative MRI measurements of human fetal brain development in utero. *NeuroImage*, 33(2), pp.463–70.

Guilherme, R. et al., 2009. Very early twin-to-twin transfusion syndrome and discordant activation of the renin-angiotensin system. *Placenta*, 30(8), pp.731–4.

Guisasola, C. et al., 2002. Biological dosimetry of magnetic resonance imaging. *Journal of Magnetic Resonance Imaging*, 15(5), pp.584–90.

Gul, A. et al., 2003. Natural history of 11 cases of twin-twin transfusion syndrome without intervention. *Twin Research*, 6(4), pp.263–6.

Habas, P. et al., 2010. A spatiotemporal atlas of MR intensity, tissue probability and shape of the fetal brain with application to segmentation. *NeuroImage*, 53(2), pp.460–70.

Habas, P. et al., 2012. Early folding patterns and asymmetries of the normal human brain detected from in utero MRI. *Cerebral Cortex*, 22(1), pp.13–25.

Habli, M., Michelfelder, E., et al., 2008. Acute effects of selective fetoscopic laser photocoagulation on recipient cardiac function in twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 199(4), pp.412.e1–6.

Habli, M. et al., 2009. Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. *American Journal of Obstetrics and Gynecology*, 201(4), pp.417.e1–7.

Habli, M., Livingston, J., et al., 2008. The outcome of twin-twin transfusion syndrome complicated with placental insufficiency. *American Journal of Obstetrics and Gynecology*, 199(4), pp.424.e1–6.

Habli, M. et al., 2010. The relationship between amniotic fluid levels of brain-type natriuretic peptide and recipient cardiomyopathy in twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 203(4), pp.404.e1–7.

Hack, K., Derks, J., et al., 2008. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. *British Journal of Obstetrics & Gynaecology*, 115(1), pp.58–67.

Hack, K., Nikkels, P., et al., 2008. Placental characteristics of monochorionic diamniotic twin pregnancies in relation to perinatal outcome. *Placenta*, 29(11), pp.976–81.

Hagmann, C.F. et al., 2008. Foetal brain imaging: ultrasound or MRI. A comparison between magnetic resonance imaging and a dedicated multidisciplinary neurosonographic opinion. *Acta Paediatrica*, 97(4), pp.414–9.

Hand, J. et al., 2006. Prediction of specific absorption rate in mother and fetus associated with MRI examinations during pregnancy. *Magnetic Resonance in Medicine*, 55(4), pp.883–93.

Hand, J., Li, Y. & Hajnal, J. V, 2010. Numerical study of RF exposure and the resulting temperature rise in the foetus during a magnetic resonance procedure. *Physics in Medicine and Biology*, 55(4), pp.913–30.

Haratz, K. et al., 2011. Morphological evaluation of lateral ventricles of fetuses with ventriculomegaly by three-dimensional ultrasonography and magnetic resonance imaging: correlation with etiology. *Archives of Gynecology and Obstetrics*, 284(2), pp.331–6.

Harkness, U. & Crombleholme, T., 2005. Twin-twin transfusion syndrome: where do we go from here? *Seminars in Perinatology*, 29(5), pp.296–304.

Has, R. et al., 2014. Stage-related outcome after fetoscopic laser ablation in twin-to-twin transfusion syndrome. *Fetal diagnosis and Therapy*, 36(4), pp.287–92.

Hatab, M., Kamourieh, S. & Twickler, D., 2008. MR volume of the fetal cerebellum in relation to growth. *Journal of Magnetic Resonance Imaging*, 27(4), pp.840–5.

Hecher, K. et al., 2000. Endoscopic laser coagulation of placental anastomoses in 200 pregnancies with severe mid-trimester twin-to-twin transfusion syndrome. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 92(1), pp.135–139.

Heerschap, A., Kok, R. & van den Berg, P., 2003. Antenatal proton MR spectroscopy of the human brain in vivo. *Child's Nervous System*, 19(7-8), pp.418–21.

Heinrichs, W. et al., 1988. Midgestational exposure of pregnant BALB/c mice to magnetic resonance imaging conditions. *Magnetic Resonance Imaging*, 6, pp.305–313.

Hernández, M. et al., 1980. Cerebral blood flow and metabolism during hypoglycemia in newborn dogs. *Journal of Neurochemistry*, 35(3), pp.622–8.

Hernandez-Andrade, E. et al., 2008. Changes in regional fetal cerebral blood flow perfusion in relation to hemodynamic deterioration in severely growth-restricted fetuses. *Ultrasound in Obstetrics and Gynecology*, 32(1), pp.71–6.

Hernandez-Andrade, E. et al., 2011. Social and economic factors influencing a fetal surgery program for complicated monochorionic twin pregnancies in a developing country. *Fetal Diagnosis and Therapy*, 29(3), pp.224–8.

Hilgetag, C. & Barbas, H., 2006. Role of mechanical factors in the morphology of the primate cerebral cortex. *PLoS Computational Biology*, 2(3), p.e22.

Hillman, S., Morris, R. & Kilby, M., 2011. Co-twin prognosis after single fetal death: a systematic review and meta-analysis. *Obstetrics and Gynecology*, 118(4), pp.928–40.

Hillman, S., Morris, R. & Kilby, M., 2010. Single twin demise: consequence for survivors. *Seminars in Fetal & Neonatal Medicine*, 15(6), pp.319–26.

Hoffmann, C. et al., 2013. Diffusion MRI findings in monochorionic twin pregnancies after intrauterine fetal death. *American Journal of Neuroradiology*, 34(1), pp.212–6.

Hoffmann, C. et al., 2010. Effect of cytomegalovirus infection on temporal lobe development in utero: quantitative MRI studies. *European Neuropsychopharmacology*, 20(12), pp.848–54.

Hu, H.-H. et al., 2009. Morphological regionalization using fetal magnetic resonance images of normal developing brains. *The European Journal of Neuroscience*, 29(8), pp.1560–7.

Huang, H., 2010. Structure of the fetal brain: what we are learning from diffusion tensor imaging. *The Neuroscientist*, 16(6), pp.634–49.

Huber, A. et al., 2006. Stage-related outcome in twin-twin transfusion syndrome treated by fetoscopic laser coagulation. *Obstetrics and Gynecology*, 108(2), pp.333–7.

Huntley, M., 1996. *The Griffiths Mental Development Scales.* M. Huntley, ed., Hogrefe Ltd.

Ishii, K. et al., 2004. Ultrasound assessment of venous blood flow before and after laser therapy: approach to understanding the pathophysiology of twin-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 24(2), pp.164–8.

Jackson, T. & Plante, E., 1996. Gyral morphology in the posterior Sylvian region in families affected by developmental language disorder. *Neuropsychology Review*, 6(2), pp.81–94.

Jacob, F. et al., 2011. Fetal hippocampal development: analysis by magnetic resonance imaging volumetry. *Pediatric Research*, 69(5), pp.425–9.

Jacobs, M. et al., 2001. Quantitative proton MR spectroscopic imaging of normal human cerebellum and brain stem. *Magnetic Resonance in Medicine*, 46(4), pp.699–705.

Jain, S. et al., 2015. Transgenic mice overexpressing human Angiotensin I receptor gene are susceptible to stroke injury. *Molecular Neurobiology*.

Jelin, A. et al., 2008. Intracranial magnetic resonance imaging findings in the surviving fetus after spontaneous monochorionic cotwin demise. *American Journal of Obstetrics and Gynecology*, 199(4), pp.398.e1–5.

Jiang, S. et al., 2007. MRI of moving subjects using multislice snapshot images with volume reconstruction (SVR): application to fetal, neonatal and adult brain studies. *IEEE Transactions on Medical Imaging*, 26(7), pp.967–80.

Johnson, A. & Moise, K., 2008. A randomized trial for the treatment of TTTS, too little to answer the question. *American Journal of Obstetrics and Gynecology*, 198(5), pp.607–8.

Johnson, S., Moore, T. & Marlow, N., 2014. Using the Bayley-III to assess neurodevelopmental delay: which cut-off should be used? *Pediatric Research.* 75(5), pp.670-4.

Johnson, S. & Marlow, N., 2006. Developmental screen or developmental testing? *Early Human Development*. 82, pp.173-183.

Jones, C., Ward, J. & Critchley, H., 2010. The neuropsychological impact of insular cortex lesions. *Journal of Neurology, Neurosurgery, and Psychiatry*, 81(6), pp.611–8.

Kanal, E. et al., 1993. Survey of reproductive health among female MR workers. *Radiology*, 187(2), pp.395–9.

Kanal, E., Shellock, F. & Talagala, L., 1990. Safety considerations in MR imaging. *Radiology*, 176(3), pp.593–606.

Kawabata, I. et al., 2003. MRI during pregnancy. *Journal of Perinatal Medicine*, 31(6), pp.449–58.

Kazan-Tannus, J. et al., 2007. MR volumetry of brain and CSF in fetuses referred for ventriculomegaly. *American Journal of Roentgenology*, 189(1), pp.145–51.

Keraudren, K. et al., 2014. Automated fetal brain segmentation from 2D MRI slices for motion correction. *NeuroImage*, 101, pp.633–43.

Kesler, S. et al., 2006. Increased temporal lobe gyrification in preterm children. *Neuropsychologia*, 44(3), pp.445–53.

Kilby, M. et al., 2001. Renin gene expression in fetal kidneys of pregnancies complicated by twin-twin transfusion syndrome. *Pediatric and Developmental Pathology*, 4(2), pp.175–9.

Kim, K. et al., 2010. Intersection based motion correction of multislice MRI for 3-D in utero fetal brain image formation. *IEEE Transactions on Medical Imaging*, 29(1), pp.146–58.

Kinney, H. et al., 1988. Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. *Journal of Neuropathology and Experimental Neurology*, 47(3), pp.217–34.

Kline-Fath, B. et al., 2007. Twin-twin transfusion syndrome: cerebral ischemia is not the only fetal MR imaging finding. *Pediatric Radiology*, 37(1), pp.47–56.

Van Klink, J. et al., 2013. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. *Fetal Diagnosis and Therapy*, 33(2), pp.81–9.

Van Klink, J. et al., 2011. Long-term neurodevelopmental outcome in monochorionic twins after fetal therapy. *Early Human Development*, 87(9), pp.601–6.

Van der Knaap, M. et al., 1996. Normal gyration and sulcation in preterm and term neonates: appearance on MR images. *Radiology*, 200(2), pp.389–96.

Knickmeyer, R. et al., 2008. A structural MRI study of human brain development from birth to 2 years. *The Journal of Neuroscience*, 28(47), pp.12176–82.

Knickmeyer, R. et al., 2011. Twin-singleton differences in neonatal brain structure. *Twin Research and Human Genetics*, 14(3), pp.268–76.

Kok, R. et al., 2004. Absence of harmful effects of magnetic resonance exposure at 1.5 T in utero during the third trimester of pregnancy: a follow-up study. *Magnetic Resonance Imaging*, 22(6), pp.851–4.

Kok, R. et al., 2002. Maturation of the human fetal brain as observed by 1H MR spectroscopy. *Magnetic Resonance in Medicine*, 48(4), pp.611–6.

Kok, R. et al., 2001. Metabolic information from the human fetal brain obtained with proton magnetic resonance spectroscopy. *American Journal of Obstetrics and Gynecology*, 185(5), pp.1011–5.

Korf, J., 2006. Is brain lactate metabolized immediately after neuronal activity through the oxidative pathway? *Journal of Cerebral Blood Flow and Metabolism*, 26(12), pp.1584–6.

Kowitt, B. et al., 2012. Long-term morbidity after fetal endoscopic surgery for severe twin-to-twin transfusion syndrome. *Journal of Pediatric Surgery*, 47(1), pp.51–6.

Kreis, R. et al., 2002. Brain metabolite composition during early human brain development as measured by quantitative in vivo 1H magnetic resonance spectroscopy. *Magnetic Resonance in Medicine*, 48(6), pp.949–58.

Kyriakopoulou, V. et al., 2014. Cortical overgrowth in fetuses with isolated ventriculomegaly. *Cerebral Cortex*, 24(8), pp.2141–50.

Larsen, K. et al., 2008. Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. *Annals of Anatomy*, 190(5), pp.421–7.

Lecanuet, J. et al., 1998. What sounds reach fetuses: biological and nonbiological modeling of the transmission of pure tones. *Developmental Psychobiology*, 33(3), pp.203–19.

Lee, G. et al., 2002. A nested case-control study of residential and personal magnetic field measures and miscarriages. *Epidemiology*, 13(1), pp.21–31.

Lee, W. et al., 1991. Transverse cerebellar diameter: a useful predictor of gestational age for fetuses with asymmetric growth retardation. *American Journal of Obstetrics and Gynecology*, 165(4), pp.1044–50.

Lenclen, R. et al., 2007. Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. *American Journal of Obstetrics and Gynecology*, 196(5), pp.450.e1–7.

Lenclen, R. et al., 2009. Neurodevelopmental outcome at 2 years in children born preterm treated by amnioreduction or fetoscopic laser surgery for twin-to-twin transfusion syndrome: comparison with dichorionic twins. *American Journal of Obstetrics and Gynecology*, 201(3), pp.291.e1–5.

Leth, H. et al., 1995. Brain lactate in preterm and growth-retarded neonates. *Acta Paediatrica*, 84(5), pp.495–9.

Lettieri, L. et al., 1992. Transverse cerebellar diameter measurements in twin pregnancies and the effect of intrauterine growth retardation. *American Journal of Obstetrics and Gynecology*, 167(4), pp.982–5.

Leveno, K. et al., 1979. Sonar cephalometry in twins: a table of biparietal diameters for normal twin fetuses and a comparison with singletons. *American Journal of Obstetrics & Gynaecology*, 135(6), pp.727–730.

Levin, M. & Ernst, S., 1997. Applied DC magnetic fields cause alterations in the time of cell divisions and developmental abnormalities in early sea urchin embryos. *Bioelectromagnetics*, 18(3), pp.255–63.

Levine, D. et al., 2001. Potential heating effect in the gravid uterus during MR HASTE imaging. *Journal of Magnetic Resonance Imaging*, 13(6), pp.856–61.

Levine, D. & Barnes, P., 1999. Cortical Maturation in Normal and Abnormal Fetuses as Assessed with Prenatal MR Imaging. *Radiology*, 210, pp.751–758.

Lewi, L. et al., 2006. Intertwin anastomoses in monochorionic placentas after fetoscopic laser coagulation for twin-to-twin transfusion syndrome: is there more than meets the eye? *American Journal of Obstetrics and Gynecology*, 194(3), pp.790–5.

Lewi, L. et al., 2008. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. *American Journal of Obstetrics and Gynecology*, 199(5), pp.514.e1–8.

Li, D.-K. et al., 2002. A population-based prospective cohort study of personal exposure to magnetic fields during pregnancy and the risk of miscarriage. *Epidemiology*, 13(1), pp.9–20.

De Lia, J. et al., 2000. Twin-to-twin transfusion syndrome--debates on the etiology, natural history and management. *Ultrasound in Obstetrics and Gynecology*, 16(3), pp.210–3.

De Lia, J., Cruikshank, D. & Keye, W., 1990. Fetoscopic Neodynium: YAG laser occlusion of placental vessels in severe twin-twin transfusion syndrome. *Obstetrics & Gynaecology*, 75(6), pp.1046–53.

Limperopoulos, C. et al., 2011. Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy. *Circulation*, 121(1), pp.1–17.

Limperopoulos, C. et al., 2013. Development and validation of a semiquantitative brain maturation score on fetal MR. *Radiology*, 268(1), pp.200–207.

Long, S. et al., 2012. Performance of 2-year old children after early surgery for congenital heart disease on the Bayley Scales of Infant and Toddler Development. *Early Human Development,* 88(8), pp.603-607.

Lopriore, E. et al., 2006. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. *American Journal of Obstetrics and Gynecology*, 194(5), pp.1215–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16647903 [Accessed February 14, 2012].

Lopriore, E., Middeldorp, J.M., et al., 2007. Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. *American Journal of Obstetrics and Gynecology*, 196(3), pp.231.e1–4.

Lopriore, E. et al., 2009. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. *Obstetrics and Gynecology*, 113(2), pp.361–6.

Lopriore, E. et al., 1995. Twin-to-twin transfusion syndrome: New perspectives. *The Journal of Pediatrics*, 127, pp.675–80.

Lopriore, E., Sueters, M., et al., 2007. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. *American journal of obstetrics and gynecology*, 196(2), pp.159.e1–5.

Lutfi, S. et al., 2004. Twin-twin transfusion syndrome: a population-based study. *Obstetrics and Gynecology*, 104(6), pp.1289–97.

Magin, R. et al., 2000. Biological effects of long-duration, high-field (4T) MRI on growth and development in the mouse. *Journal of Magnetic Resonance Imaging*, 12(1), pp.140–9.

Mahieu-Caputo, D. et al., 2005. Paradoxic activation of the renin-angiotensin system in twin-twin transfusion syndrome: an explanation for cardiovascular disturbances in the recipient. *Pediatric Research*, 58(4), pp.685–8.

Mahieu-Caputo, D. et al., 2000. Twin-to-twin transfusion syndrome. Role of the fetal renin-angiotensin system. *The American Journal of Pathology*, 156(2), pp.629–36.

Malamateniou, C. et al., 2013. Motion-compensation techniques in neonatal and fetal MR imaging. *American Journal of Neuroradiology*, 34(6), pp.1124–36.

Manto, M. & Jissendi, P., 2012. Cerebellum: links between development, developmental disorders and motor learning. *Frontiers in Neuroanatomy*, 6, p.1.

Martin, E. et al., 1988. Developmental stages of human brain: an MR study. *Journal of Computer Assisted Tomography*, 12(6), pp.917–22.

Maschke, C. et al., 2010. Growth after intrauterine laser coagulation for twin-twin transfusion syndrome. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 95(2), pp.115–7.

Maschke, C. et al., 2011. Long-term outcome after intrauterine laser treatment for twin – twin transfusion syndrome. *Prenatal Diagnosis*, 31, pp.647–53.

Merhar, S. et al., 2013. Fetal and postnatal brain MRI in premature infants with twin-twin transfusion syndrome. *Journal of Perinatology*, 33(2), pp.112–8.

Mevissen, M., Buntenkötter, S. & Löscher, W., 1994. Effects of static and time-varying (50-Hz) magnetic fields on reproduction and fetal development in rats. *Teratology*, 50(3), pp.229–37.

Meyer, W. et al., 1993. The fetal transverse cerebellar diameter/abdominal circumference ratio: a gestational age-independent method of assessing fetal size. *Journal of Ultrasound in Medicine*, 12(7), pp.379–82.

Michel, S. et al., 2003. Fetal cardiographic monitoring during 1.5-T MR imaging. *American Journal of Roentgenology*, 180(4), pp.1159–64.

Michelfelder, E. et al., 2007. Early manifestations and spectrum of recipient twin cardiomyopathy in twin-twin transfusion syndrome: relation to Quintero stage. *Ultrasound in Obstetrics and Gynecology*, 30(7), pp.965–71.

Middeldorp, J. et al., 2007. Fetoscopic laser surgery in 100 pregnancies with severe twin-to-twin transfusion syndrome in the Netherlands. *Fetal Diagnosis and Therapy*, 22(3), pp.190–4.

Milne, S., et al. 2015. Alternate scoring of the Bayley-III improves prediction of performance on Griffiths Mental Development Scales before school entry in preschoolers with developmental concerns. *Child: care, health and development.* 41(2), pp. 203-12.

Mirmiran, M. et al., 2004. Neonatal brain magnetic resonance imaging before discharge is better than serial cranial ultrasound in predicting cerebral palsy in very low birth weight preterm infants. *Pediatrics*, 114(4), pp.992–8.

Moise, K. et al., 2013. The benefit of laser therapy for severe twin-twin transfusion: which metaanalysis do you pick? *American Journal of Obstetrics and Gynecology*, 209(2), pp.158–9.

Moon-Grady, A., 2014. Fetal echocardiography in twin-twin transfusion syndrome. *American Journal of Perinatology*, 1(212), pp.S31–S38.

Moore, E. et al., 2013. Angiotensin-(1-7) prevents radiation-induced inflammation in rat primary astrocytes through regulation of MAP kinase signaling. *Free Radical Biology & Medicine*, 65, pp.1060–8.

Moore, T., et al. 2012. Relationship between test scores using the second and third editions of the bayley scales in extremely preterm children. *Journal of Pediatrics.* 160(4), pp. 553-8.

Moreira de Sa, R. et al., 2005. Analysis of fetal growth after laser therapy in twin-to-twin transfusion syndrome. *Journal of Ultrasound in Medicine*, 24(9), pp.1213–9.

Morin, L. & Lim, K., 2011. Ultrasound in twin pregnancies. *Journal of Obstetrics and Gynaecology Canada*, 33(6), pp.643–56.

Morris, R. et al., 2010. Fetoscopic laser coagulation for severe twin-to-twin transfusion syndrome: factors influencing perinatal outcome, learning curve of the procedure and lessons for new centres. *British Journal of Obstetrics & Gynaecology*, 117(11), pp.1350–7.

Moxon-Lester, L. et al., 2007. Increased cerebral lactate during hypoxia may be neuroprotective in newborn piglets with intrauterine growth restriction. *Brain Research*, 1179, pp.79–88.

Mulder, E. et al., 2012. Fetal behavior in normal dichorionic twin pregnancy. *Early Human Development*, 88(3), pp.129–34.

Murakoshi, T. et al., 2008. Validation of Quintero stage III sub-classification for twin-twin transfusion syndrome based on visibility of donor bladder: characteristic differences in pathophysiology and prognosis. *Ultrasound in Obstetrics and Gynecology*, 32(6), pp.813–8.

Myers, C. et al., 1998. Failure to detect intrauterine growth restriction following in utero exposure to MRI. *The British Journal of Radiology*, 71(845), pp.549–51.

Nageotte, M. et al., 1989. Atriopeptin in the twin transfusion syndrome. *Obstetrics and Gynecology*, 73(5), pp.867–70.

Naidich, T. et al., 1994. The developing cerebral surface. Preliminary report on the patterns of sulcal and gyral maturation--anatomy, ultrasound, and magnetic resonance imaging. *Neuroimaging Clinics of North America*, 4(2), pp.201–40.

Nakahara, T. et al., 2002. Effects of exposure of CHO-K1 cells to a 10-T static magnetic field. *Radiology*, 224(3), pp.817–22.

Nakata, M. et al., 2009. Modified sequential laser photocoagulation of placental communicating vessels for twin-twin transfusion syndrome to prevent fetal demise of the donor twin. *The journal of obstetrics and gynaecology research*, 35(4), pp.640–7.

Nakayama, S. et al., 2012. Perinatal outcome of monochorionic diamniotic twin pregnancies managed from early gestation at a single center. *The Journal of Obstetrics and Gynaecology Research*, 38(4), pp.692–7.

Narra, V. et al., 1996. Effects of a 1.5-Tesla static magnetic field on spermatogenesis and embryogenesis in mice. *Investigative Radiology*, 31(9), pp.586–90.

Neilson, J. & Kilby, M., 2008. *Management of monochorionic twin pregnancy. RCOG Green-top Guideine 51.*, London, UK.

Nie, J. et al., 2012. Axonal fiber terminations concentrate on gyri. *Cerebral Cortex*, 22(12), pp.2831–9.

Nieuwenhuys, R., 2012. The insular cortex: a review. *Progress in Brain Research*, 195, pp.123–63.

Nikkels, P., Hack, K. & van Gemert, M., 2008. Pathology of twin placentas with special attention to monochorionic twin placentas. *Journal of Clinical Pathology*, 61(12), pp.1247–53.

O'Donoghue, K. et al., 2007. Stage I twin-twin transfusion syndrome: rates of progression and regression in relation to outcome. *Ultrasound in Obstetrics and Gynecology*, 30(7), pp.958–64.

O'Donoghue, K. et al., 2009. Transfusional fetal complications after single intrauterine death in monochorionic multiple pregnancy are reduced but not prevented by vascular occlusion. *British Journal of Obstetrics & Gynaecology*, 116(6), pp.804–12.

Oberg, K. et al., 1999. Renal tubular dysgenesis in twin-twin transfusion syndrome. *Pediatric and Developmental Pathology*, 2(1), pp.25–32.

Ong, S. et al., 2002. The creation of twin centile curves for size. *British Journal of Obstetrics & Gynaecology*, 109, pp.753–758.

Ortibus, E. et al., 2009. The pregnancy and long-term neurodevelopmental outcome of monochorionic diamniotic twin gestations: a multicenter prospective cohort study from the first trimester onward. *American Journal of Obstetrics and Gynecology*, 200(5), pp.494.e1–8.

Padilla, N. et al., 2011. Differential effects of intrauterine growth restriction on brain structure and development in preterm infants: a magnetic resonance imaging study. *Brain Research*, 1382, pp.98–108.

De Paepe, M. et al., 2010. Placental markers of twin-to-twin transfusion syndrome in diamniotic-monochorionic twins: A morphometric analysis of deep artery-to-vein anastomoses. *Placenta*, 31(4), pp.269–76.

Palmen, S. et al., 2005. Increased gray-matter volume in medication-naive high-functioning children with autism spectrum disorder. *Psychological Medicine*, 35(4), pp.561–70.

Palmen, S. et al., 2004. Larger brains in medication naive high-functioning subjects with pervasive developmental disorder. *Journal of Autism and Developmental Disorders*, 34(6), pp.603–13.

Pan, H., 1996. The effect of a 7T magnetic field on the egg hatching of Heliothis Virescens. *Magnetic Resonance Imaging*, 14(6), pp.673–677.

Panahpour, H. & Dehghani, G., 2010. Inhibition of central angiotensin-converting enzyme with enalapril protects the brain from ischemia/reperfusion injury in normotensive rat. *Daru*, 18(1), pp.35–40.

Papile, L. et al., 1978. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *The Journal of Pediatrics*, 92(4), pp.529–34.

Paramasivam, G. et al., 2010. Radiofrequency ablation for selective reduction in complex monochorionic pregnancies. *British Journal of Obstetrics & Gynaecology*, 117(10), pp.1294–8.

Patel, S. et al., 2012. Prevalence of noncardiac structural anomalies in twin-twin transfusion syndrome. *Journal of Ultrasound in Medicine*, 31(4), pp.555–560.

Pellerin, L. et al., 1998. Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Developmental Neuroscience*, 20(4-5), pp.291–9.

Persson, P. & Grennert, L., 1979. The intrauterine growth of the biparietal diameter of twins. *Acta Genet Med Gemellol*, 28(4), pp.273–277.

Peterson, B. et al., 2000. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. *Journal of the American Medical Association*, 284(15), pp.1939–47.

Picciolini, O., et al. 2015. Neurodevelopmental outcome of extremely low birth weight infants at 24 months corrected age: a comparison between Griffiths and Bayley scales. *BMC Pediatrics.* 15, pp. 139-148.

Pier, D. et al., 2011. Magnetic resonance volumetric assessments of brains in fetuses with ventriculomegaly correlated to outcomes. *Journal of Ultrasound in Medicine*, 30(5), pp.595–603.

Pighini, M. et al., 2014. Learning from parents' stories about what works in early intervention. *International journal of psychology*, 49(4), pp.263–70.

Pistorius, L. et al., 2008. Fetal neuroimaging: Ultrasound, MRI or both? *Obstetrical and Gynecological Survey*, 63(11), pp.733–45.

Poutamo, J. et al., 1998. MRI does not change fetal cardiotocographic parameters. *Prenatal Diagnosis*, 18(3), pp.1149–54.

Price, D. et al., 2001. Investigation of acoustic noise on 15 MRI scanners from 0.2 T to 3 T. *Journal of Magnetic Resonance Imaging*, 13(2), pp.288–93.

Quarello, E., Molho, M. & Ville, Y., 2007. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. *The Journal of Maternal-Fetal & Neonatal Medicine*, 20(8), pp.589–97.

Quintero, R. et al., 2000. Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 16(3), pp.230–6.

Quintero, R. et al., 2007. Sequential selective laser photocoagulation of communicating vessels in twin-twin transfusion syndrome. *The Journal of Maternal-Fetal & Neonatal Medicine*, 20(10), pp.763–8.

Quintero, R. et al., 1999. Staging of twin-twin transfusion syndrome. *Journal of Perinatology*, 19(8), pp.550–5.

Quintero, R., 2003. Twin–twin transfusion syndrome. *Clinics in Perinatology*, 30(3), pp.591–600.

Quintero, R. & Chmait, R., 2009. The con trial. *American Journal of Obstetrics and Gynecology*, 200(3), pp.e14–5.

Quintero, R., Chmait, R. & Kontopoulos, E., 2013. Laser treatment of twin-twin transfusion syndrome: what do the data really show? *American Journal of Obstetrics and Gynecology*, 209(2), pp.157–8.

Raboisson, M.J. et al., 2004. Early intertwin differences in myocardial performance during the twin-to-twin transfusion syndrome. *Circulation*, 110(19), pp.3043–3048.

Rajagopalan, V., Scott, J. & Habas, P., 2011. Local tissue growth patterns underlying normal fetal human brain gyrification quantified in utero. *The Journal of Neuroscience*, 31(8), pp.2878–87.

Ramsay, M. & Fitzhardinge, P., 1977. A comparative study of two developmental scales: the Bayley and the Griffiths. *Early Human Development*, 1(2), pp.151–7.

Reece, E. et al., 1991. A prospective longitudinal study of growth in twin gestations compared with growth in singleton pregnancies. *Journal of Ultrasound Medicine*, 10(8), pp.439–43.

Reeves, M. et al., 2010. Neonatal cochlear function : measurement after exposure to acoustic noise during in utero MR imaging. *Radiology*, 257(3), pp.802–9.

Richards, D. et al., 1992. Sound levels in the human uterus. *Obstetrics and Gynecology*, 80(2), pp.186–90.

Robbins, M. et al., 2011. Renin-angiotensin system blockers and modulation of radiation-induced brain injury. *Current Drug Targets*, 11(11), pp.1413–22.

Robert, E., 1999. Intrauterine effects of electromagnetic fields - (low frequency, mid-frequency RF, and microwave): review of epidemiologic studies. *Teratology*, 59, pp.292–8.

Roberts, D. et al., 2014. Interventions for the treatment of twin-twin transfusion syndrome. *Cochrane Database of Systematic Reviews*,

Roberts, D., Gates, S., et al., 2008. Interventions for twin-twin transfusion syndrome: a Cochrane review. *Ultrasound in Obstetrics and Gynecology*, 31(6), pp.701–11.

Robertson, E. & Neer, K., 1983. Placental injection studies in twin gestation. *American Journal of Obstetrics and Gynecology*, 147(2), pp.170–4.

Robinson, J. et al., 2004. Detection of fetal lactate with two-dimensional-localised proton magnetic resonance spectroscopy. *Obstetrics & Gynaecology*, 104(5), pp.1208–10.

Robyr, R. et al., 2006. Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 194(3), pp.796–803.

Robyr, R., Yamamoto, M. & Ville, Y., 2005. Selective feticide in complicated monochorionic twin pregnancies using ultrasound-guided bipolar cord coagulation. *British Journal of Obstetrics & Gynaecology*, 112(10), pp.1344–8.

Rodegerdts, E. et al., 2000. In vitro evaluation of teratogenic effects by time-varying MR gradient fields on fetal human fibroblasts. *Journal of Magnetic Resonance Imaging*, 12(1), pp.150–6.

Roelants-van Rijn, A., van der Grond, J., et al., 2004. Cerebral structure and metabolism and long-term outcome in small-for-gestational-age preterm neonates. *Pediatric Research*, 56(2), pp.285–90.

Roelants-van Rijn, A., Groenendaal, F., et al., 2004. Lactate in the foetal brain: detection and implications. *Acta Paediatrica*, 93(7), pp.937–40.

Rossi, C. & D'Addario, V., 2009a. Comparison of donor and recipient outcomes following laser therapy performed for twin-twin transfusion syndrome : A meta-analysis and review of literature. *Journal of Perinatology*, 1(212), pp.27–32.

Rossi, C. & D'Addario, V., 2008. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome: a metaanalysis and review of literature. *American Journal of Obstetrics and Gynecology*, 198(2), pp.147–52.

Rossi, C. & D'Addario, V., 2013. Survival outcomes of twin-twin transfusion syndrome stage I: a systematic review of literature. *American Journal of Perinatology*, 30(1), pp.5–10.

Rossi, C. & D'Addario, V., 2009b. The efficacy of Quintero staging system to assess severity of twin-twin transfusion syndrome treated with laser therapy : a systematic review with meta-analysis. *Journal of Perinatology*, 1(212), pp.537–44.

Rouch, L. et al., 2015. Antihypertensive drugs, prevention of cognitive decline and dementia: a systematic review of observational studies, randomized controlled trials and meta-analyses, with discussion of potential mechanisms. *CNS drugs*, 29(2), pp.113–30.

Ruano, R. et al., 2013. Fetoscopic laser ablation of placental anastomoses in twin-twin transfusion syndrome using "Solomon technique". *Ultrasound in Obstetrics and Gynecology*, 42(4), pp.434–9.

Rustico, M. et al., 2012. Fetal and maternal complications after selective fetoscopic laser surgery for twin-to-twin transfusion syndrome: a single-center experience. *Fetal Diagnosis and Therapy*, 31(3), pp.170–8.

Rutherford, M. et al., 2008. MR imaging methods for assessing fetal brain development. *Developmental Neurobiology*, 68(6), pp.700–11.

Rutherford, M. a, 2009. Magnetic resonance imaging of the fetal brain. *Current Opinion in Obstetrics & Gynecology*, 21(2), pp.180–6.

Rychik, J. et al., 2007. The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease. *American Journal of Obstetrics and Gynecology*, 197(4), pp.392.e1–8.

Sadovsky, E. et al., 1986. Incidence of spontaneous and evoked fetal movements. *Gynecologic and Obstetric Investigation*, 21(4), pp.177–81.

Sago, H. et al., 2010. The outcome and prognostic factors of twin-twin transfusion syndrome following fetoscopic laser surgery. *Prenatal Diagnosis*, 30, pp.1185–91.

Sallet, P. et al., 2003. Reduced cortical folding in schizophrenia: an MRI morphometric study. *The American Journal of Psychiatry*, 160(9), pp.1606–13.

Salomon, L. et al., 2005. Growth discrepancy in twins in the first trimester of pregnancy. *Ultrasound in Obstetrics and Gynecology*, 26(5), pp.512–6.

Salomon, L. et al., 2010. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 203(5), pp.444.e1–7.

Samuelsen, G. et al., 2007. Severe cell reduction in the future brain cortex in human growth-restricted fetuses and infants. *American Journal of Obstetrics and Gynecology*, 197(1), pp.56.e1–7.

Santini, M. et al., 1994. A static magnetic field does not affect the dielectric properties of chick embryo myoblast membranes. *International Journal of Radiation Biology*, 65(2), pp.277–84.

Sanz-Cortes, M. et al., 2014. Brainstem and cerebellar differences and their association with neurobehavior in term small-for-gestational-age fetuses assessed by fetal MRI. *American Journal of Obstetrics and Gynecology*, 210(5), pp.452.e1–8.

Sanz-Cortes, M. et al., 2015. Proton Magnetic Resonance Spectroscopy Assessment of Fetal Brain Metabolism in Late-Onset “Small for Gestational Age” versus “Intrauterine Growth Restriction” Fetuses. *Fetal Diagnosis and Therapy*, 37(2), pp.108–16.

Sato, K. et al., 1992. Growth of human cultured cells exposed to a non-homogeneous static magnetic field generated by Sm-Co magnets. *Biochimica et biophysica acta*, 1136(3), pp.231–8.

Saunders, N., Snijders, R. & Nicolaides, K., 1991. Twin-twin transfusion syndrome during the 2nd trimester is associated with small intertwin hemoglobin differences. *Fetal Diagnosis and Therapy*, 6(1-2), pp.34–6.

Savitz, D.A., 2002. Magnetic fields and miscarriage. *Epidemiology*, 13(1), pp.1–3.

Schatz, F., 1882. Eine besondere Art von einseitiger Polyhydramnie mit anderseitiger Oligohydramnie bei eineiigen Zwillingen. *Arch Gynakol*, 19, pp.329–369.

Schenck, J., 2000. Safety of strong, static magnetic fields. *Journal of Magnetic Resonance Imaging*, 12(1), pp.2–19.

Scher, A. et al., 2002. The risk of mortality or cerebral palsy in twins : a collaborative population-based study. *Pediatric Research*, 52(5), pp.671–81.

Schierlitz, L. et al., 2001. Three-dimensional magnetic resonance imaging of fetal brains. *The Lancet*, 357, pp.1177–8.

Schmahmann, J.D. & Sherman, J.C., 1998. The cerebellar cognitive affective syndrome. *Brain : a journal of neurology*, 121 (Pt 4, pp.561–79.

Schonhaut, L. et al., 2013. Validity of the ages and stages questionnaires in term and preterm infants. *Pediatrics,* 131(5), pp.1468-74.

Schroder, H. & Power, G., 1997. Engine and radiator: Fetal and placental interactions for heat dissipation. *Experimental Physiology*, 82, pp.403–414.

Scott, J. et al., 2012. 3D morphometric analysis of human fetal cerebellar development. *Cerebellum*, 11(3), pp.761–70.

Scott, J. et al., 2013. Volumetric and surface-based 3D MRI analyses of fetal isolated mild ventriculomegaly: brain morphometry in ventriculomegaly. *Brain Structure and Function*, 218(3), pp.645–55.

Scott, J., Habas, P. & Kim, K., 2011. Growth trajectories of the human fetal brain tissues estimated from 3D reconstructed in utero MRI. *International Journal of Developmental Neuroscience*, 29(5), pp.529–36.

Sebire, N. et al., 2000. Early prediction of severe twin-to-twin transfusion syndrome. *Human Reproduction*, 15(9), pp.2008–10.

Senat, M.-V. et al., 2004. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. *The New England Journal of Medicine*, 351(2), pp.136–44.

Sepulveda, W. et al., 2007. Endoscopic laser surgery in severe second-trimester twin-twin transfusion syndrome: a three-year experience from a Latin American center. *Prenatal Diagnosis*, 27(11), pp.1033–8.

Shek, N., Hillman, S. & Kilby, M., 2014. Single-twin demise: pregnancy outcome. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, 28(2), pp.249–63.

Shih, M. et al., 2004. Brain lesions with elevated lactic acid peaks on magnetic resonance spectroscopy. *Current Problems in Diagnostic Radiology*, 33(2), pp.85–95.

Shimizu, T., Gaudette, S. & Nimrod, C., 1992. Transverse cerebellar diameter in twin gestations. *American Journal of Obstetrics & Gynaecology*, 167(4), pp.1004–8.

Simpson, L., 2013. Twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 208(1), pp.3–18.

Skupski, D. et al., 2010. Preoperative predictors of death in twin-to-twin transfusion syndrome treated with laser ablation of placental anastomoses. *American Journal of Obstetrics and Gynecology*, 203(4), pp.388.e1–11.

Slagle, T., Oliphant, M. & Gross, S., 1989. Cingulate sulcus development in preterm infants. *Pediatric Research*, 26(6), pp.598–602.

Smith, F., 1983. NMR imaging in pregnancy. *The Lancet*, 321(8314), pp.61–62.

Smith, F. & MacLennan, F., 1984. NMR imaging in human pregnancy: a preliminary study. *Magnetic Resonance Imaging*, 2(1), pp.57–64.

Snijders, R., De Courcy-Wheeler, R. & Nicolaides, K., 1994. Intrauterine growth retardation and fetal transverse cerebellar diameter. *Prenatal Diagnosis*, 14(12), pp.1101–5.

Spencer-Smith, M., et al., 2015. Bayley-III Cognitive and Language scales in preterm children. *Pediatrics,* 135(5), pp.1258-65.

Spittle, A. et al., 2007. Early developmental intervention programs post hospital discharge to prevent motor and cognitive impairments in preterm infants. *The Cochrane Database of Systematic Reviews*, 2.

Spittle, A., et al. 2013. Does the Bayley-III Motor Scale at 2 years predict motor outcome at 4 years in very preterm children? *Developmental Medicine and Child Neurology,* 55(5), pp.448-52.

Springer, S. et al., 2014. Congenital heart disease in monochorionic twins with and without twin-to-twin transfusion syndrome. *Prenatal Diagnosis*, 34(10), pp.994–9.

Spruijt, M. et al., 2012. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. *Obstetrics and Gynecology*, 120(1), pp.15–20.

Squires, J. & Bricker, D., 2009. *Ages & Stages Questionnaires.* 3rd ed., Brookes Publishing Co.

Stamilio, D., Fraser, W. & Moore, T., 2010. Twin-twin transfusion syndrome: an ethics-based and evidence-based argument for clinical research. *American Journal of Obstetrics and Gynecology*, 203(1), pp.3–16.

Staudt, M., 2010. Reorganization after pre- and perinatal brain lesions. *Journal of Anatomy*, 217(4), pp.469–74.

Steinberg, L. et al., 1990. Acute polyhydramnios in twin pregnancies. *The Australian & New Zealand Journal of Obstetrics & Gynaecology*, 30(3), pp.196–200.

Stephani, C. et al., 2011. Functional neuroanatomy of the insular lobe. *Brain Structure and Function*, 216(2), pp.137–149.

Stirnemann, J. et al., 2010. Profiling fetal cardiac function in twin-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 35(1), pp.19–27.

Story, L. et al., 2011. Brain metabolism in fetal intrauterine growth restriction: a proton magnetic resonance spectroscopy study. *American Journal of Obstetrics and Gynecology*, 205(5), pp.483.e1–8.

Story, L. et al., 2013. Myo-inositol metabolism in appropriately grown and growth-restricted fetuses: a proton magnetic resonance spectroscopy study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 170(1), pp.77–81.

Sueters, M. et al., 2006. Timely diagnosis of twin-to-twin transfusion syndrome in monochorionic twin pregnancies by biweekly sonography combined with patient instruction to report onset of symptoms. *Ultrasound in Obstetrics and Gynecology*, 28(5), pp.659–64.

Sueters, M. & Oepkes, D., 2014. Diagnosis of twin-to-twin transfusion syndrome, selective fetal growth restriction, twin anaemia-polycythaemia sequence, and twin reversed arterial perfusion sequence. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, 28(2), pp.215–26.

Sur, M. & Rubenstein, J.L.R., 2005. Patterning and plasticity of the cerebral cortex. *Science*, 310(5749), pp.805–10.

Sutcliffe, A., Soo, A. & Barnes, J., 2010. Predictive value of developmental testing in the second year for cognitive development at five years of age. *Pediatric Reports,* 2(2), pp.48-50.

Sutcliffe, G. et al., 2001. Outcome for children born after in utero laser ablation therapy for severe twin-to-twin transfusion syndrome. *British Journal of Obstetrics & Gynaecology*, 108(12), pp.1246–50.

Suzuki, Y. et al., 2003. Inflammation and angiotensin II. *The International Journal of Biochemistry & Cell Biology*, 35(6), pp.881–900.

Tarui, T. et al., 2012. Altered fetal cerebral and cerebellar development in twin-twin transfusion syndrome. *American Journal of Neuroradiology*, 33(6), pp.1121–6.

Taylor, M. et al., 2002. Validation of the Quintero staging system for twin-twin transfusion syndrome. *Obstetrics and Gynecology*, 100(6), pp.1257–65.

Taylor-Clarke, M.C. et al., 2013. Ventricular strain changes in monochorionic twins with and without twin-to-twin transfusion syndrome. *American journal of obstetrics and gynecology*, 208(6), pp.462.e1–6.

Thach, W., et al. 2007. On the mechanism of cerebellar contributions to cognition. *Cerebellum*, 6(3), pp.163–167.

Tilea, B. et al., 2009. Cerebral biometry in fetal magnetic resonance imaging: new reference data. *Ultrasound in Obstetrics and Gynecology*, 33(2), pp.173–81.

Toi, A., Lister, W. & Fong, K., 2004. How early are fetal cerebral sulci visible at prenatal ultrasound and what is the normal pattern of early fetal sulcal development? *Ultrasound in Obstetrics and Gynecology*, 24(7), pp.706–15.

Tolsa, C. et al., 2004. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatric Research*, 56(1), pp.132–8.

Toro, R. & Burnod, Y., 2005. A morphogenetic model for the development of cortical convolutions. *Cerebral cortex*, 15(12), pp.1900–13.

Trompoukis, P. et al., 2012. Fetal MRI: is it really helpful? *Journal of maternal, fetal and neonatal medicine.*, 25(11), pp.2363–8.

Tsakiris, M. et al., 2007. Neural signatures of body ownership: a sensory network for bodily self-consciousness. *Cerebral cortex*, 17(10), pp.2235–44.

Tyndall, D. & Sulik, K., 1991. Effects of magnetic resonance imaging on eye development in the C57BL/6J mouse. *Teratology*, 43(3), pp.263–75.

Ulfig, N., Nickel, J. & Saretzki, U., 1998. Alterations in myelin formation in fetal brains of twins. *Pediatric Neurology*, 19(4), pp.287–293.

Umur, a et al., 2002. Monochorionic twins and twin-twin transfusion syndrome: the protective role of arterio-arterial anastomoses. *Placenta*, 23(2-3), pp.201–9.

Umur, A., van Gemert, M. & Nikkels, P., 2003. Monoamniotic-versus diamniotic-monochorionic twin placentas: anastomoses and twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 189(5), pp.1325–9.

Urenjak, J. et al., 1993. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *The Journal of Neuroscience*, 13(3), pp.981–9.

Vadeyar, S. et al., 2000. Effect of fetal magnetic resonance imaging on fetal heart rate patterns. *American Journal of Obstetrics and Gynecology*, 182(3), pp.666–9.

Vanderbilt, D. et al., 2014. Predictors of 2-year cognitive performance after laser surgery for twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 211(4), pp.388.e1–7.

Vanderbilt, D.L. et al., 2012. Prevalence and risk factors of cerebral lesions in neonates after laser surgery for twin-twin transfusion syndrome. *American Journal of Obstetrics and Gynecology*, 207(4), pp.320.e1–320.e6.

Vannucci, R. & Vannucci, S., 2000. Glucose metabolism in the developing brain. *Seminars in Perinatology*, 24(2), pp.107–15.

Veldhuizen, S. et al., 2015. Concurrent validity of the Ages and Stages Questionnaires and Bayley Developmental Scales in a general population sample. *Academic Pediatrics,* 15(2), pp.231-7.

Villapol, S. et al., 2015. Neurorestoration after traumatic brain injury through angiotensin II receptor blockage. *Brain*, 138(11), pp. 3299-315.

Villapol, S. & Saavedra, J., 2015. Neuroprotective effects of angiotensin receptor blockers. *American Journal of Hypertension*, 28(3), pp.289–99.

Ville, Y. et al., 1998. Endoscopic laser coagulation in the management of severe twin-to-twin transfusion syndrome. *British Journal of Obstetrics & Gynaecology*, 105(4), pp.446–53.

Ville, Y. et al., 1995. Preliminary experience with endoscopic laser surgery for severe twin-twin transfusion syndrome. *The New England Journal of Medicine*, 332(4), pp.224–7.

Ville, Y. et al., 1992. Successful outcome after Nd:YAG laser separation of chorioangiopagus-twins under sonoendoscopic control. *Ultrasound in Obstetrics & Gynecology*, 2, pp.429–31.

De Villiers, S. et al., 2012. Arterio-arterial vascular anastomoses in monochorionic placentas with and without twin-twin transfusion syndrome. *Placenta*, 33(8), pp.652–4.

Volpe, J., 2009. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *The Lancet Neurology*, 8(1), pp.110–24.

De Vries, L., Eken, P. & Dubowitz, L., 1992. The spectrum of leukomalacia using cranial ultrasound. *Behavioural Brain Research*, 49(1), pp.1–6.

Wagner, M. et al., 2009. Short-and long-term outcome in stage 1 twin-to-twin transfusion syndrome treated with laser surgery compared with conservative management. *American Journal of Obstetrics & Gynecology*, 201(3), pp.286.e1–6.

Walsh, C. & McAuliffe, F., 2012. Recurrent twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a systematic review of the literature. *Ultrasound in Obstetrics and Gynecology*, 40(5), pp.506–12.

Weaver, A., 2005. Reciprocal evolution of the cerebellum and neocortex in fossil humans. *Proceedings of the National Academy of Sciences of the United States of America*, 102(10), pp.3576–80.

Weir, P., Ratten, G. & Bescher, N., 1979. Acute polyhydramnios: a complication of monozygous twin pregnancy. *British Journal of Obstetrics and Gynaecology*, 86, pp.849–53.

Welker, W., 1990. Why does cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In *Comparative structure and evolution of cerebral cortex, part II.* New York: Plenum Press, pp. 3–136.

Whitby, E. et al., 2004. Comparison of ultrasound and magnetic resonance imaging in 100 singleton pregnancies with suspected brain abnormalities. *British Journal of Obstetrics & Gynaecology*, 111(8), pp.784–92.

Wieacker, P. et al., 1992. Pathophysiology of polyhydramnios in twin transfusion syndrome. *Fetal Diagnosis and Therapy*, 7(2), pp.87–92.

Wiskirchen, J. et al., 1999. Long-term effects of repetitive exposure to a static magnetic field (1.5 T) on proliferation of human fetal lung fibroblasts. *Magnetic Resonance in Medicine*, 41(3), pp.464–8.

Wolfberg, A. et al., 2007. Identification of fetal cerebral lactate using magnetic resonance spectroscopy. *American Journal of Obstetrics and Gynecology*, 196(1), pp.e9–11.

Woodward, B. et al., 2011. Use of the Ages and Stages Questionnaire and Bayley Scales of Infant Development-II in neurodevelopmental follow-up of extremely low birth weight infants. *Journal of Perinatology*, 31(10), pp.641–6.

Wright, R. et al., 2014. Automatic quantification of normal cortical folding patterns from fetal brain MRI. *NeuroImage*, 91, pp.21–32.

Xu, D. et al., 2011. MR spectroscopy of normative premature newborns. *Journal of Magnetic Resonance Imaging*, 33(2), pp.306–11.

Yamamoto, M. et al., 2005. Incidence and impact of perioperative complications in 175 fetoscopy-guided laser coagulations of chorionic plate anastomoses in fetofetal transfusion syndrome before 26 weeks of gestation. *American Journal of Obstetrics and Gynecology*, 193(3), pp.1110–6.

Yamamoto, M. et al., 2007. Intertwin discordance in umbilical venous volume flow: a reflection of blood volume imbalance in twin-to-twin transfusion syndrome. *Ultrasound in Obstetrics and Gynecology*, 29(3), pp.317–20.

Yip, Y. et al., 1994. Effects of MR exposure at 1.5 T on early embryonic development of the chick. *Journal of Magnetic Resonance Imaging*, 4(5), pp.742–748.

Yip, Y.P., Capriotti, C. & Yip, J.W., 1995. Effects of MR exposure on axonal outgrowth in the sympathetic nervous system of the chick. *Journal of magnetic resonance imaging : JMRI*, 5(4), pp.457–62.

Zeisel, S.H., 2000. Choline: an essential nutrient for humans. *Nutrition (Burbank, Los Angeles County, Calif.)*, 16(7-8), pp.669–71.

Zhao, D.P. et al., 2013. Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. *Placenta*, 34(7), pp.589–93.

Zhao, D.P. et al., 2014. The role of veno-venous anastomoses in twin-twin transfusion syndrome. *Placenta*, 35(5), pp.334–6.

Zikulnig, L. et al., 1999. Prognostic factors in severe twin-twin transfusion syndrome treated by endoscopic laser surgery. *Ultrasound in Obstetrics and Gynecology*, 14(6), pp.380–7.

Zilles, K. et al., 1988. The human pattern of gyrification in the cerebral cortex. *Anatomy and embryology*, 179(2), pp.173–9.

Ziskin, M.C. & Morrissey, J., 2011. Thermal thresholds for teratogenicity, reproduction, and development. *International journal of hyperthermia*, 27(4), pp.374–87.