

**Pathophysiology of fetal
intrauterine central shunts in
high-risk pregnancies: a
prospective observational
Doppler study**

**Nayana Anupam Parange
MBBS MS (Medical Sonography)**

A thesis submitted for the degree of
Doctor of Philosophy
Department of Obstetrics and Gynaecology
University of Adelaide
September 2008



**PATHOPHYSIOLOGY OF FETAL
INTRAUTERINE CENTRAL SHUNTS IN
HIGH RISK PREGNANCIES:
A PROSPECTIVE OBSERVATIONAL
DOPPLER STUDY**

Nayana Anupam Parange



*This thesis is dedicated to all the families
affected by adverse pregnancy outcomes*

TABLE OF CONTENTS

ABSTRACT	vii
DECLARATION	ix
ACKNOWLEDGEMENTS	x
ABBREVIATIONS	xiii
ORIGINAL CONTRIBUTIONS AND SCIENTIFIC PRESENTATIONS RELATED TO THIS THESIS	xvi
TABLES AND FIGURES	xviii
CHAPTER 1	1
Introduction	1
<i>When to deliver the compromised fetus?</i>	2
<i>How to recognise and monitor the compromised fetus?</i>	3
Traditional clinical techniques	4
Monitoring technologies	4
Invasive tests	5
<i>Traditional Clinical techniques</i>	6
Maternal weight gain	6
Abdominal palpation and symphysis-fundal height (SFH)	6
Fetal movement record	7
<i>Monitoring technologies</i>	8
Ultrasonography	8
Biophysical profile	10
Doppler ultrasound.....	12
Fetal Heart Monitoring	13
Intermittent auscultation	13
CTG.....	13
Fetal ECG.....	15
Pulse oximetry	15
Fetal Blood Sampling (FBS).....	16
Fetal lactate	16
Vibro-acoustic stimulation test (VAST)	16
Near red infraspectroscopy (NIRS)	16
<i>Summary</i>	17
CHAPTER 2	19
Evaluation of the fetal circulation: A literature review	19
(2.1) Doppler ultrasound of maternal -fetal circulation in fetal hypoxia	20
<i>Introduction</i>	20
<i>Doppler Interpretation: qualitative and quantitative methods of evaluation</i>	21
Arterial Doppler Indices	21
Venous Doppler Indices.....	23
<i>Fetal circulation</i>	24
<i>Fetal arterial circulation in UPI</i>	26
<i>Fetoplacental circulation evaluation with Umbilical artery Doppler</i>	26

<i>Uteroplacental circulation: Uterine artery Doppler</i>	28
<i>Cerebral circulation</i>	31
<i>Cerebroplacental ratio (CPR)</i>	35
<i>Other arterial vessels</i>	35
<i>Fetal venous circulation in IUGR</i>	36
<i>Fetal cardiac Doppler in IUGR</i>	38
<i>Longitudinal studies in IUGR: Temporal sequence of events in progressive fetal compromise in UPI</i>	40
<i>Doppler safety in clinical practice</i>	46
(2.2) Evaluation of intrauterine fetal shunts	50
<i>Fetal circulation and intrauterine shunts: Historical perspective</i>	50
<i>Comparative anatomy of fetal shunts</i>	54
<i>Shunts and fetal heart</i>	56
<i>Pulmonary circulation and the heart</i>	58
<i>Shunts and cerebral flows in congenital cardiac disease</i>	58
<i>Ductus Arteriosus</i>	59
Embryology.....	59
Anatomy.....	59
Histology.....	60
Physiology.....	60
Sensitivity to vasomediators	60
Doppler examination.....	61
<i>Foramen ovale</i>	62
Embryology.....	62
Histology.....	62
Physiology.....	62
Doppler exam.....	63
<i>Ductus venosus</i>	64
Anatomy.....	65
Histology.....	65
Physiology.....	66
(2.3) Introduction to fundamental concepts in fetal cardiophysiology	68
<i>Cardiac output</i>	68
<i>Stroke volume</i>	69
<i>Cardiac preload</i>	69
<i>Cardiac contractility (inotropy), Frank Starling law and myocardial stiffness</i> ...	70
<i>Cardiac afterload</i>	71
<i>Cardiac compliance</i>	71
<i>Distribution of fetal cardiac output</i>	72
<i>Cardiac compliance and myocardial rigidity</i>	74
<i>Summary of chapter 2</i>	75
CHAPTER 3	76
Study design and general methodology	76
<i>Rationale of the research</i>	77
<i>Hypotheses and specific aims</i>	78
<i>Study Design</i>	80
<i>Recruitment strategies</i>	80
<i>Patient classification</i>	81
Low risk classification	81

High risk classification	81
Study 1: Normograms of fetal central shunts and other parameters.....	82
Inclusion criteria: Study 1	82
Exclusion criteria: Study 1	82
Variables for study 1	83
Statistical methods for study 1	86
Study 2 : Fetal shunts and acute adaptive mechanisms- haemodynamics before and after Intra-Uterine Transfusion (IUT).	87
Inclusion criteria	87
Exclusion criteria	87
Fetal transfusion procedure.....	87
Variables for study 2	88
Statistical methods for Study 2	90
Study 3 and 4.....	91
Inclusion criteria for study 3 and study 4.....	91
Exclusion criteria	91
Sample size calculations for study 3	92
Definitions for clinical outcomes	100
Definitions for placental outcomes.....	102
Statistical methods for study 3 and 4	103
Data entry.....	104
Statistical methods for analysis of categorical data	106
Statistical analysis methods for longitudinal data.....	107
Mixed linear models	107
Quality assurance and quality control measures	108
Strategies to minimise errors and bias	108
Strategies to address confounding factors.....	109
DOPPLER METHODOLOGY	111
UMBILICAL ARTERY DOPPLER PROTOCOL.....	111
MIDDLE CEREBRAL ARTERY DOPPLER PROTOCOL.....	113
UTERINE ARTERY PROTOCOL.....	115
DUCTUS VENOSUS PROTOCOL.....	117
DUCTUS ARTERIOSUS PROTOCOL	119
FORAMEN OVALE PROTOCOL.....	121
CHAPTER 4.....	123
Study 1: Normograms of fetal central shunts and other parameters	123
Introduction.....	124
Hypothesis	126
Aim.....	126
Methods and study design.....	126
Statistical analysis	128
Results.....	131
NORMOGRAMS OF CENTRAL SHUNTS.....	132
Foramen Ovale.....	132
FO PSV	133
FO EDV	134
FO PI.....	135
Ductus Arteriosus.....	136
DA PSV	137

DA EDV.....	138
DA PI.....	139
<i>NORMOGRAMS OF VENOUS INTRAUTERINE SHUNT: DUCTUS</i>	
<i>VENOSUS.....</i>	<i>141</i>
DV Preload Index	142
DV S/a ratio	143
DV Pulsatility Index	145
<i>NORMOGRAMS FOR UTEROPLACENTAL AND FETOPLACENTAL</i>	
<i>CIRCULATION.....</i>	<i>147</i>
Mean Uterine RI	148
Umbilical artery PI.....	150
Uteroplacental ratio.....	151
<i>CEREBRAL FLOW WAVEFORMS NORMOGRAMS</i>	<i>152</i>
MCA RI	153
Cerebroplacental ratio.....	154
<i>PLACENTAL THICKNESS AND BIOMETRY NORMOGRAMS.....</i>	<i>156</i>
Placental thickness	156
Fetal biometric measurements	158
BPD.....	159
HC.....	160
AC.....	161
FL.....	162
EFW	163
<i>Key findings</i>	<i>164</i>
<i>CHAPTER 5.....</i>	<i>166</i>
Study 2: Fetal shunts and acute adaptive mechanisms: haemodynamics before and after intrauterine transfusion (IUT)	166
<i>Introduction</i>	<i>167</i>
<i>Hypothesis</i>	<i>168</i>
<i>Aim</i>	<i>168</i>
<i>Methods</i>	<i>168</i>
<i>Statistical analysis</i>	<i>168</i>
<i>Results</i>	<i>170</i>
<i>Key findings</i>	<i>176</i>
<i>CHAPTER 6.....</i>	<i>177</i>
Study 3: Fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in uteroplacental insufficiency.....	177
<i>Introduction</i>	<i>178</i>
<i>Hypotheses and aims</i>	<i>180</i>
<i>Methods and Study design</i>	<i>181</i>
Sample size	181
Study population	181
Data collection	181
Endpoint.....	185
Outcome measures for statistical analysis	185
Statistical analysis	186
<i>RESULTS</i>.....	<i>187</i>
<i>(A) CLINICAL OUTCOMES</i>.....	<i>187</i>

Pregnancy outcomes in high risk and control pregnancies	187
Severity of adverse clinical outcomes.....	187
Comparison of outcomes according to the clinical history.....	189
Comparison of outcomes with South Australian state data	189
Birth weight and gestational age in adverse clinical outcomes	190
(B) LONGITUDINAL ANALYSIS OF ALL VARIABLES IN ADVERSE	
PREGNANCY OUTCOMES	193
UPI.....	193
Sequence of observed significant haemodynamic changes in UPI.....	196
IUGR.....	200
Sequence of observed significant haemodynamic changes in IUGR	203
PREECLAMPSIA.....	209
Sequence of observed significant haemodynamic changes in Preeclampsia....	212
<i>Key findings</i>	218
CHAPTER 7.....	219
Study 4: Fetal and Maternal Doppler flow haemodynamics: correlation with	
adverse clinical and placental outcomes	219
<i>Introduction</i>	220
<i>Hypothesis</i>	222
<i>Aims</i>	222
<i>Study design</i>	222
<i>Scan variables for study 4</i>	222
<i>Endpoint</i>	223
<i>Statistical analysis</i>	226
<i>Results</i>	228
(A) Placental histopathology in adverse clinical outcomes including UPI, PE and	
IUGR.....	228
UPI and placental outcomes.....	228
Preeclampsia and placental outcomes.....	229
IUGR and placental outcomes	230
Preterm birth and placental outcomes.....	230
(B) Fetal and maternal Doppler flow haemodynamics in the presence of	
abnormal placental histopathology	231
Uteroplacental flow haemodynamics in adverse placental outcomes.....	231
Fetoplacental flow haemodynamics in adverse placental outcomes.....	232
Placental thickness	233
Fetal cerebral circulation in adverse placental outcomes	234
Cerebroplacental ratio (CPR).....	235
Intrauterine fetal shunts in adverse placental outcomes	236
Fetal Ductus arteriosus and placental outcomes	236
Fetal Foramen ovale and placental outcomes	237
Doppler haemodynamics and Placental bed biopsies	238
Thromboprophylaxis and placental outcomes	240
<i>Key findings</i>	241
Chapter 8.....	243
<i>Introduction</i>	244
<i>Results of hypothesis testing</i>	248
<i>Discussion</i>	252
Clinical adverse outcomes and placental histopathology in UPI.....	252

<i>Doppler flow velocity waveforms of maternal-fetal circulation and UPI</i>	252
<i>PATHOPHYSIOLOGY OF FORAMEN OVALE</i>	254
Anatomical basis for pathophysiology of abnormal flows through FO.....	256
Pathophysiology of flows through FO in relation to preload-afterload interactions	256
Pathophysiology of abnormal flows through FO based on aortic biomechanics	258
Pseudonormalisation of FO PI.....	260
<i>PATHOPHYSIOLOGY OF DUCTUS ARTERIOSUS</i>	262
Pathophysiology of flows through DA based on morphological characteristics	264
Pathophysiology of flows through DA in relation to preload-afterload interactions.....	264
Pseudonormalisation of DA PI.....	266
<i>PATHOPHYSIOLOGY OF DUCTUS VENOSUS</i>	270
<i>Clinical significance of the study</i>	271
<i>Diagnosis of IUGR</i>	271
<i>Ultrasound parameters in IUGR</i>	272
<i>Central shunts and IUGR</i>	272
<i>Uteroplacental circulation</i>	274
<i>Placental thickness</i>	274
<i>“Adapt, Get out or Die” Hypothesis in adverse maternal-fetal outcomes</i>	278
<i>Strength and limitations</i>	279
<i>Future research and practice</i>	280
<i>Conclusion</i>	281
Contents of the enclosed CD ROM: APPENDIX	283
BIBLIOGRAPHY	284

ABSTRACT

The primary objective of antenatal assessment and monitoring is to ensure wellbeing of the fetus and the mother. There are different methods of assessment during pregnancy and in labour. Doppler ultrasound is one of the tests widely used in clinical practice in the evaluation of pregnancies that are at a greater risk of developing maternal or fetal complications due to uteroplacental insufficiency.

Doppler ultrasound enables evaluation of sequential changes in circulatory haemodynamics in the fetus by evaluation of the fetus for signs of brain sparing and severity of redistribution of circulation. Recognition of abnormal Doppler flow patterns helps the clinician to optimise the appropriate timing of delivery.

Identification of the 'high risk' fetus, before any changes of fetal compromise become evident, still remains one of the major dilemmas in contemporary clinical practice.

This thesis seeks to explore the role of Doppler monitoring fetal intrauterine central shunts as a method of identifying the 'high-risk' fetus before any other established parameters, such as, fetal biometry, fetal weight or flow waveforms in umbilical artery become abnormal. This thesis also evaluates the role of serial Doppler monitoring of fetal central shunts in those fetuses where IUGR has been established.

This is based on the premise that the intrauterine shunts are present in fetal circulation to work closely with the placenta to ensure appropriate nutrition and oxygenation of the fetus, bypassing the lungs.

Four prospective longitudinal studies were designed to evaluate the role of fetal intrauterine shunts in adaptive response mechanisms in cardiovascular stress. Two models were taken into consideration: an 'acute cardiovascular stress' model and a 'chronic cardiovascular stress' model.

To study the 'response to acute cardiovascular stress' in high-risk fetuses, a cohort of mothers undergoing fetal intrauterine transfusion for fetal anaemia were selected. These fetuses were scanned immediately before and after transfusion, and Doppler flows through all the intrauterine shunts were documented and compared with fetoplacental and cerebral circulation.

To study the 'response to chronic cardiovascular stress', a prospective longitudinal observational study was designed and the sequence of changes in Doppler ultrasound of the fetal central shunts studied and compared with the Doppler flow waveforms of

normal pregnancies with a group of pregnancies complicated by uteroplacental insufficiency.

Normograms were designed for all the Doppler parameters and flows from adverse pregnancy outcomes were compared to the normogram.

The pregnancy outcomes in the longitudinal study were correlated with placental pathology.

Our study showed that although changes were demonstrated in the flow patterns within central shunts, these changes were not statistically significant in the 'acute cardiovascular stress model', suggesting that there may be other haemodynamic alterations in acute cardiovascular stress.

However, in the 'chronic cardiovascular stress model', the results suggest that the intrauterine cardiac shunts may play an important role in redistribution of fetal flows in early stages of growth restriction, suggesting that Doppler ultrasound monitoring of foramen ovale can be potentially used as a screening tool to identify high-risk fetuses as early as 16 weeks.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

.....

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

Nayana Anupam Parange

ACKNOWLEDGEMENTS

At times our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us.

Albert Schweitzer 1875-1965

I am indebted to many, more than I can acknowledge, most of all, my family, friends, and my teachers.

I would like to specially mention:

Anupam, my beloved husband, my soul mate, and my best friend – thank you for your unfailing support. You have been my tower of strength. You make me want to be the best I can be. I wouldn't know what I would do without you.

Anurag, my 14 year old son, the joy of my life - I am blessed to have you, thank you for being my very own, 'in-house IT expert'! Thanks to you, I can now make my figures 'tight' or 'beveled' or get them to stay 'in line with text', where I want them to stay !

I am sorry for all the matches and great cricket catches I have missed-- I promise I'll make it up to you!

My supervisor Prof Gustaaf Dekker. Thank you for giving me this opportunity, your tremendous support and patient guidance. Your enthusiasm is inspiring—you have challenged me time and again, to do better professionally and personally. It has been a privilege to work with you.

My co-supervisor Dr Chris Wilkinson. Thank you. Words seem inadequate in expressing my deep gratitude for all your help. Thank you for your kindness, advice and tireless support.

Prof Jeffrey Robinson. Your knowledge is awe-inspiring; I have learnt a lot from you, thank you for the brainstorming sessions. It is humbling to be in your presence.

Paediatric cardiologist Terry Robertson. Thank you for all the brainstorming in the corridors of WCH.

Statisticians Prof Peter Baghurst and Craig Hirte. Thanks Craig, for your invaluable help and statistics advice, as well as a 'fast track crash course' in statistical analysis and advanced modelling of longitudinal data.

Dr Christina Eira, lecturer Integrated Bridging program. Thank you, not only for your guidance with academic writing, but also for being such a great mentor; you have become a dear friend.

NIH grants funding, this helped fund most of this work through the WCH postgraduate PhD scholarship.

All the patients who were scanned for this research for the pivotal role they played to make this thesis happen. Thank you everyone, for placing your trust in my scanning abilities and sharing your hopes, fears and anxieties regarding your pregnancy and your unborn child.

Staff of WCH as well as the research midwives of the NIH Study—Denise Healy, Sally Sieger, Deniece Priess and Paula Picot. Thank you for all your help with recruiting patients and collecting clinical data. Michelle Cox, a big thank you to you, not only for your administrative and organisational abilities, but also for your bubbly and happy personality, that kept everyone going.

All my fellow graduate students -Denise Furness, Rachel Nowak, Jui Ho for the endless 'coffee and cake' sessions, thanks for sharing the roller-coaster 'PhD journey' of hardship, misery and procrastination, to frenzied writing and jubilation; thanks for the laughter, hugs, support. Denise, thanks to you, I learnt to enjoy being a student again!

My new academic colleagues at the University of South Australia, Ms Wendy Barber, Ms Maureen Phillips and Dr Kerry Thoires - thank you for making me feel welcome. Kerry, thank you for your friendship, advice, as well as for reading my chapters and helping me edit my writing----I know it was a chore!!

My ultrasound 'gurus' from Chennai, India, Dr S. Suresh and Dr Indrani Suresh.

Thank you for offering me the ultrasound fellowship in 1996. You taught me all the skills I needed to practice advanced, tertiary-level obstetrics ultrasound. Your encouragement inspired me to make the move from clinical work to academics and research. I would like to express my sincere appreciation for motivating me and converting me from a budding

obstetrician to a die-hard ultrasound enthusiast. Indrani, your love and passion for fetal echocardiography have rubbed off on me!

My friends, many of whom are my present and past work colleagues, who gave me continued love and support, and helped me cope with the stress of migrating and starting all over again in a new country. Julie, Chiara, Yasna, Sean McPeake, Malcolm and Kevin, a big thank you!

My mother- in- law, father- in- law and the extended Parange family-you are a fantastic clan! Thank you all for your constant support, understanding, and encouragement to help me go beyond cultural constraints, to follow my dream.

My wonderful parents. You have set high professional and personal standards for yourselves and taught me to do the same. You have instilled in me a love for learning, a determination to follow my dreams, and taught me never to quit. Thank you. I am blessed to be your daughter.

Finally, in loving memory of my dearest, one and only kid brother. Nitin, you have been gone for more than 10 years now, but I have missed you every day. I know you would have been proud of me today.

Contribution from colleagues towards this thesis:

The intrauterine fetal transfusions in chapter 5 were performed by the Maternal Fetal medicine consultants Dr Chris Wilkinson and Dr Peter Muller and the MFM registrar Dr Joseph Thomas at WCH.

The placental histopathologist Dr Yee Khong and his team performed placental bed and placental histopathology.

I am also grateful to all the consultants and registrars in Obstetrics and Gynaecology who helped with the collection of placental bed biopsies during Caesarian Sections.

ABBREVIATIONS

UPI	Uteroplacental insufficiency
GRIT	Growth Restriction Intervention Trial
TRUFFLE	Trial of Umbilical and Fetal Flow in Europe
SGA	Small for gestational age
AGA	Appropriate for gestational age
CSA	Constitutionally small for age
IUGR	Intrauterine growth restriction
ASSHP	Australasian Society for the study of Hypertension in Pregnancy
RANZCOG	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
ASUM	Australasian Society of Ultrasound in Medicine
RCOG	Royal College of Obstetricians and Gynaecologists
NICE	National Institute for Clinical Excellence
ACOG	American college of Obstetricians and Gynaecologists
ISUOG	International society of ultrasound in obstetrics and gynaecology
CTG	Cardiotocography
SFH	Symphysio Fundal Height
DFMR	Daily fetal movement record
VAST	Vibroacoustic stimulation test
ECG	Electrocardiography
USG	Ultrasonography
BPP	biophysical profile
APGAR	Criteria used to evaluate the newborn baby based on the baby's Appearance, Pulse, Grimace, Activity, Respiration
FBS	fetal blood sampling
NIRS	Near infrared spectroscopy
HbO ₂	oxyhaemoglobin
dHb	deoxyhaemoglobin
Hb	haemoglobin
PO ₂	partial pressure of oxygen in the plasma phase of arterial blood
AEDV	absent end diastolic velocity
REDV	reverse end diastolic velocity
NICU	neonatal intensive care unit
IVH	intraventricular haemorrhage

HIE	hypoxic ischaemic encephalopathy
BPD	Biparietal diameter
HC	Head circumference
AC	Abdominal circumference
FL	Femur length
EFW	estimated fetal weight
UA	Umbilical artery
UAD	Uterine artery Doppler
MCA	Middle cerebral artery
DV	Ductus venosus
IVC	Inferior vena cava
Dao	Descending aorta
PA	Pulmonary artery
DA	Ductus arteriosus
FO	Foramen ovale
SVC	Superior vena cava
IVC	Inferior vena cava
RA	Right atrium
LA	Left atrium
RV	Right ventricle
LV	Left ventricle
RI	resistance index
PI	pulsatility index
S/D Ratio	Systolic/ Diastolic ratio
S/a ratio	ratio to systolic to 'a' wave
PIV	pulsatility index of veins
PVIV	peak velocity index for veins
E/A	ratio of early to late diastolic filling
VTI	velocity time integrals
TAMx	time averaged maximum velocity
TI	thermal index
MI	mechanical index
ALARA	as low as reasonably acceptable
CPR	Cerebroplacental ratio
RI	resistance index
PI	pulsatility index
S/D Ratio	systolic/diastolic ratio

S/a ratio	ratio of systolic to 'a' wave
PIV	pulsatility index of veins
PVIV	peak velocity index for veins
E/A	ratio of early to late diastolic filling
VTI	velocity time integrals
TAMx	time averaged maximum velocity
TI	thermal index
MI	mechanical index
ALARA	As Low As Reasonably Acceptable or achievable
SV	stroke volume
CO	cardiac output
CCO	combined cardiac output
RCO	right cardiac output
LCO	left cardiac output
TR	tricuspid regurgitation
CW	continuous wave
PD	Pulsed Doppler
BMI	body mass index
PCOS	polycystic ovarian syndrome
RMC	recurrent miscarriage
IUT	intrauterine transfusion
WCH	Women's and Children's hospital, Adelaide
SA	South Australia

ORIGINAL CONTRIBUTIONS AND SCIENTIFIC PRESENTATIONS RELATED TO THIS THESIS

Parange N. A., Wilkinson C., Dekker G. A., Romero R. OC118: Doppler flow velocity waveforms assessment of fetal central shunts - a potential predictive tool for Intrauterine growth restriction in high risk pregnancies. *Ultrasound in Obstetrics and Gynecology*. Volume 32, Issue 3, Date: August 2008, Pages: 281-282. Presented at ISUOG, Chicago, August 2008.

Parange NA. Fetal intrauterine central shunts: embryological vestigial remnants or a brilliant evolutionary strategy for efficient oxygenation? ASA Conference, Perth, May 2008.

Parange NA, Wilkinson C., Dekker GA. OP04.15: Nomograms of fetal intrauterine shunts. *Ultrasound in Obstetrics and Gynecology*. Volume 28, Issue 4, Date: September 2006, Pages: 442-443. Presented at ISUOG, London, August 2006

Parange NA, Wilkinson C., Dekker GA. OP06.10: Acute fetal cardiac and other hemodynamic redistribution after intrauterine transfusion for treatment of severe red blood cell alloimmunisation. *Ultrasound in Obstetrics and Gynecology*. Volume 28, Issue 4, Date: September 2006, Pages: 455. Presented at ISUOG, London, August 2006

Parange N., Furness D., Fenech M., Wilkinson C., Dekker G. Role of uterine artery Doppler and the folate metabolic pathway in prediction of uteroplacental insufficiency. *American Journal of Obstetrics and Gynecology*, 2006, Volume 195, Issue 6, Pages S207-S207-Abstract, presented at SMFM San Francisco 2007.

Furness D., **Parange N.**, Dekker G., Fenech M. (2006) Role of Genome Damage and Uterine Artery Doppler in Prediction of Uteroplacental Insufficiency. *American Journal of Obstetrics and Gynecology*, 2006, Dec; 195(6):S221 – Abstract, presented at SMFM San Francisco 2007.

Parange NA. Uterine Artery Doppler: a) The clinicopathologic Classification of abnormal waveform Pattern. b) Recent research evidence of its usefulness. *38th International Annual Meeting of the Society for the Study of the Pathophysiology of Pregnancy (Organisation Gestosis)*. Presented at Adelaide 2006, South Australia.

Parange NA. Doppler Monitoring of High Risk Pregnancies. PSANZ 2005, Presented at Obstetric Medicine workshop, in Adelaide.

Parange NA. Fetal Doppler assessment of IUGR. Presented at 35th Annual Scientific Meeting 2004, Adelaide, South Australia.

TABLES AND FIGURES

Table 1 Clinical evidence for tests for assessment of fetal wellbeing	4
Table 2 Modified Manning’s biophysical profile scoring	11
Table 3 Longitudinal studies in evaluation of temporal sequence of events in UPI...42	
Table 4 Statements on safety of ultrasound endorsed by accrediting bodies worldwide	49
Table 5 Distribution of Blood Flow Expressed as Percent of Combined (Biventricular) Cardiac Output:	73
Table 6 Number of observations for Ultrasound and Doppler variables in Study 1...85	
Table 7 Number of observations for Ultrasound and Doppler variables in Study 2...90	
Table 8 Classification of high risk	91
Table 9 Number of observations for Ultrasound and Doppler variables in Study 3...95	
Table 10 Number of observations at every time point for study 3.	96
Table 11 Number of observations for Ultrasound and Doppler variables in Study 4.99	
Table 12 Placental outcomes evaluated for histopathology.....	103
Table 13 Number of observations for Ultrasound and Doppler variables in Study 1	130
Table 14 Reference ranges for Foramen Ovale Peak Systolic Velocity.....	133
Table 15 Reference ranges for Foramen Ovale End Diastolic Velocity.....	134
Table 16 Reference ranges for Foramen Ovale Pulsatility Index	135
Table 17 Reference ranges for Ductus Arteriosus Peak Systolic Velocity.....	137
Table 18 Reference ranges for Ductus Arteriosus End Diastolic Velocity	138
Table 19 Reference ranges for Ductus Arteriosus Pulsatility Index	139
Table 20 Reference ranges for Ductus venosus Preload Index.....	142
Table 21 Reference ranges for Ductus Venosus S/a ratio.....	143
Table 22 Reference ranges for Ductus Venosus Peak velocity Index	144
Table 23 Reference ranges for Ductus Venosus Pulsatility Index.....	145
Table 24 Reference ranges for Mean Uterine RI.....	148
Table 25 Reference ranges for Mean Umbilical Artery Pulsatility Index	150
Table 26 Reference ranges for uteroplacental ratio	151
Table 27 Reference ranges for Middle Cerebral Artery Resistance Index	153
Table 28 Reference ranges for Cerebroplacental ratio	154
Table 29 Reference ranges for placental thickness.....	157
Table 30 Reference ranges for biparietal diameter	159
Table 31 Reference ranges for head circumference.....	160
Table 32 Reference ranges for abdominal circumference	161
Table 33 Reference ranges for femur length.....	162
Table 34 Reference ranges for estimated fetal weight.....	163
Table 35 Transfusion details of fetuses undergoing transfusion	170
Table 36 Doppler results and p values before and after IUT	171
Table 37 Classification of clinical history of high risk patients at first visit	182
Table 38 Number of Ultrasound and Doppler observations in study 3	184
Table 39 Number of observations at every time point for study 3	185
Table 40 Severity of outcomes in UPI and preeclampsia	187
Table 41 Severity of outcomes in IUGR.....	188
Table 42 Clinical Histories and outcomes	189
Table 43 Comparison of IUGR outcomes with South Australian data.....	190
Table 44 Birth Weight in adverse clinical outcomes	191

Table 45 Gestational age in adverse clinical outcomes	192
Table 46 Comparison of all Doppler and ultrasound variables in UPI versus normal outcomes	197
Table 47 Ultrasound and Doppler parameters in Uteroplacental insufficiency (UPI)	199
Table 48 Comparison of all Doppler and ultrasound variables in IUGR versus normal outcomes	204
Table 49 Ultrasound and Doppler variables in all IUGR versus normal outcomes..	205
Table 50 Uteroplacental and fetoplacental haemodynamics and placental thickness in IUGR with differing severity	207
Table 51 Cerebral circulation, 'Brain sparing' and central shunts in IUGR with differing severity	208
Table 52 Comparison of all Doppler and ultrasound variables in Preeclampsia versus normal outcomes	213
Table 53 Comparison of all Doppler and ultrasound variables in late-onset Preeclampsia versus normal outcomes	214
Table 54 Comparison of all Doppler and ultrasound variables in all Preeclampsia versus normal outcomes	215
Table 55 Uteroplacental and fetoplacental haemodynamics and placental thickness in Preeclampsia with differing severity	216
Table 56 Cerebral circulation, 'Brain sparing' and central shunts in preeclampsia with differing severity	217
Table 57 Number of observations for Ultrasound and Doppler variables in Study 4	227
Table 58 Placental outcomes evaluated for histopathology	227
Table 59 Uteroplacental Insufficiency and placental outcomes	228
Table 60 Preeclampsia and placental outcomes	229
Table 61 IUGR and placental outcomes	230
Table 62 Uterine artery RI and placental outcomes	231
Table 63 Umbilical artery PI and placental outcomes	232
Table 64 Placental thickness and placental outcomes	233
Table 65 Middle cerebral artery RI and Placental outcomes	234
Table 66 Cerebroplacental ratio and placental outcomes	235
Table 67 Ductus arteriosus PI and placental outcomes	236
Table 68 Foramen ovale PI and Placental outcomes	237
Table 69 Longitudinal analysis of all ultrasound and Doppler variables in adverse placental outcomes	239

Figure 1 The Doppler effect.....	21
Figure 2 Arterial Doppler waveform	22
Figure 3 Venous Doppler waveform.....	23
Figure 4 Fetal circulation	24
Figure 5 Fetoplacental circulation	26
Figure 6 Umbilical artery Doppler.....	27
Figure 7 Trophoblastic invasion	29
Figure 8 Uterine artery Doppler.....	30
Figure 9 Middle Cerebral artery Doppler	32
Figure 10 MCA demonstrating increased PSV in fetal anaemia, PSV reduced after intrauterine transfusion.	33
Figure 11 Ductus venosus Doppler waveform.....	37
Figure 12 Summary of changes in fetal compromise as observed on fetal monitoring.	45
Figure 13 Placenta, umbilical cord, liver, and ductus venosus (from Fabricius'	51
Figure 14 Heart, lung, great vessels, and ductus arteriosus (E) (from Fabricius'	51
Figure 15 Classic illustration of fetal and neonatal circulation by Dawes (1954).....	53
Figure 16 Central shunts and the fetal heart.	56
Figure 17 Ductus venosus, foramen ovale and the hepatic circulation.....	64
Figure 18 Stroke volume.....	69
Figure 19 Cardiac preload.....	70
Figure 20 Flow chart describing study design for Study 1	84
Figure 21 Flow chart describing the study design for Study 2	89
Figure 22 Flow chart describing study design for Study 3	94
Figure 23 Flow chart describing study design for Study 4	98
Figure 24 Umbilical artery Doppler flow patterns.....	112
Figure 25 Middle cerebral artery Doppler flow patterns	114
Figure 26 Uterine artery Doppler flow patterns.....	116
Figure 27 Ductus venosus Doppler flow patterns.....	118
Figure 28 Ductus arteriosus Doppler flow velocity patterns	120
Figure 29 Foramen ovale Doppler flow patterns	122
Figure 30 Flow chart describing the study design for Study 1	129
Figure 31 Foramen Ovale Doppler(FO) Methodology	132
Figure 32 Reference ranges for Foramen Ovale Peak Systolic Velocity.....	133
Figure 33 Reference ranges for Foramen Ovale End- diastolic Velocity.....	134
Figure 34 Reference ranges for Foramen Ovale Pulsatility Index.....	135
Figure 35 Ductus Arteriosus Doppler (DA) Methodology	136
Figure 36 Reference ranges for Ductus Arteriosus Peak Systolic Velocity	137
Figure 37 Reference ranges for Ductus Arteriosus End-diastolic Velocity.....	138
Figure 38 Reference ranges for Ductus Arteriosus Pulsatility Index	139
Figure 39 Ductus venosus Doppler (DV) Methodology	141
Figure 40 Reference ranges for Ductus venosus Preload Index	142
Figure 41 Reference ranges for Ductus Venosus S/a ratio.	143
Figure 42 Reference ranges for Ductus venosus Peak velocity Index.....	144
Figure 43 Reference ranges for Ductus Venosus Pulsatility Index	145
Figure 44 Uterine arteries Doppler (Ut RI) Methodology	147
Figure 45 Reference ranges for Mean Uterine RI.....	148
Figure 46 Umbilical artery Doppler (Umb A) Methodology.....	149
Figure 47 Reference ranges for Umbilical Artery Pulsatility Index	150

Figure 48 Reference ranges for uteroplacental ratio.....	151
Figure 49 Middle Cerebral Artery Doppler (MCA) Methodology.....	152
Figure 50 Reference ranges for Middle Cerebral Artery Resistance Index.....	153
Figure 51 Reference ranges for Cerebroplacental ratio.....	154
Figure 52 Placental thickness Methodology.....	156
Figure 53 Reference ranges for placental thickness.	157
Figure 54 Sonographic planes for biometric measurements.....	158
Figure 55 Reference ranges for biparietal diameter.....	159
Figure 56 Reference ranges for head circumference.....	160
Figure 57 Reference ranges for abdominal circumference.....	161
Figure 58 Reference ranges for femur length.....	162
Figure 59 Reference ranges for estimated fetal weight.....	163
Figure 60 Flow diagram for study design for Study 2.....	169
Figure 61 Umbilical artery PI before and after fetal intrauterine transfusion.....	171
Figure 62 MCA PSV and RI before and after fetal intrauterine transfusion.	172
Figure 63 DV PIV before and after fetal intrauterine transfusion.	173
Figure 64 DA PSV, EDV, and PI before and after fetal intrauterine transfusion.....	174
Figure 65 FO PSV, EDV and PI before and after fetal intrauterine transfusion.....	175
Figure 66 Flow diagram describing the study design for Study 3.....	183
Figure 67 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in uteroplacental insufficiency and normal pregnancy outcome.....	194
Figure 68 Longitudinal evaluation of fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in uteroplacental insufficiency and normal pregnancy outcome.....	195
Figure 69 Schematic representation of the sequence of changes in UPI.....	197
Figure 70 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in IUGR and normal pregnancy outcome.....	201
Figure 71 Longitudinal evaluation of estimated fetal weight, fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in IUGR and normal pregnancy outcome.....	202
Figure 72 Schematic representation of the sequence of changes in IUGR.....	204
Figure 73 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in preeclampsia and normal pregnancy outcome.....	210
Figure 74 Longitudinal evaluation of estimated fetal weight, fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in preeclampsia and normal pregnancy outcome.....	211
Figure 75 Schematic representation of the sequence of changes in preeclampsia	213
Figure 76 Schematic representation of the sequence of changes in late onset preeclampsia.....	214
Figure 77 Flow chart describing study design for study 4.....	225
Figure 78 Longitudinal evaluation of uterine artery Doppler RI: profile plots of estimated marginal means in adverse placental and normal placental outcome.....	231
Figure 79 Longitudinal evaluation of umbilical artery Doppler PI: profile plots of estimated marginal means in adverse placental and normal placental outcome.....	232
Figure 80 Longitudinal evaluation of sonographic placental thickness: profile plots of estimated marginal means in adverse placental and normal placental outcome.....	233

Figure 81 Longitudinal evaluation of middle cerebral artery RI : profile plots of estimated marginal means in adverse placental and normal placental outcome.....	234
Figure 82 Longitudinal evaluation of cerebroplacental ratio : profile plots of estimated marginal means in adverse placental and normal placental outcome	235
Figure 83 Longitudinal evaluation of ductus arteriosus PI: profile plots of estimated marginal means in adverse placental and normal placental outcome	236
Figure 84 Longitudinal evaluation of foramen ovale PI: profile plots of estimated marginal means in adverse placental and normal placental outcome.	237
Figure 85 Summary of all ultrasound and Doppler measurements in the present thesis	246
Figure 86 Sequence of changes in ultrasound parameters in UPI	253
Figure 87 Hypothetical pathophysiological mechanisms for increased Doppler pulsatility index in foramen ovale in fetal compromise associated with uteroplacental insufficiency.....	255
Figure 88 Pathophysiology of abnormal flows through Ductus arteriosus.....	263
Figure 89 Results of ductus arteriosus PI in 25 SGA fetuses plotted on reference range: a study by Mari and colleagues. [232]	269
Figure 90 Doppler ultrasound of central fetal shunts.....	273
Figure 91 Speculated sequence of events in UPI leading to increasing severity of fetal compromise.....	275
Figure 92 Proposed clinical monitoring algorithm for fetal monitoring for adaptation into clinical trials.....	277

APPENDIX: Supplementary files enclosed in the CD-ROM

S File 1: File name: SUPPLEMENTARY DATA FOR CHAPTER 6.xls	283
S File 2: File name: SUPPLEMENTARY DATA FOR CHAPTER 7.xls	283

CHAPTER 1

Introduction

The principal aim of fetal monitoring during pregnancy and intrapartum is to assess fetal growth and well being, to ensure a safe delivery and normal outcome for the mother and the fetus. Extensive research over the last four decades has provided an increasing body of knowledge as well as advanced technology to enable monitoring of mothers and fetuses throughout pregnancy as well as in labour. Fetal monitoring is aimed at identification of fetuses at risk for hypoxia-asphyxia, with the assumption that an early recognition of fetal compromise may enable timely intervention and prevent fetal mortality and reduce fetal morbidity.

One of the major causes of maternal and fetal morbidity and mortality is uteroplacental insufficiency (UPI), i.e. preeclampsia, intrauterine growth restriction (IUGR), placental abruption, and many cases of preterm labour. The national perinatal statistics of Australia [1] indicate that 6.4% of live born babies were of low birth weight (less than 2,500 grams), 8.1% were preterm (less than 37 weeks gestation), 15.5% of live born babies were admitted to a special care nursery or neonatal intensive care unit, and the perinatal death rate was 10.5 per 1,000 births of which the fetal death rate was 7.3 per 1,000 births and the neonatal death rate was 3.2 per 1,000 live births. It is therefore necessary to evaluate the high risk fetus for well being and growth disorders, as untreated, progressive compromise may lead to fetal myocardial dysfunction and even death. These national perinatal statistics demonstrate that UPI remains a major concern even in developed countries. The maternal and fetal morbidity and mortality can be minimised by early identification of the mother and fetus at risk for compromise, instituting appropriate monitoring protocols and tests in fetal wellbeing and optimising the time to deliver the compromised fetus. Appropriate implementation of these often time consuming and costly fetal surveillance techniques remains as major challenge in modern obstetrics

When to deliver the compromised fetus?

The results of a large randomised trial, the GRIT [2] demonstrated that nowadays obstetricians are able to recognise severe fetal hypoxia with existing tests, and are able to deliver the fetuses at an optimum time, to prevent mortality in the majority of cases. However, the GRIT trial also demonstrated that with the available tests, obstetricians are not able to deliver the fetus in time to reduce fetal morbidity. Thus, obstetricians are still in a dilemma as to when to deliver the premature IUGR fetus. If the fetus is severely growth restricted and the baby is delivered too early, the

complication of prematurity is added to the IUGR. On the other hand, a decision to prolong pregnancy to reduce the added risk of prematurity may in fact be detrimental to the fetus. The dilemma of the timing of delivery as shown by the results of the GRIT trial is being addressed in the TRUFFLE trial (Trial of Umbilical and Fetal flow in Europe) [3] . The TRUFFLE trial is a major international randomised trial, evaluating the role of fetal Doppler versus CTG to decide the timing of delivery, to reduce adverse perinatal outcome. It is hoped that the results of this trial will provide answers regarding the timing of delivery in a prematurely growth restricted fetus.

How to recognise and monitor the compromised fetus?

Fetal growth monitoring in clinical practice has been aimed at identification of a ‘small’ fetus, or ‘small for gestational age (SGA)’ fetus. However, not all small fetuses are necessarily growth restricted or hypoxic due to UPI, and therefore at risk for the complications associated with IUGR. Conversely, there is a proportion of fetuses who are of average birth size, but [4, 5] might have not achieved their genetic potential and have been affected by UPI and are technically speaking, IUGR. This group of AGA fetuses would be at risk for chronic hypoxia similar to the IUGR fetuses, but would have escaped detection based on birth weight alone. These chronically hypoxic, ‘normal sized’ fetuses could present with birth asphyxia, fetal distress, or even ‘unexplained’ fetal death in utero. It is therefore necessary to design appropriate clinical tests to improve identification of these ‘normal sized’ high- risk fetuses. In addition, there is a paucity of data demonstrating the early changes and possible compensatory mechanisms, which might occur in a fetus at risk for fetal compromise. Broadly, this thesis seeks to explore the early changes in these ‘normal sized’ and small fetuses affected by UPI.

Fetal wellbeing and growth is assessed by different tests in clinical practice, they include traditional clinical techniques as well as modern monitoring technology, all of which have their advantages and limitations. The clinical evidence supporting or refuting the use of these tests in clinical practice is summarised in the table below (Table 1). This chapter provides an overview of the different intrapartum as well as antenatal tests for fetal wellbeing and discusses the evidence obtained from clinical trials.

Table 1 Clinical evidence for tests for assessment of fetal wellbeing

Tests	Evidence	Source of evidence
Traditional clinical techniques		
Maternal weight gain	Routine maternal weight gain assessment is no longer recommended as it provides no added benefit; on the other hand, may cause unnecessary anxiety to the mother	NICE 2003 [4]
Abdominal palpation	Abdominal palpation has limited diagnostic accuracy to predict IUGR, detecting only 30 % of IUGR.	RCOG guidelines no 31, Nov 2002 [5]
Symphysiofundal height (SFH)	No obvious advantages to abdominal palpation or any differences in detection of adverse pregnancy outcome with routine use of SHF was routinely used during antenatal care	Cochrane Database Syst Rev 2000 [6]
	Serial SFH measurements fortnightly recommended.	NICE 2003 [4]
	Insufficient evidence to recommend the routine use of SFH before 24 weeks.	NICE 2008 [7]
	Customised antenatal growth charts may be useful but more prospective research needed to establish diagnostic effectiveness.	RCOG 2002, [5] NICE 2008. [7]
Daily fetal movement record	Reporting of reduced fetal movements is associated with an increased likelihood of fetal death but there was insufficient evidence to determine the impact of FMR on outcomes in clinical practice one way or other.	Cochrane Database of Systematic Reviews 2007 [8]
Monitoring technologies		
Ultrasound	Routine ultrasound, in unselected populations, after 24 weeks of pregnancy did not improve the perinatal mortality	Cochrane Database of Systematic Reviews 2000 [9]
	There is fair evidence to suggest that routine ultrasound in early pregnancy is useful in for detection of viability , dating the pregnancy , identify multiple pregnancies as well as in detection of many major lethal abnormalities at a gestation when medical termination of pregnancy is possible	Cochrane Database of Systematic Reviews 2000 [10]
	All women should be offered an ultrasound at 18-19 weeks gestation after counselling them about the limitations as well as advantages of ultrasound.	RANZCOG statement. [11]
Biophysical profile	There was insufficient evidence to support the use of biophysical profile as a test of fetal wellbeing	Cochrane rev 2000 [12]
Doppler ultrasound	Doppler can improve obstetric outcomes in high- risk pregnancies. The use of Doppler showed a reduction in perinatal deaths, fewer inductions of labour and fewer admissions to hospital, without reports of adverse effects. The reviewers, however, did not find any difference for fetal distress in labour or caesarean delivery. There is insufficient evidence so far, for the use of Doppler in an unselected population.	Cochrane rev 2000 [13, 14]
Fetal vibro-acoustic stimulation test.	There are no randomised trials and therefore there is insufficient evidence to recommend the use of VAST as a test of fetal wellbeing in labour.	Cochrane Database of Systematic Reviews 2005

		[15]
Intermittent auscultation	For a woman who is healthy and has had an otherwise uncomplicated pregnancy, intermittent auscultation should be offered and recommended in labour to monitor fetal wellbeing.	NICE 2001, RANZCOG Guidelines .[16]
Cardiotocography	There are no internationally agreed practice recommendations . However, various authorities such as ACOG, RCOG, NICE and RANZCOG have published guidelines. CTG is performed when there is a suspicion of fetal compromise . Daily CTG In the preterm fetus a non-reactive CTG tracing indicates the need for more detailed biophysical monitoring In the mature fetus a non-reactive CTG tracing may be an indication for delivery .	RANZCOG Guidelines. [16]
	Currently a systematic review of all randomised trials is underway to compare admission CTG with intermittent auscultation of the heart rate on maternal and infant outcomes.	Cochrane rev 2005. [17]
Fetal ECG and ST segment analysis	An adjunctive use of ST segment analysis in labour was associated with fewer babies with metabolic acidosis at birth and fewer babies with neonatal encephalopathy although there was no difference in the Caesarian rate, APGAR5 less than 7 at five minutes or number of admissions to special care units. The advantages have to be weighed against the disadvantages of using an internal electrode after the membranes have been ruptured.	Cochrane Database of Systematic Reviews [17-19]
Fetal pulse oximetry	Routine use did not show any difference in Caesarian rate or any difference in maternal or newborn outcomes; however, an addition of pulse oximetry to CTG did show a reduction in the number of caesareans in those pregnancies where the fetuses were already suspected to be compromised.	Cochrane Database of Systematic Reviews 2004 [20]
	Further trials are needed before it can be recommended in routine intrapartum monitoring, especially to evaluate adverse outcomes, especially in relation to falsely reassuring data	ACOG Committee opinion No. 258. Obstet Gynecol 2001[21]
Invasive tests		
Fetal scalp blood sampling	Fetal blood sampling is recommended in UK and Canada the presence of an uninterpretable or non-reassuring cardiotocograph (CTG) traces, When fetal scalp blood sampling is used in combination with CTG monitoring, both false positive and false negative CTGs are reduced, however in Australia and New Zealand, the review group recognises that it is not practical for all hospitals to offer FBS.	RCOG 2001[22]; SOGC 2002 [23] RANZCOG 2006. [16]
	Currently, a randomised trial is underway in Netherlands, to compare the role of CTG plus ST segment analysis with CTG plus FBS, and will also evaluate the role of FBS in intrapartum assessment	BMC Pregnancy and Childbirth 2007 [24]
Fetal scalp lactate measurement	Animal studies and observational studies in the fetus seem to suggest that fetal lactate measurements are good predictors of fetal asphyxia . Samples as little as 5 ml are enough for analysis. A systematic review of fetal lactate is now being conducted to ensure the appropriate use of this test in clinical practice	(Protocol) Cochrane Database of Systematic Reviews 2006 [25]
Other investigational tests		
Near infrared spectroscopy	There is insufficient evidence to recommend use of this technique in clinical practice.	Cochrane Reviews 2000 [26]

Traditional Clinical techniques

Traditional clinical techniques are widely used in clinical practice and have the advantage of being simple to perform and do not need expensive and technology. A brief discussion of the research evidence regarding performance and limitations of these tests is described below.

Maternal weight gain

Maternal weight gain monitoring in pregnancy was first proposed as a clinical indicator of maternal nutrition [27] and several studies have demonstrated that maternal malnutrition in early gestation [28, 29], and more recently, in periconception, can be an important determinant of IUGR [30].

However, routine measurement of maternal weight gain has low sensitivity and positive predictive value (PPV) for adverse pregnancy outcome and has not demonstrated any benefit to the mother or the child [31]. Routine maternal weight gain assessment is therefore no longer recommended as it provides no added benefit; on the other hand, it may cause unnecessary anxiety to the mother [4].

Abdominal palpation and symphysio-fundal height (SFH)

Abdominal palpation and SFH measurements are established method of clinical monitoring in most obstetric services worldwide. In many developing countries, serial SFH measurements remain the only available method for assessment of IUGR as they are inexpensive, easy to perform and do not require sophisticated technology.

Abdominal palpation is performed when the clinician or midwife assesses the growth of the fetus by gently feeling and pressing the outside of the uterus, to determine the fetal position, lie and liquor volume. Evidence suggests that abdominal palpation has limited diagnostic accuracy to predict IUGR, detecting only 30 % of IUGR [5].

Abdominal palpation is therefore accompanied by SFH measurement.

The SFH measurement is made using an inelastic measuring tape, with the woman in a supine position, legs extended and bladder empty. The distance between the top of the symphysis pubis and the top of the uterine fundus is measured, with the tape lying in contact with the anterior abdominal wall. The SFH was initially considered to be approximately equal to the week of gestation, and therefore taught accordingly. However, this concept has been proved erroneous and studies have shown that SFH

has varying sensitivities (27 % to 86 %) although specificities are high (80 to 93 %) [5].

A systematic review by Neilson et al., found only one trial involving 1639 women, which found no obvious advantages to abdominal palpation or any differences in detection of adverse pregnancy outcome when SHF was routinely used during antenatal care [32]. However, a prospective, controlled, non randomised, population based study involving 1272 women showed that serial plotting of SFH measurements on individually customised charts incorporating fetal weight can significantly improve detection of fetal growth abnormalities and reduces the number of unnecessary interventions and can therefore potentially be a useful cost-effective screening tool [33]. The RCOG guidelines now recommend the use of plotting serial SFH measurements fortnightly [5] and customised antenatal growth charts [5]. A recent systematic review states that SFH must be measured and recorded during every visit from 24 weeks onwards, however, there is insufficient evidence to recommend the routine use of SFH before 24 weeks or the use of customised fetal growth charts as a screening procedure in routine practice. There is a need for more prospective research to evaluate the diagnostic value and effectiveness of SFH and customised fetal growth charts [7].

Fetal movement record

Fetal movement record (FMR) is a method where the mother is asked to keep a record of the number of times she can feel the movements or kicks of the fetus and several techniques have been described to document the fetal movements. FMR is based on the premise that a compromised fetus will have decreased fetal movements.

A sudden decrease in fetal movements may occur before fetal death [34]. A major limitation of this test is that it is subjective and depends on the perception of the mother and can be unreliable because there may be absence of movements in many other states in absence of fetal compromise, such as maternal exercise and medication [34]. A meta-analysis of randomised controlled trials of FMR in low-risk pregnancies has shown that the reporting of reduced fetal movements is associated with an increased likelihood of fetal death but there was insufficient evidence to determine the impact of FMR on outcomes in clinical practice one way or other. It was therefore recommended that further research was necessary “ to determine the sensitivity and

the specificity of fetal movement counting in detecting fetal compromise; its effectiveness in decreasing the perinatal mortality in high-risk and low-risk women; its acceptability to women; how easy it is for women; and the best fetal movement counting method.” [8]. The NICE guidelines [7] do not recommend the routine use of formal fetal movement counting as a method of preventing fetal death.

Limitations of traditional techniques therefore led to a quest for other methods of assessment of fetal wellbeing, leading to a technological advancement in different monitoring technologies.

Monitoring technologies

The limitations of traditional techniques have fuelled research for other methods of surveillance. Recent advances in monitoring technologies have enabled different non-invasive as well as invasive tests for antenatal as well as intrapartum assessment of fetal growth and behaviour, perfusion as well as fetal heart rate assessment. One of the more widely used non-invasive methods in pregnancy is ultrasonography and Doppler technology.

Ultrasonography

Ultrasonography (USG), also known as ultrasound, is a technique where high frequency sound waves beyond the audible range, between 3.5 MHz to 5 MHz (3.5 to 5 million cycles per second) are emitted from a probe, also called the transducer, which is placed on the maternal abdomen and sometimes transvaginally using real-time scanning equipment. These sound waves interact with the tissues and some sound waves are reflected back to the transducer as echoes. These echoes are then digitally converted to create a real-time image of the fetus in utero. USG is useful in non-invasive evaluation of organs and tissues and has especially gained wide acceptance in obstetric practice.

A systematic review of nine trials including 34245 women suggested that there is fair evidence to suggest that routine ultrasound in early pregnancy is useful in for detection of viability, dating the pregnancy, identify multiple pregnancies as well as in detection of many major lethal abnormalities at a gestation when medical termination of pregnancy is possible [9]. In addition, ultrasound in pregnancy has also been

thought to provide an increased psychological support to the parents during pregnancy [35, 36].

An earlier meta-analysis of nine trials including 25,036 women concluded that routine ultrasound, in unselected populations, after 24 weeks of pregnancy did not improve the perinatal mortality [14]. These results have been influenced by several significant factors. Firstly, the primary reports of the European trials Helsinki [37], Trondheim [38], Alesund [39] and Stockholm [40] did demonstrate a greater reduction in perinatal mortality (49 %) as opposed to the American RADIUS (Routine Antenatal Diagnostic Ultrasound Screening) trial [41]. The London trial [42] had to exclude 30 % of the results as the code had to be broken due to clinical concerns, leading to intervention during pregnancy. This had a negative impact upon the meta-analysis.

The RADIUS trial did not show any difference in perinatal mortality in the women who had routine ultrasounds, as opposed to those who had ultrasounds for clinical indication. This can be attributed to the low detection rate of congenital anomalies (13% to 35%), depending upon the experience of the sonographers. Besides, the RADIUS trial had much larger numbers when compared with the European trials. 55,744 women were registered in the RADIUS trial of which, 33, 317 (58%) were excluded, as they had known clinical indications and only 15,530 low risk women were randomised, either to a two-stage ultrasound program or to an ultrasound exam for medical reasons which developed after randomisation. Despite the exclusion of 58% of women registered, the RADIUS trial did have the largest number of women included in the meta-analysis, thereby influencing the results of the Cochrane review [14].

Even though the meta-analysis concluded that routine ultrasound does not improve the perinatal mortality, these trials brought home several important aspects of clinical practice. Most importantly, it highlighted the need for rigorous scanning protocols, proper training and accreditation of sonographers, sonologists and maternal-fetal specialists to improve the detection rate of congenital anomalies. More intensive scanning protocols have since been implemented globally, to evaluate all the major structural aspects of the fetus, including the fetal spine, brain, heart, abdomen, face and limbs [43, 44]. This has led to an improvement in quality of clinical ultrasound practice.

An important argument in favour of ultrasound is that, should the parents decide to continue the pregnancy after detection of an anomaly, the perinatal mortality may not change, but, the parents will be better prepared to plan for the appropriate measures to be instituted during and after the delivery. Another aspect to remember is that all these trials were conducted in the late 1970s and 1980s. In contemporary practice, ultrasound machines have much better resolution; there is availability of quality training, as well as a greater body of knowledge gained due to the dedicated effort of researchers over the last couple of decades.

The RCOG UK guidelines now recommend that every woman must have at least one scan during pregnancy [45]. The RANZCOG Clinical Guidelines Development group, with an aim towards developing best practice guidelines in the Australia and New Zealand setting, recommend that all women should be offered an ultrasound at 18-19 weeks gestation after counselling them about the limitations as well as advantages of ultrasound [46]. It would therefore be reasonable to conclude that ultrasound does have great potential as an investigative tool in pregnancy and therefore warrants more research regarding its role in fetal wellbeing.

A single ultrasound before 24 weeks of pregnancy detects less than one-third of fetuses with IUGR [47] and therefore has not been useful so far, to predict IUGR. Serial scans however, reveal a slowing, cessation or acceleration of growth, and are therefore being used in clinical practice in high-risk fetuses to identify fetuses at risk for compromise due to IUGR. Growth monitoring is now possible by measurements of BPD, HC, AC, FL and estimated fetal weight. Standardised centile charts are present for the appropriate population and a reduction in $AC < 5^{\text{th}}$ centile or $EFW < 10^{\text{th}}$ centile has now been defined as a criteria for SGA [48]. However small fetuses could be small because they are constitutionally small, not necessarily growth restricted. Therefore serial growth scans need to be performed to observe the fetal growth trajectory to observe a slowing or absence of growth, before labelling a fetus as IUGR. Fetal growth monitoring is also accompanied by an assessment of fetal behaviour with ultrasound, also known as biophysical profile evaluation.

Biophysical profile

Biophysical profile (BPP) is a test initially conceived and validated by Manning [49]. It attempts to assess different fetal behavioural responses to placental insufficiency with the help of ultrasound. It is a composite scoring system and combines dynamic

fetal variables such as fetal movements, tone, breathing with amniotic fluid volume and fetal heart rate monitoring (Table 2).

Table 2 Modified Manning's biophysical profile scoring

Biophysical variable	Normal score (score =2)	Abnormal score (score = 0)
Fetal Breathing movement	At least one episode of fetal breathing of at least 30 sec duration.	Absent fetal breathing, or no episode of more than 30 sec.
Gross fetal body movement	At least three discrete body/limb movements.	Two or fewer body limb movements.
Fetal tone	At least one episode of active extension with return to flexion of fetal limbs or trunk, includes opening or closing of the hand.	Show extension with return to partial flexion or limb movements without flexion or absent fetal movement.
Fetal heart rate	Below 26 weeks of gestation: at least two accelerations of \geq 10 beat accelerations of \geq 10s duration; 26-36 weeks gestation: at least two episodes of $>$ 10 beat accelerations of $>$ 15s duration; Beyond 36 weeks gestation: at least two episodes of $>$ 20 beat accelerations of $>$ 20s duration	Less than two episodes of acceleration and durations as specified.
Amniotic fluid volume	At least one amniotic fluid pocket of 2 x 2 cm in perpendicular planes	No amniotic fluid pocket of 2 x 2 cm in perpendicular planes.
* All parameters are examined in a 30-min monitoring interval. Manning. [49]		

Vintzelios et al observed that a sequential loss of variables, i.e., abnormalities of heart rate trace, followed by a loss of breathing, then amniotic fluid volume, then

movements and finally tone, correlates with worsening acidaemia, hypoxaemia and hypercapnoea [50]. Therefore this test seemed to be promising in the detection of a fetus with progressive compromise. However, interpretation of BPP is fraught with limitations due to inter-observer variability in NST [51]. In addition, fetal behavioural patterns may be varied, with differences in behavioural patterns of fetal quiescence and breathing cycles [52]. A Cochrane systematic review of five randomised and quasi-randomised trials including 2974 women suggested that there was insufficient evidence to support the use of biophysical profile as a test of fetal wellbeing [12]. A less subjective and more valid technique is therefore necessary to examine fetal wellbeing. It is also necessary to observe any alteration in blood flow haemodynamics seen in IUGR, as progressive fetal compromise can lead to myocardial dysfunction and death. With the advancement in ultrasound and Doppler technology, it has now become possible to non-invasively evaluate changes in maternal and fetal circulation in an IUGR fetus.

Doppler ultrasound

The Doppler principle is defined as a perceived change in frequency of the sound wave with relative motion between the sound source and observer and was first proposed by Christian Doppler in 1842 [53]. A detailed review about current practice in Doppler monitoring in high-risk pregnancies is provided in the next chapter.

A systematic review of eleven randomised trials including seven thousand women demonstrated that Doppler can improve obstetric outcomes in high risk pregnancies. The use of Doppler showed a reduction in perinatal deaths (odds ratio 0.71, 95% confidence interval 0.50 to 1.01), fewer inductions of labour (odds ratio 0.83, 95% confidence interval 0.74 to 0.93) and fewer admissions to hospital (odds ratio 0.56, 95% 0.43 to 0.72), without reports of adverse effects. The reviewers, however, did not find any difference for fetal distress in labour (odds ratio 0.81, 95% confidence interval 0.59 to 1.13) or caesarean delivery (odds ratio 0.94, 95% 0.82 to 1.06). [13] The reviewers also concluded that there is insufficient evidence so far, for the use of Doppler in an unselected population.

Fetal Heart Monitoring

Fetal heart rate assessment is commonly performed by intermittent auscultation and cardiotocography, or CTG. More recently, ECG analysis is also being explored as a method of fetal heart assessment is done in labour.

Intermittent auscultation

Intermittent auscultation is a test where a stethoscope or a Doppler ultrasound device is used to assess the fetal heart rate at predetermined intervals. It can accurately assess the fetal heart rate at that defined time of auscultation and it is perceived to help reassure the patient. The RANZCOG guidelines recommend the use of intermittent auscultation, preferable with a Doppler device on speaker mode, as a minimum for all women with a low risk for development of compromise at the onset of labour, every 30 minutes during the first stage of labour, and every 5 minutes in the active stage in the absence of pushing and after every contraction in the presence of active pushing [16].

One of the major limitations of this method is that it does not allow for assessment of beat-to-beat variability or periodic changes and evidence suggests that it adds no predictive value besides the knowledge of the fact that the fetus is alive [54]. Furthermore, the women apparently find the pressure exerted by intermittent auscultation quite uncomfortable [55].

Findings on auscultation suggesting abnormalities of heart rate such as bradycardia, (decrease in heart rate less than 110 beats per minute), tachycardia (increase in heart rate above 160 beats per minute) or an audible deceleration of heart beat prompts further evaluation of the fetal heart electronically using CTG.

CTG

CTG is a test to electronically monitor the fetal heart intermittently or continuously and identify changes in heart rate and heart beat patterns as well as their temporal relationship with uterine contractions. CTG is now accepted as a standard method of fetal heart assessment in high risk pregnancies in most of obstetrics units antenatally as well as in labour. It is performed with a CTG machine, either externally, with the Doppler probe strapped to the woman's abdomen, or internally, where the electrode is

applied to the fetal scalp in labour. The heart rate and pattern is documented as a trace on paper.

Accurate interpretation of CTG and identification of a normal heart rate and heart pattern seen on intrapartum CTG is reassuring to the clinician as it suggests a better outcome for the neonate, whereas ominous fetal trace patterns suggest that intervention is necessary. A systematic Cochrane review of twelve trials, which included two trials of high quality, revealed that continuous CTG during labour is associated with a reduction in neonatal seizures, but no significant differences in cerebral palsy, infant mortality or other standard measures of neonatal well-being, while the use of CTG is associated with an increase in caesarean births and instrumental birth [17]. CTG also has a low positive predictive value for prediction of acidaemia or adverse neurological outcome [22].

The RANZCOG clinical guidelines group concluded that CTG visual interpretation is subjective and therefore has its shortcomings. It further recommended that institutions undertaking antepartum care must ensure that the clinicians have adequate understanding of the pathophysiology, must demonstrate competence in interpretation of the CTG tracings and that the CTG machines have to be standardised [16]. In the presence of any factors suggesting fetal compromise, continuous CTG has been recommended, or intervention in labour be considered, as the case may be. The subjective nature of interpretation with a wide inter-observer variation between midwives and doctors [53, 58] has been of concern and attempts have been made to standardise terminology for clinical analysis with the help of computerised programs [56].

Currently, a systematic review of all randomised trials is underway to compare admission CTG with intermittent auscultation of the heart rate on maternal and infant outcomes [57]. Electronic heart monitoring does not identify the cause of abnormality of heart tracings, such as congenital abnormalities, cardiac arrhythmias or cardiac dysfunction due to placental insufficiency. As an adjunct to CTG, further developments in fetal cardiac monitoring technology have led to assessment of fetal cardiac function with fetal ECG.

Fetal ECG

Fetal ECG is a method to evaluate electrical activity of fetal heart and is performed with an electrode attached to fetal head through maternal cervix in labour. A change in the PQRST complex with an elevation of the ST segment has been observed in hypoxaemia. A systematic review of four randomised clinical trials including 9829 women comparing fetal ECG to electronic fetal monitoring suggests that an adjunctive use of ST segment analysis in labour was associated with fewer babies with metabolic acidosis at birth and fewer babies with neonatal encephalopathy although there was no difference in the caesarean section rate, APGAR scores less than 7 at five minutes or number of admissions to special care units [18]. The reviewers concluded that the advantages have to be weighed against the disadvantages of using an internal electrode after the membranes have been ruptured.

This method is still investigational and is not yet used in clinical practice in Australia. Other investigational modalities such as pulse oximetry have been investigated to improve the detection of fetal stress in fetuses with non-reassuring CTG traces.

Pulse oximetry

Pulse oximetry is a method being investigated to improve detection of fetal compromise and monitor fetal acid-base status directly. It is performed by placing a probe on the fetal scalp, temple or cheek within the maternal cervix after the membranes are ruptured.

An analysis of five published trials including 7424 pregnancies comparing fetal pulse oximetry and CTG with CTG alone did not show any difference in caesarean section rate or any difference in maternal or newborn outcomes [20]. However, an addition of pulse oximetry to CTG did show a reduction in the number of caesareans in those pregnancies where the fetuses were already suspected to be compromised. Further trials are needed before it can be recommended in routine intrapartum monitoring, especially to evaluate adverse outcomes, especially in relation to falsely reassuring data [21]. Additional tests for fetal wellbeing in labour include more invasive tests such as fetal blood sampling, fetal lactate, fetal vibroacoustic stimulation and near-red infrared spectroscopy.

Fetal Blood Sampling (FBS)

Saling pioneered the technique of FBS in the 1960s, where 30 to 50 μl of fetal scalp blood is sampled to evaluate the actual scalp pH. FBS remains the standard for further investigating-non-reassuring CTG traces. However, FBS has its limitations, as it is invasive, difficult to perform in labour, requiring the membranes to be ruptured, fetal presenting part to be easily accessible and provides information regarding the fetal condition at one moment in time [58], necessitating repeated invasive measurements to make it more reliable. Currently, a randomised trial is underway in the Netherlands, to compare the role of CTG plus ST segment analysis with CTG plus FBS, and will also evaluate the role of FBS in intrapartum assessment [24].

Fetal lactate

Fetal lactate measurements are performed using smaller samples, as little as 5 μl , to evaluate fetal compromise. Animal studies [59] and observational studies in the fetus [60] seem to suggest that fetal lactate measurements are good predictors of fetal asphyxia. A recent Swedish RCT demonstrated that there were no significant differences in rate of acidaemia at birth after use of lactate analysis or pH analysis of fetal scalp blood samples to determine hypoxia during labour [61]. A systematic review of fetal lactate is now being conducted to ensure the appropriate use of this test in clinical practice [25].

Vibro-acoustic stimulation test (VAST)

Fetal VAST is a test where the fetus is stimulated with a predetermined level of sound for a few seconds with the help of a device placed on the maternal abdomen. A demonstration of the startle reflex in the fetus with an acceleration of the fetal heart rate is assumed to be a sign of fetal wellbeing. A systematic review of nine trials including 4838 women suggests that VAST offers benefits by decreasing the incidence of non-reactive cardiotocography and reducing the testing time [15]. The Cochrane reviewers, however, concluded that there are no randomised trials and therefore there is insufficient evidence to recommend the use of VAST as a test of fetal wellbeing in labour [62] .

Near red infraspectroscopy (NIRS)

NIRS is a method of assessing cerebral haemodynamics, cerebral tissue oxygenation and perfusion in the fetal brain by the use of 'near infrared' light. 750 to 1000 nm of

light is transmitted through a fibre optic cable into the fetal head through the maternal cervix after rupture of membranes and a spectrophotometric analysis of fetal haemoglobin (Hb), oxyhaemoglobin (HbO₂), deoxyhaemoglobin (dHb) cerebral oxygen saturation and cerebral flow volume. However, there is insufficient evidence to recommend use of this technique in clinical practice [26]. One observational study suggested normal changes in Hb and HbO₂ where, in fact, the fetus was non viable [63].

Summary

The primary objective of antenatal assessment and monitoring is to ensure wellbeing of the fetus and the mother. There are many tests in clinical practice to assess fetal wellbeing and identify a small fetus antenatally as well as in labour. Some tests are still at an investigational stage. Clinical tests such as ultrasound and Doppler have been widely accepted in clinical practice. Serial ultrasound can identify a slowing or cessation of growth. Doppler ultrasound is useful in evaluating uteroplacental and fetoplacental haemodynamics in uteroplacental insufficiency (UPI). Evidence, as summarised in table 1, suggests that Doppler ultrasound is useful in monitoring high risk pregnancies in a compromised fetus; however, attempts to identify early stages of compromise with the existing tests have been disappointing. In particular, there still remains a lack of data in literature regarding tests before 24 weeks of pregnancy to enable early identification of fetuses at risk for fetal compromise, before overt manifestation of IUGR.

Evidence, as summarised in table 1, suggests that Doppler monitoring of high risk pregnancy in high risk pregnancies can improve the perinatal outcome. However, early changes in fetal hypoxia are poorly defined and none of the existing Doppler and ultrasound markers has been clinically useful as predictors of adverse maternal or fetal outcome. It would be clinically useful to identify the early changes occurring in the fetal and maternal vascular haemodynamics before fetal compromise has an adverse effect on the fetus. This research attempts to address this gap in knowledge and explore early changes in Doppler parameters in both normal and compromised fetuses, as well as test their usefulness as predictors in adverse outcomes.

Outline of the thesis

The general aim of this thesis is to explore early ultrasound and Doppler changes in maternal-fetal haemodynamics in UPI.

Chapter 1 has discussed the existing tests for fetal wellbeing and the evidence obtained from clinical trials and has highlighted the gaps in knowledge, regarding the early diagnosis of fetal compromise.

Chapter 2 is a review of ultrasound and Doppler particularly with reference to antenatal assessment of maternal and fetal circulation, as Doppler has evolved as a useful clinical tool in assessment of UPI. A brief discussion of all the longitudinal Doppler studies in IUGR has been included, which places the current thesis in appropriate historical context. This is followed by a discussion of common concepts in fetal cardiovascular physiology as well as a focus on intrauterine shunts in fetal circulation.

In Chapter 3, the rationale for the research is discussed, followed by the aims and hypotheses and research methodology, including statistical methods.

Four prospective longitudinal case-cohort studies were undertaken.

Chapter 4 describes the results of Study 1: Normograms of fetal central shunts and other parameters

Chapter 5 provides the results of Study 2: Fetal shunts and acute adaptive mechanisms- haemodynamics before and after intra-uterine transfusion (IUT).

Chapter 6 demonstrates the results of Study 3, a study of fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in UPI

Chapter 7 includes the observations of Study 4, a study of fetal and maternal Doppler flow haemodynamics – a correlation with adverse clinical and placental outcomes.

Chapter 8 is the concluding chapter with a discussion of results and implications for future research and clinical practice.

CHAPTER 2

Evaluation of the fetal circulation: A literature review

(2.1) Doppler ultrasound of maternal -fetal circulation in fetal hypoxia

Introduction

Doppler surveillance has emerged as a useful tool in the diagnosis of circulatory abnormalities in clinical practice. Doppler effect was first described by Christian Doppler [53] and is defined as a perceived change in frequency of the sound wave with relative motion between the sound source and observer. With the rapid advancement in ultrasound and Doppler technology, it has now become possible to non-invasively evaluate the status of the maternal and fetal circulation. Colour flow mapping, or colour Doppler can identify a specific vessel and demonstrate changes in the colour flow direction and average flow velocity by colour coding. A change in flow channels and hues, also known as aliasing, can also denote pathology within that vessel or the organ it supplies. Once the vessel is mapped with colour Doppler, pulsed flow Doppler evaluation is performed.

Pulsed Doppler is performed by using a sampling gate, once the specific vessel and sampling site is chosen. An ultrasound beam is then used to insonate that vessel, using an optimum frequency. The frequency shift, that is, the difference between the source frequency and the received frequency, is displayed as a graph, with velocities in cardiac systole and diastole, being displayed over time. This graph is known as the spectral flow velocity waveform and used to interpret the vascular impedance within the organ, resistance in the vessel, as well as flow velocity changes proximal or distal to the vessel. The velocity in the vessel is inversely proportional to downstream impedance. (Figure 1). Therefore the frequency shift, is directly dependent on the velocity of flow in the vessels as well as directly proportional to the cosine of the angle the beam makes with the blood vessel

In cases where the vessels are tortuous, using absolute velocities as a measure of flow changes in the vessel may be erroneous. Therefore, in clinical practice, to avoid errors, different qualitative and quantitative methods are used to interpret a waveform.

NOTE:

This figure is included on page 21 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1 The Doppler effect

Source: http://www.centrus.com.br/DiplomaFMF/SeriesFMF/doppler/capitulos-html/chapter_01.htm accessed on 10th May 2007.

Doppler Interpretation: qualitative and quantitative methods of evaluation

Different arterial and venous vessels have been examined and Doppler indices developed to interpret impedance and resistance offered by the regional circulation being examined. These indices are described below (Figure 2).

Arterial Doppler Indices

The arterial Doppler indices and parameters commonly used include RI (Resistance Index), PI (Pulsatility Index), S/D ratio (Systolic/Diastolic velocity ratio), PSV (Peak Systolic Velocity), EDV (End-Diastolic Velocity), however, there is no consensus as to which is the best parameter to evaluate the respective vessel, as none of the indices provide continual description of flow in all circumstances.

Pulsatile flow requires averaging over several cardiac cycles in order to get a mean flow. Pulsatility Index is expressed as $\text{Systolic flow} - \text{Diastolic flow} / \text{Mean Velocity}$, the mean velocity being Time averaged maximum velocity over the cardiac cycle.

Mathematical models of flow in haemodynamics have been constructed and described to explain laminar flow profiles as well as complex and dynamic flows.

Flow PI have been shown to be sensitive to impedance to pulsatile flows as well as pressure pulsatility, in addition to the vascularity of the organ [64]. Flow PI has been further defined by Adamson [65] as

$$\text{flow PI} = \text{Pressure PI} \times \frac{\text{Resistance}}{\text{Fundamental Impedance}}$$

Fundamental Impedance

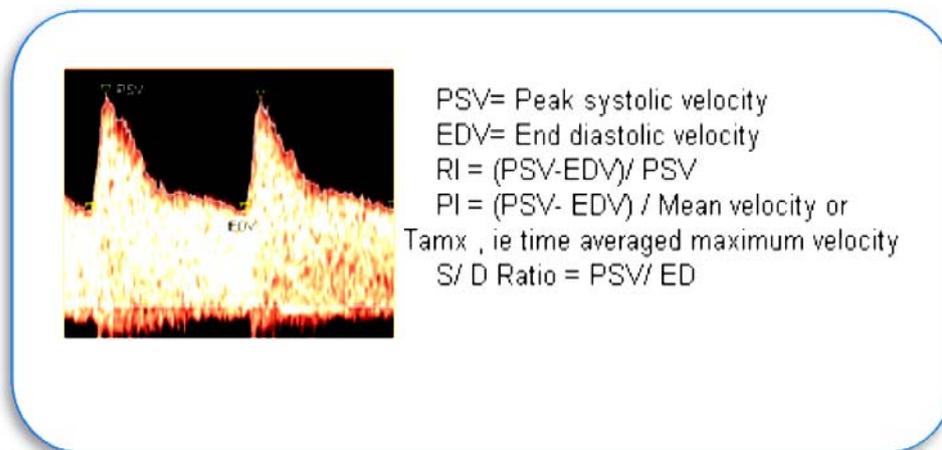


Figure 2 Arterial Doppler waveform

Venous Doppler Indices

Different venous vessels have been investigated and Doppler indices have been described. The central veins are close to the heart, and therefore reflect pulsatile flow of blood returning to the heart. These include the hepatic veins, ductus venosus, inferior vena cava, superior vena cava and pulmonary veins.

The venous Doppler indices also evaluate the pulsatility of the flow velocity waveform. (Figure 3)

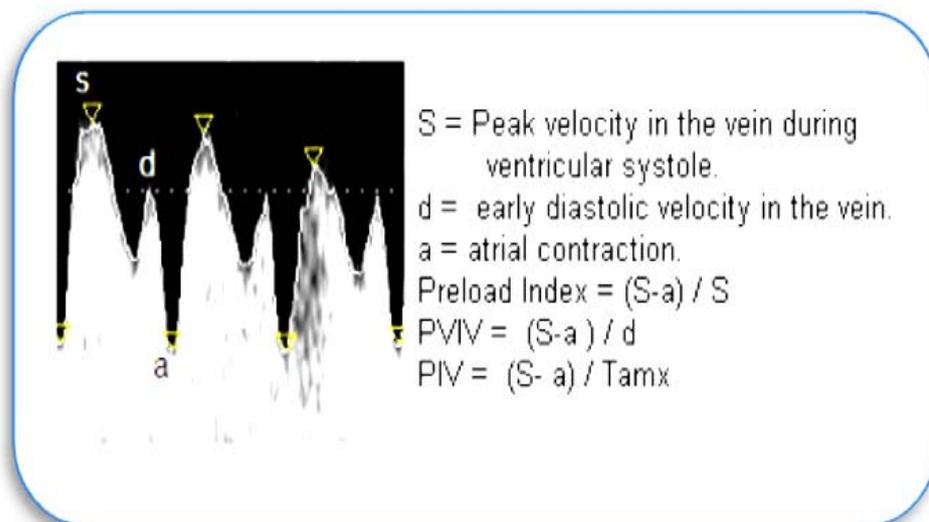


Figure 3 Venous Doppler waveform

Fetal circulation

NOTE:

This figure is included on page 24 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4 Fetal circulation

©2007 UpToDate® www.uptodate.com accessed on March 28, 2007

Fetal circulation is different from neonatal circulation because the oxygenation and nutrient exchange in the fetus is carried out in the placenta, as the lungs do not have a role in oxygen-nutrient exchange. There are three intrauterine shunts, viz. the foramen ovale, the ductus venosus and the ductus arteriosus, which close after birth.

Classical fetal lamb experiments using techniques of fetal exteriorisation [66] , radionucleotide labelled microsphere studies [67], and chronic arterial and venous catheterisation with pressure/flow catheters in the 1960s by Dawes and colleagues [68, 69], described the direction and distribution of blood flow in the fetus and ascertained that the right and left side of the fetal heart works in parallel. In the last decade, Doppler studies in the human fetus have confirmed the same [70]. Blood flow through the fetus occurs via two pathways – where almost 60 % oxygenated flow from the placenta is shunted through the ductus venosus into the inferior vena cava through the foramen ovale via the left sided pathway into the left ventricle, perfusing the upper part of the body (Figure 4). The remaining 40 % of the oxygenated flow passes through the portal vein, combines with the flows within the hepatic veins and follows the right-sided pathway through the right ventricle into the ductus arteriosus and thence to the descending aorta, supplying the lower half of the body [71]. Deoxygenated blood returns to the placenta via the umbilical arteries arising from the internal iliac arteries near the fetal bladder.

Fetal arterial circulation in UPI

Fetoplacental circulation evaluation with Umbilical artery Doppler

Umbilical artery was the first vessel to be evaluated with Doppler ultrasound in the early 1970s described by Fitzgerald [72] and is the easiest vessel to image. In uncomplicated pregnancies, the umbilical artery shows good diastolic flows with decreasing impedance with advancing gestation. This reduced impedance has been attributed to an increase in vascularisation of the placenta in late gestation as well as decrease in placental resistance (Figure 5). The umbilical artery is thought to represent this increased vascularity and downstream resistance [65]. Umbilical artery Doppler has been shown to be clinically useful to evaluate placental compromise [13].

NOTE:

This figure is included on page 26 of the print copy of the thesis held in the University of Adelaide Library.

Figure 5 Fetoplacental circulation

©2007 UpToDate® www.uptodate.com accessed on March 28, 2007

Normal flow pattern with low impedance in placenta is reflected as an increased diastolic flow (fig 2.6) whereas increasing resistance of flow is seen with increasing placental resistance, progressing to absent end diastolic flows and even reverse end diastolic flows (Figure 6) in intrauterine growth restriction (IUGR) [73]. This increased impedance is associated with low birth weight, low Apgar score, low pH at birth and increased morbidity. The absent diastolic flow velocity waveform pattern (AEDV) has been associated with IUGR, severe preeclampsia hypertension [74], increased risk of fetal acidosis [75] and perinatal death.

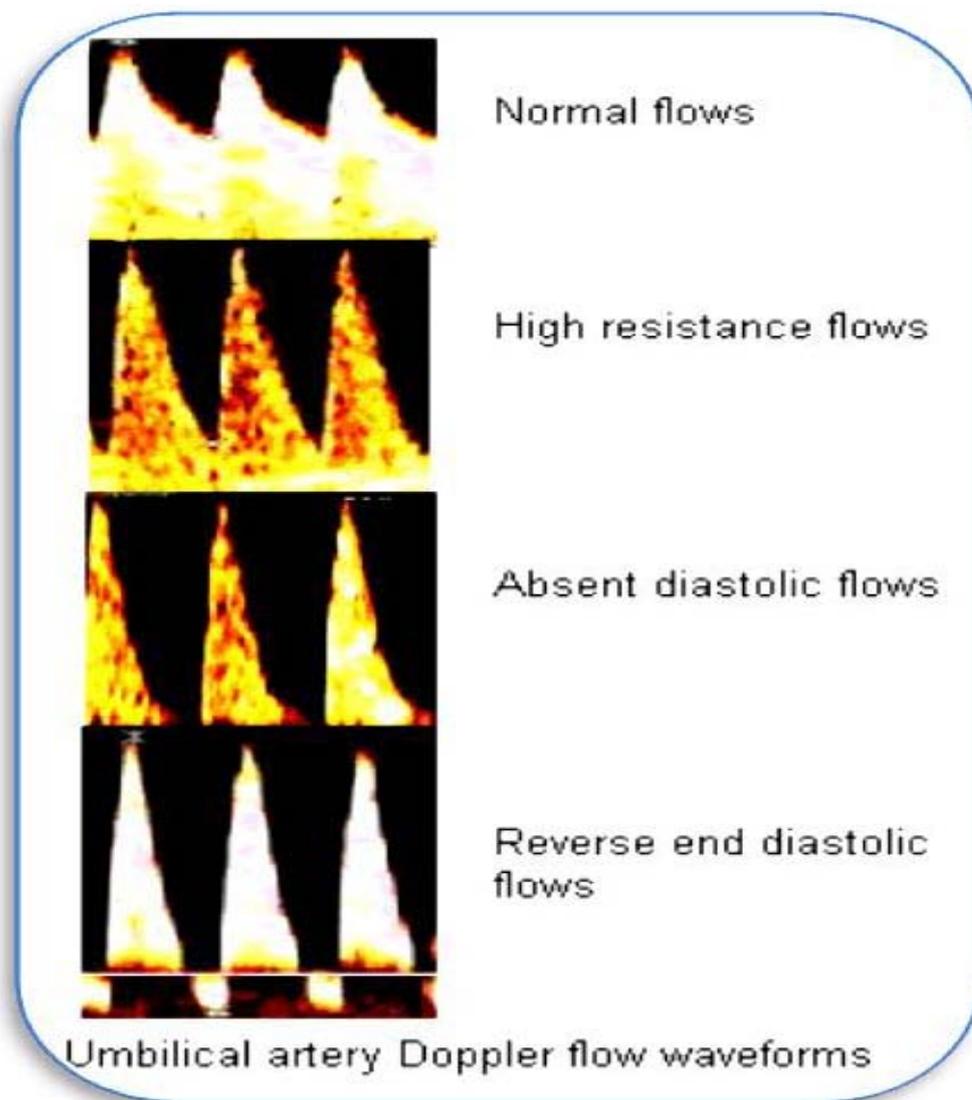


Figure 6 Umbilical artery Doppler

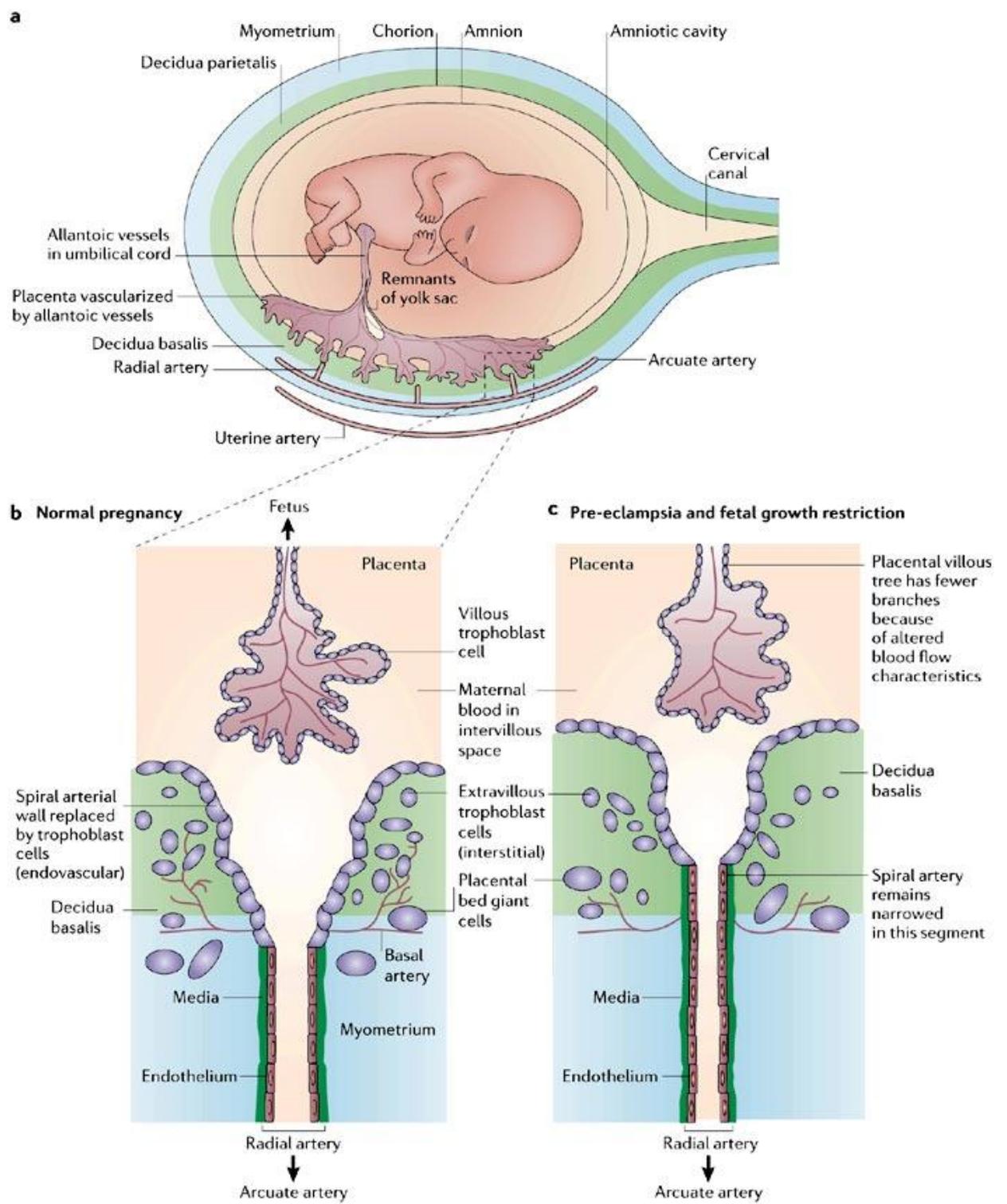
Umbilical artery screening has not been found useful as a predictor in development of complications in low-risk pregnancies [76], but has been found to be useful as a

predictor of adverse outcome and admission to NICU after 34 weeks [77]. Umbilical artery Doppler velocimetry has been evaluated extensively in randomised controlled trials and the Cochrane Pregnancy and Childbirth Group in Liverpool [13] concluded that among high-risk patients, Doppler was helpful in reducing perinatal mortality. Appropriate use of umbilical artery Doppler evaluation was associated with a significant reduction in the number of antenatal admissions and inductions of labour associated with Doppler use. A study comparing fetal heart-rate monitoring, biophysical profile and umbilical artery Doppler found that only umbilical artery Doppler had value in predicting poor perinatal outcomes in SGA fetuses [78].

High impedance in umbilical artery flow waveforms was first seen in placental vascular lesions [79]. Umbilical artery flow indices measure resistance to flow at placental level and depend upon the extent of placental disease mostly due to abnormalities in spiral arteries [80]. It has been demonstrated that 60% of the intraplacental fetal vasculature has to be occluded before significant changes in umbilical waveform occur [81]. Therefore, an abnormality detected in umbilical artery flows may be reflected late in the evolution of placental disease.

Uteroplacental circulation: Uterine artery Doppler

Uterine artery Doppler has shown promise in evaluating uteroplacental circulation in placental disease. During pregnancy, the uteroplacental flow increases almost 17 folds - from 40 ml/ minute in the follicular phase to 750 ml at term. This increase in flow is due to a decrease of resistance caused by invasion of cytotrophoblast into the spiral arteries [80, 82], as well as blood volume and cardiac output changes [83]. Cardiac output increases by 30 to 35%, with the uteroplacental circulation contributing to 25% of the total increase in maternal output [84]. In uncomplicated pregnancies, spiral arteries undergo a series of changes that convert them from small diameter, high resistance vessels into low-resistance vessels. Normal placental development involves good endovascular cytotrophoblastic invasion which has been deemed necessary for good pregnancy outcome (Figure 7). Histological studies of placentas in pregnancies with established preeclampsia and fetal growth restriction have often, but not always, shown a defective or superficial cytotrophoblast invasion [85]. This process starts with conception and stops by the end of 2nd trimester. This increasing flow through the placenta can be indirectly evaluated with uterine artery Doppler evaluation.



Copyright © 2006 Nature Publishing Group
 Nature Reviews | Immunology

Figure 7 Trophoblastic invasion

With permission from Nature publishing group doi:10.1038/nri1897 [86]

In the presence of normal cytotrophoblastic invasion, normal uterine artery Doppler waveforms are seen, which can be described as a low resistance pattern (Figure 8) with steadily increasing diastolic flows throughout pregnancy. In situations where trophoblastic invasion is inadequate, narrower, undistended uterine arteries will demonstrate a high resistance pattern (Figure 8). An increase in placental resistance may also be associated with an early diastolic notch, thought to be caused by a waveform interference between outgoing and reflected waves [87].

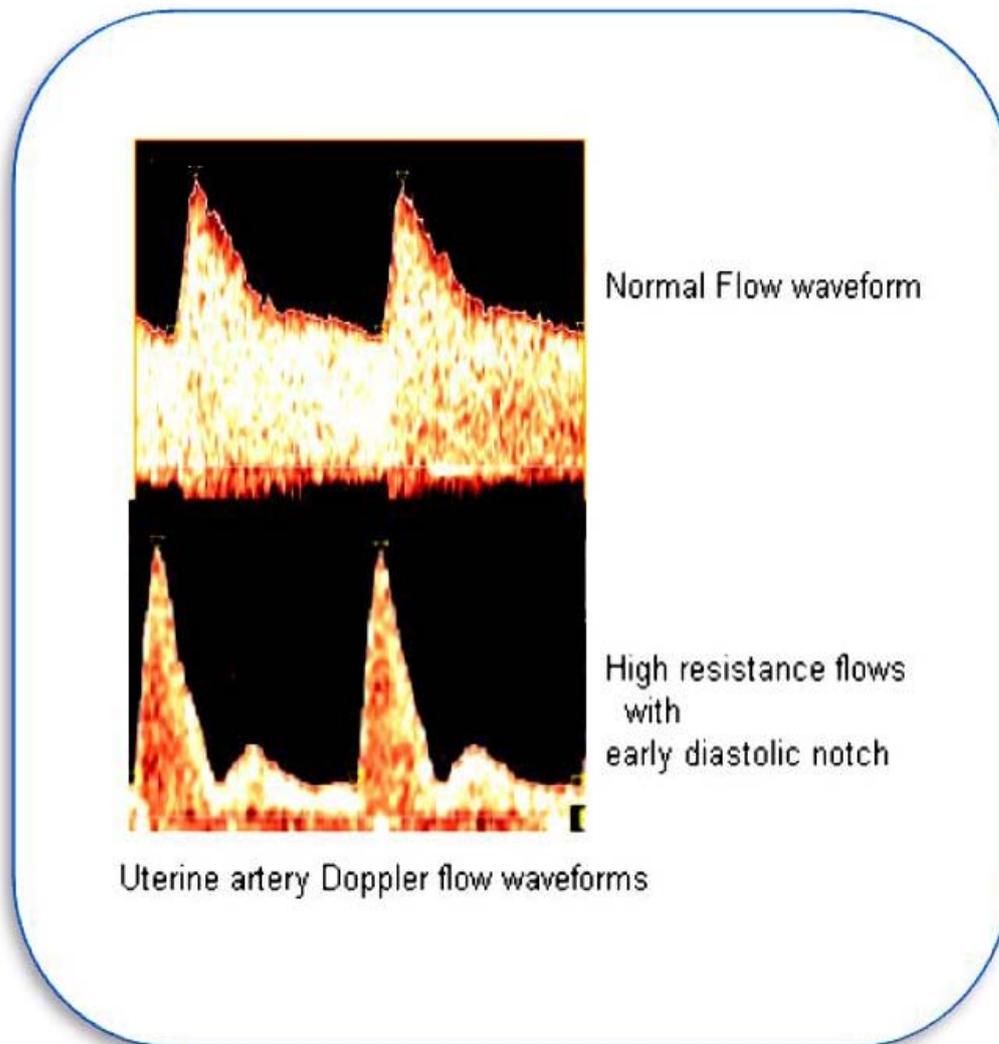


Figure 8 Uterine artery Doppler

There is a lack of randomised controlled trials evaluating the utility of uterine Doppler in evaluating complications such as preeclampsia and IUGR. In a meta-analysis of 27 clinical studies of uterine artery Doppler (UAD), Chien et al observed that UAD had a limited role in low risk women [88] although these data showed that the risk for uteroplacental insufficiency (UPI) was about 6 times higher when compared to patients with normal uteroplacental Doppler results. A review of 19 studies [89]

demonstrated that UAD can be useful in identifying women at risk for developing complications of UPI and may help in stratifying care. This review showed that abnormal UAD has a better predictive value in severe forms of the disease, with a likelihood ratio of 6 for preeclampsia, 3.7 for IUGR and 2.4 for perinatal death. Furthermore, the reviewers concluded that UAD could identify women in whom serum biochemical markers could be measured, to develop screening tests in pregnancy. The same authors in a recent review [90] concluded that UAD could predict almost two thirds of preterm stillbirths and a combination of UAD in the first trimester along with serum markers can potentially increase the detection rates of UPI related complications.

Cerebral circulation

Cerebral circulation has been extensively researched in the high- risk fetus. Middle cerebral artery (MCA) Doppler imaging has been the chosen cerebral artery for research as well as in clinical practice, as it is easy to image, hardly needs angle correction and reproducibility and repeatability have been well established [91]. In addition, the site of insonation has been chosen as proximal rather than mid region or distal region, as the proximal area is supposedly less influenced by behavioural patterns [92].

The MCA in the fetus normally has high resistance flows in early pregnancy and second trimester. Studies have found that there is a decrease in the impedance after 30 weeks of gestation [93, 94]. This could be probably due to increased oxygen demands and metabolism in the third trimester [93]. It has now been established that the fetus at risk for hypoxia preserves the oxygen supply to its brain by increasing blood flow; a phenomenon called ‘brain sparing’.

Brain sparing was first described by Saling in 1966 and has subsequently been demonstrated in human fetuses as well [95]. In a growth restricted fetus, there is redistribution of cardiac output in favour of the critical organs such as the heart, brain and adrenals, all showing vasodilation, leading to an increase in diastolic flow and a progressive reduction in PI in the MCA Doppler waveforms, (Figure 9) also called the ‘brain-sparing effect’ [96, 97].

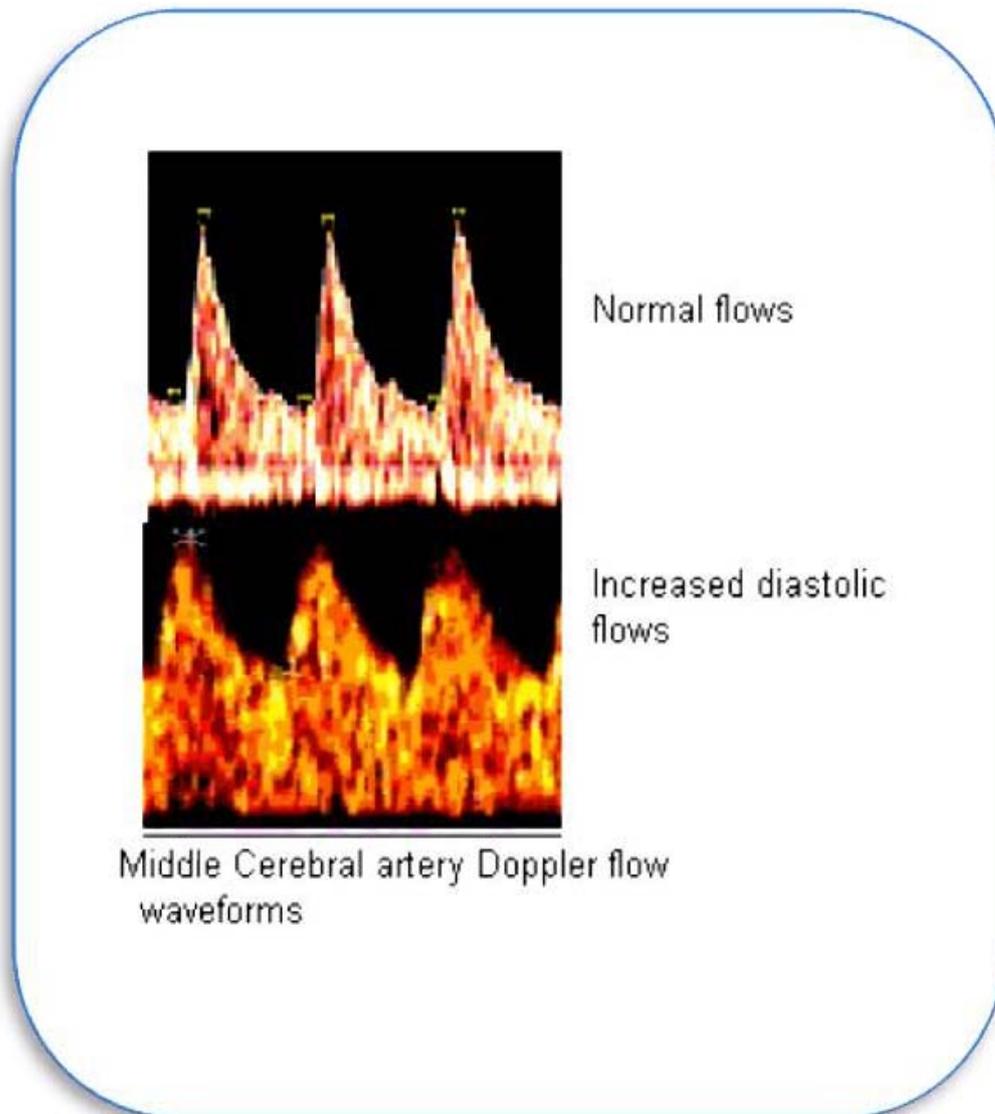


Figure 9 Middle Cerebral artery Doppler

When the fetus has become too compromised to compensate for the hypoxia any longer, the brain sparing disappears. The increased diastolic flows start diminishing, progressing to high resistance flows, absent diastolic and then reverse diastolic flows, usually associated with abnormalities in the CTG tracing [98-100], which could be an ominous sign for impending fetal demise [101]. It has been suggested that this brain sparing is brought about by autoregulation in regional circulation. The commonest conditions where cerebral evaluation is performed for brain-sparing in the fetus include fetal anaemia and IUGR.

However, it has been demonstrated that this ‘brain-sparing’ could be transitory and there is a ‘normalization’ of cerebral flows in severely hypoxic fetuses probably due to cerebral oedema, cardiac insufficiency or failure of cerebral auto regulation [93, 102]. There has been no significant association demonstrated between signs of ‘brain sparing’ and perinatal outcome [103] to support the clinical experience. MCA has emerged as a useful and sensitive predictive tool in fetal anaemia [104]. Anaemic fetuses demonstrate an increase in peak systolic velocity, which dramatically decreases after intrauterine fetal transfusion [104] (Figure 10).

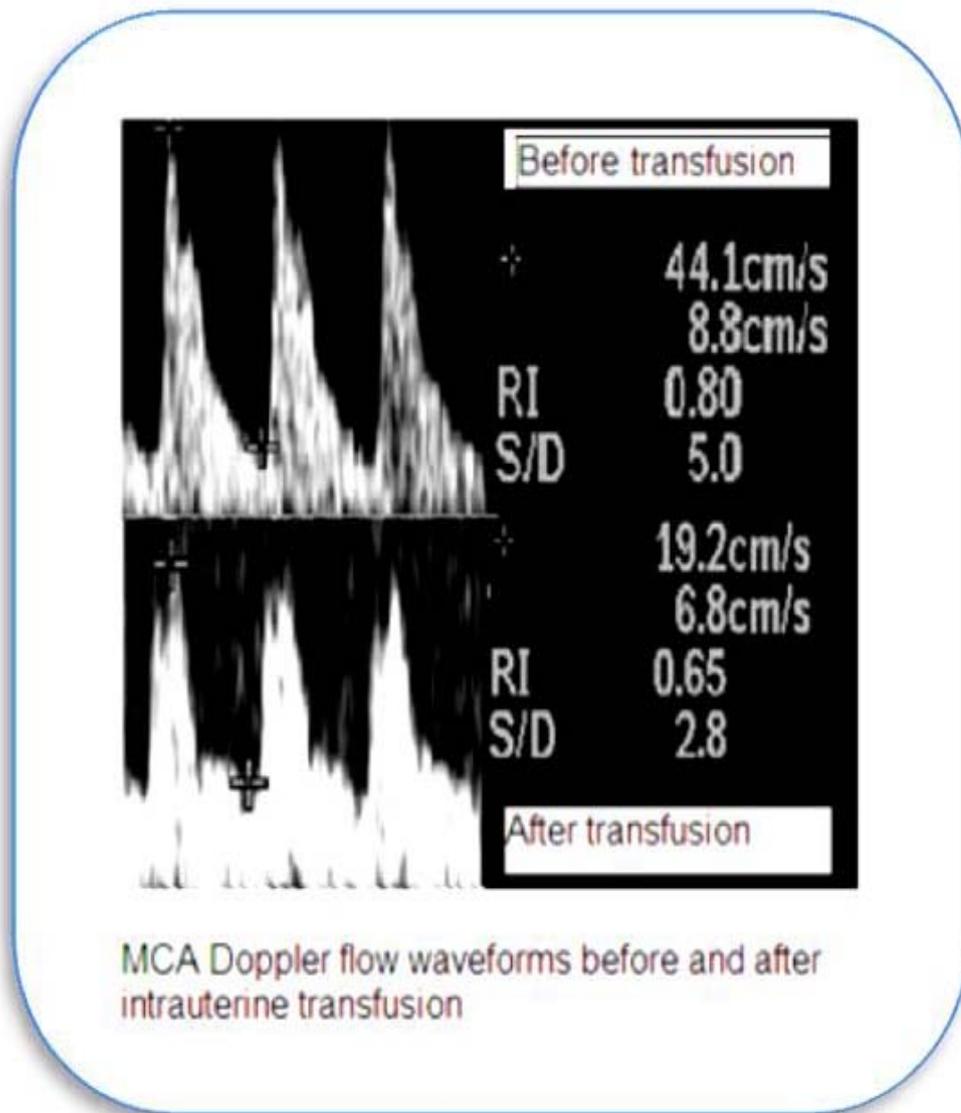


Figure 10 MCA demonstrating increased PSV in fetal anaemia, PSV reduced after intrauterine transfusion.

MCA has also been utilised to evaluate an IUGR fetus [105]. MCA flows in a compromised fetus demonstrate an increase in EDV, followed by a reduction in MCA RI and PI, and with progressive fetal compromise, the peak systolic velocities also increase in IUGR [106]. It has also been observed that in severely compromised fetuses there may be decompensation and loss of autoregulation, associated with cerebral oedema, causing the RI and PI to rise again, therefore causing a 'pseudonormalisation' of RI and PI, which in fact could be a preterminal event [101]. Forouzan et al [107] reported that in the progressively compromised fetus, MCA flows become abnormal earlier in gestation than other parameters such as the biophysical profile, non-stress and contraction stress tests, used to monitor the status of the growth-restricted fetus.

Van den Wijngaard et al, in 1989, demonstrated that there was no difference in the flow velocity waveforms obtained from anterior cerebral arteries, posterior cerebral, middle cerebral and internal carotid arteries in IUGR, suggesting that all the major intracranial arteries show similar changes in fetal hypoxia [108]. Cerebellar arteries have also been investigated and have shown similar brain sparing effect [109]. Other cerebral arteries included the anterior cerebral artery, the pericallosal artery and the posterior cerebral artery, although it has been suggested that there are minor differences in regional circulation with redistribution [110]. Baschat et al [111] evaluated the role of cerebral flows in predicting intraventricular haemorrhage and had specific definitions for 'brain sparing', 'centralisation' of flow and 'redistribution of flow'. 'Brain sparing' was defined as MCA PI more than 2 SD below the gestational age mean, 'centralization' was defined as the ratio of MCA /umbilical artery pulsatility indices (cerebroplacental ratio) more than two 2 SD below the gestational age mean, and 'redistribution' was considered to be absent or reversed umbilical artery end-diastolic velocity. Baschat et al found no correlation between brain sparing or centralisation and development of IVH although they found an increased association of absent/reversed end diastolic velocity in the umbilical artery of the IUGR fetus. Brain sparing has been suggested as a protective mechanism in reduction of severity of brain injury in growth restricted children [112]. However, in a study of 23 full term newborns, Kirimi et al [113] found that brain-sparing effect did have a significant correlation with the development of perinatal asphyxia and hypoxic ischemic encephalopathy (HIE) in the first 12 hours of their lives. Therefore, the role of cerebral flow Doppler needs to be investigated further with large sample sizes, to

confirm or refute the 'brain sparing effect' as a possible predictor or a protective mechanism against severe brain damage.

Brain sparing has further been evaluated by evaluating ratios between different vessels. MCA Doppler was further combined with umbilical artery Doppler to arrive at the so-called cerebroplacental ratio [114]. Several studies have been performed to compare cerebral and placental resistance [111, 114]. This has been thought to help in identifying placental disease and then evaluating the extent of haemodynamic consequences.

Cerebroplacental ratio (CPR)

CPR has been constructed using different vessels such as cerebral, carotid and descending aorta [115] and umbilical arteries [116]. More recently, CPR has been constructed using the ratio of MCA to UA PI or RI. It has the potential advantage of evaluation of increasing placental resistance as well as the redistribution of cerebral flows with MCA Doppler flow waveforms. In lamb fetuses, the CPR closely reflected changes in PO₂ [117]. This ratio is thought to evaluate fetal haemodynamics and has been shown to be better in detecting fetal adaptation and compromise when compared with umbilical artery or MCA alone [77, 114, 118], even when umbilical flows are in the normal range [119]. It has therefore been considered superior to umbilical Doppler pulsatility in predicting adverse fetal outcome [120]. CPR values below the threshold of 1.08, or, alternatively, below the 5th centile for gestational age, has been considered a good predictor of adverse perinatal outcomes in growth-restricted fetuses [121]. Abnormal fetoplacental flow and CPR have not only shown to correlate with higher mortality and morbidity but also abnormal cognitive development in an age range of 3 to 6 years [122].

Other arterial vessels

Other arterial vessels have been investigated in IUGR, such as internal carotid [123], renal [124] and adrenal arteries [125], mesenteric [126, 127], hepatic [128] and peripheral arteries such as femoral [129] and internal iliac artery [130]. These studies have demonstrated effects of preferential sparing, where there was lowered resistance in adrenals and internal carotid arteries, and increased resistance in splanchnic, renal and other peripheral arteries, but have not proved useful as predictors of adverse pregnancy outcomes.

Fetal venous circulation in IUGR

In the presence of abnormal flow patterns in umbilical arteries, Doppler assessment of fetal venous circulation has been found useful to establish the severity of fetal compromise. Fetal venous Doppler waveforms reflect changes in fetal central venous pressure and the events occurring in the fetal atria, especially during ventricular systole and the opening and closing of atrioventricular valves.

In uncomplicated pregnancies, fetal veins closer to the heart, such as the IVC, Ductus venosus, SVC and hepatic veins demonstrate a pulsatile waveform whereas those veins further away from the heart, such as the umbilical vein, as well as veins in the portal circulation demonstrate a continuous, non pulsative wave flow pattern in the normal pregnancies. Significant alterations in fetal venous haemodynamics have been observed in compromised fetuses and have been shown to correlate with abnormalities in CTG [131, 132] and hypoxaemia. The abnormal waveforms in Ductus venosus are depicted as an increased pulsatility of waveform, absent 'a' wave and even a reversal of 'a' wave (Figure 11).

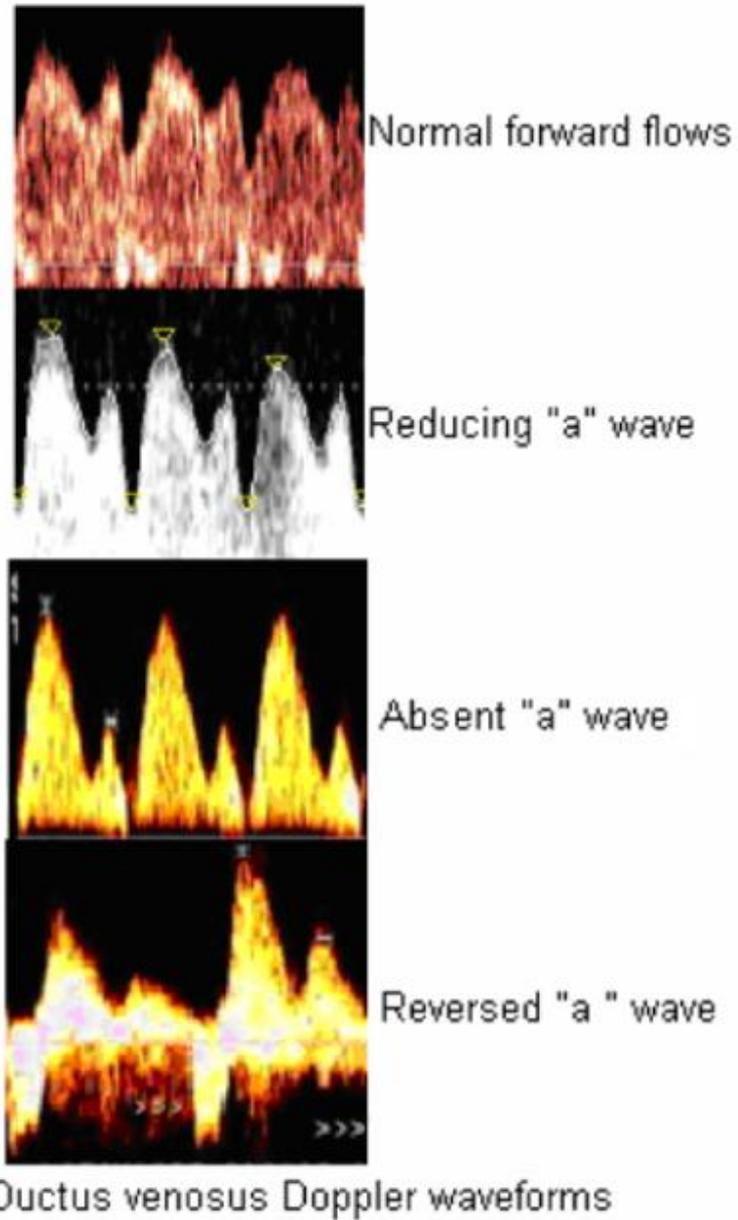


Figure 11 Ductus venosus Doppler waveform

Increased shunting has been observed through DV in hypoxaemia and IUGR [133]. The DV and IVC have shown an increased pulsatility [134], when there was

AEDV/REDV in umbilical artery, suggesting severe compromise [135]. This increase in venous pulsatility has been attributed to augmented atrial contraction with myocardial dysfunction. Late changes in venous waveforms have been documented in progressive fetal compromise, which correlate with the degree of acidaemia [136] and even perinatal death [137].

Reversed flow in IVC has been demonstrated to be associated with acidaemia and adverse perinatal outcome [135, 138]. In a study on fetuses with absent end diastolic velocities in umbilical artery, a reciprocal shift was seen between the IVC and SVC velocity waveforms characterized by a flow profile in the IVC which resembles that of a normal SVC profile and vice versa. These changes have been thought to be additional parameters reflecting fetal blood flow redistribution as a response to hypoxia [139].

Umbilical vein flows are normally continuous flows, while pulsatile flows have been seen in severely compromised fetuses [140]. This has been attributed to cardiac decompensation, reflected in reverse flows from ductus venosus and IVC extending to umbilical veins.

Anecdotal evidence and evidence from observational studies suggests that appearance of abnormalities in venous parameters indicates that the fetus is severely compromised and therefore should warrant timely intervention by the obstetrician [141]. A large randomised trial (TRUFFLE trial) is underway to determine if DV waveforms can be utilised as a parameter to decide the optimum time of delivery of a compromised fetus[3].

Fetal cardiac Doppler in IUGR

IUGR is associated with several changes in fetal cardiac waveforms. Severe uteroplacental insufficiency leads to a redistribution of fetal cardiac output in favour of the left ventricle [142]. Progressing increase in right ventricle afterload leads to progressive cardiac dilatation and cardiac decompensation and can lead to cardiomegaly and tricuspid regurgitation [143]. Other cardiac changes observed have been a reduction in peak systolic velocities in aortic valves, pulmonary valves,

increase in tricuspid and pulmonary volume flows, dilatation of the coronary arteries [144] and a reversal of flow through the aortic isthmus [123, 145-150].

Fetal cardiovascular circulation in relation to fetal shunts is discussed in further detail in the next section.

Longitudinal studies in IUGR: Temporal sequence of events in progressive fetal compromise in UPI

Serial ultrasound and Doppler evaluation of the fetus enable the clinician to optimise the management in high-risk pregnancies. This has been made possible by dedicated work by numerous researchers in this field. A literature search was conducted to find longitudinal studies evaluating the sequence of haemodynamic changes in UPI. This search was performed to determine what sequential changes occur in the fetus as a response to UPI and if there is any evidence that these changes are effective predictors of adverse perinatal outcome in IUGR.

A comprehensive database search for longitudinal studies in Doppler ultrasound and adverse pregnancy outcome, with the search terms, “ longitudinal studies , sequence of changes, Doppler ultrasound, preeclampsia, fetal growth restriction, adverse pregnancy outcome”, revealed seven landmark studies, the key findings in the major studies has been summarised in the table below (Table 3).

This literature search showed that, so far, no Doppler or ultrasound study in clinical practice had been able to identify any early responses to UPI, i.e. any abnormal fetal flow patterns recognisable before IUGR set in. Most of the studies were aimed at identification of worsening parameters of fetal compromise, once IUGR was suspected in the fetus.

In established IUGR, several key changes in the ultrasound parameters were seen.

These results can be summarised as follows.

- A review of 60 studies [151] has shown that fetal abdominal circumference (AC) , especially with serial measurements [152], and estimated fetal weight (EFW)[152] have been the best indicator of birth weight < 10th centile .
- Early changes in UPI as observed so far, are a reduction in fetal size, which can be identified with a reduction in fetal AC [153], this is associated with an increasing resistance in Umbilical Doppler and a reduction in MCA resistance, and a reduction in CPR [118, 154, 155]. When this happens, the IUGR is by now clinically recognisable in the fetus.

- Further worsening of Doppler parameters in an IUGR fetus includes deterioration in cardiac function, which is seen as increased pulsatility in fetal venous waveforms [156], that may be leading to absent or even reversal in flows in the ductus venosus [137, 157]. This maybe associated with CTG changes as well as reduction in peak velocities through AV valves, pulmonary valves and aortic valves [137]. If there is no intervention, it may lead to tricuspid regurgitation, and fetal death[158]. However, again, these changes are observed in later changes of compromise.

Table 3 Longitudinal studies in evaluation of temporal sequence of events in UPI

Authors	Year	Reference	N	Parameters observed	Key findings
Soothill PW, Ajayi RA, Campbell S, Nicolaides KH. [159]	1993	BJOG Int J O & G, Volume 100 Issue 8 Page 742-745, August 1993	191	Fetal AC,CTG, BPP, and umbilical artery pulsatility index(CW)	AC --> predicts SGA. Umbilical Doppler PI -->predicts fetal morbidity. fetal heart rate variability and the biophysical profile score did not predict morbidity. no parameter was effective in predicting morbidity in AGA fetuses.
K. Harrington , M. O. Thompson , R. G. Carpenter , M. Nguyen TS. Campbell [118]	1999	BJOG 1999, 106' 453-466	292	The AC, Umb A PI , MCA PI and Tamx and the Thoracic aorta PI and Tamx.	Fetal AC-->Umbilical PI-->MCA PI-->Thoracic aorta PI and Tamx
Senat MV, Schwarzler P, Alcais A, Ville Y. [155]	2000	Ultrasound Obstet Gynecol 2000; 16: 19–24	75	Umb a, MCA, Descending Aorta, MCA, Transverse cerebral sinus, DV	Changes in cerebral transverse sinus waveforms correlated with DV and haemodynamic changes correlated with decreasing variability preceding distress.
Baschat AA, Gembruch U, Harman CR. [154]	2001	Ultrasound Obstet Gynecol 2001; 18: 571–77	236	BPP, Umbilical PI, MCA, IVC, Umbilical vein,	Sequential deterioration of arterial and venous flows usually preceded abnormal biophysical profile score.

Ferrazzi E, Bozzo M, Rigano S, Bellotti M, Morabito A, Pardi G, Bettaglia FC, Galan HL. [137]	2002	Ultrasound Obstet Gynecol	26	2002;19: 140–6	Umb a PI, AEDV, REDV, Middle cerebral artery (UA MCA PI) Ductus venosus Abnormal S/ a ratio; (DV S/a), reverse flow Pulmonary outflow tracts Abnormal peak velocity aortic outflow tracts abnormal peak velocity	60 % of fetuses with abnormal CTGs, did not have venous abnormalities or any other late changes such as changes in ao or pa flows.. Early changes --> peripheral vessels (umbilical and middle cerebral arteries; Late changes --> umbilical artery reverse flow, and abnormal changes in the ductus venosus reversed flows, reduced peak velocities aortic and pulmonary outflow tracts. late changes were significantly associated with perinatal death (P < 0.01).
Gardiner H, Brodzki J, Eriksson A, Marsál K [148]	2002	Ultrasound Med Biol.	20	2002 Sep;28(9):1107-13	longitudinal physiology and Ao pulse wave velocity in the thoracic descending aorta and IVC using an ultrasonic phase-locked echo-tracking system; brain-sparing defined as a MCA/UA PI ratio of < 1.08. M Mode through mitral valve-shortening fraction, Doppler through AV valves, pulmonary valves, aortic valves-‘e’ and ‘a’ waveforms, mean velocity time integrals (VTIs), including the ratio of flow velocity during the ‘a’ wave over the total	Growth restriction is associated with reduced aortic wall pulsations and lower mean blood flow velocities and pulse wave velocity in DAo secondary to increased placental impedance. In both age- and weight-matched analyses, the growth-restricted fetuses showed significantly reduced values reflecting the chronic fetal ventriculovascular and possible adaptive responses to increased placental impedance.

				VTI, acceleration and ejection times were measured	
Figueras, Puerto, 2003 Martinez, Cararach and Vanrell [158]	European Journal of Obstetrics & Gynecology and Reproductive Biology, Volume 110, Issue 2, 10 October 2003, Pages 159-163	22 IUGR	Pulsatility indices of fetal arterial and venous Doppler waveforms, systolic peak velocity in the aorta and pulmonary artery, right and left ventricular shortening fraction and atrioventricular flow E/A ratio were assessed at each monitoring session	Cardiac function monitoring of fetuses with growth restriction was performed. The authors found an earlier and more pronounced right than left and diastolic than systolic fetal cardiac function deterioration in growth restricted fetuses monitored longitudinally. Umbilical artery pulsatility index was the first variable to become abnormal, followed by the middle cerebral artery, right diastolic indices (right E/A, ductus venosus), right systolic indices and, finally, both diastolic and systolic left cardiac indices.	

All the changes as observed on serial fetal monitoring, have since been summarised by Baschat as follows [160] (Figure 12).

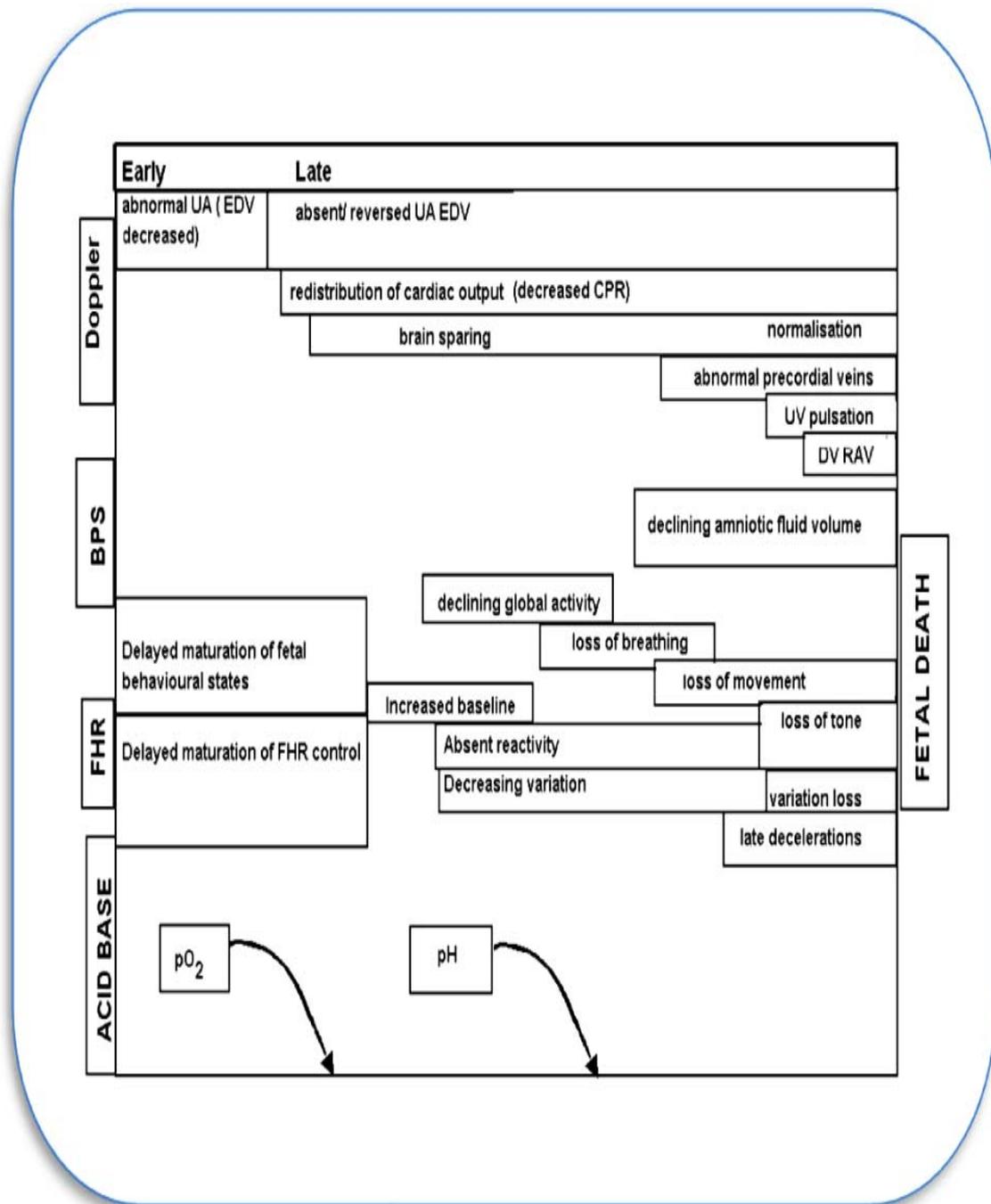


Figure 12 Summary of changes in fetal compromise as observed on fetal monitoring.

Source: Baschat., 2005 [160]

Doppler safety in clinical practice

Ultrasound scanning has now become increasingly routine in the management of low risk as well as high-risk pregnancies. However, it is necessary to address all concerns regarding the safety of ultrasound practice in obstetric care and to ensure that safe scanning practices are in place. This section discusses the position of the different ultrasound accreditation bodies worldwide regarding safety in obstetric scanning, as well as the guidelines to ensure safety for the fetus while performing diagnostic ultrasound.

Ultrasound scanning is achieved by insonating the tissue with sound waves, the reflection of which is then interpreted by the machine to an image on the screen.

Different bio-effects have been described, in relation to ultrasound and Doppler exams in the experimental scenario. These effects include tissue heating when ultrasound is absorbed by the tissue, cavitation effects being caused by gas bubbles in negative pressure, and mechanical effects due to radiation of streaming fluids [161]. However, these bioeffects have been induced in animals under experimental conditions, using ultrasound in much higher frequencies and for a longer time, than what is used in clinical practice.

In clinical practice, the ALARA principle is used, which is 'As Low As Reasonably Achievable' [162]. This implies that the ultrasound should be performed by using the minimum acoustic power necessary to perform the scan. The examiner must also be qualified to scan in the minimum time, and be trained to use the appropriate settings, i.e. the appropriate ultrasound frequency, the use of appropriate mode of scanning, i.e. B-Mode, Colour or Pulsed Doppler, scan and focus, as any changes in these technical specifications can easily alter the intensity setting. The ISUOG Safety statements [163, 164] provide details regarding the regulations in relation to the measurements output display, [165] where the output measurement is displayed as Thermal Index (TI) and Mechanical Index.(MI). The TI of 1 indicates a power leading to a temperature increase of 1⁰ C. With regard to thermal effects of ultrasound, experimental data has shown that in the scientific laboratory conditions, where the transducer and target are immobile and

there is little heat dispersion, bio-effects are seen, when the temperature increase of 5°C . for more than 5 minutes continuously [163, 164, 166]. However, in real-time scanning, heating to this magnitude is unlikely, especially in obstetrics, as the fetus is continuously moving, so is the examiner's hand with the transducer, as well as changing maternal position. In addition, the amniotic fluid helps dissipate the energy. Furthermore, the machine settings are usually set at an output (spatial peak temporal velocity) of $25\text{ mW} / \text{cm}^2$, most of the readings are in fact obtained at levels as low as $5\text{ mW} / \text{cm}^2$, which are well below the threshold level of $100\text{ mW} / \text{cm}^2$ level determined by the American Institute of Ultrasound in Medicine, below which no demonstrable adverse effects have been demonstrated in mammalian tissue [161, 162, 167]. Besides, most of the measurements can be done offline, thus restricting time of ultrasound exposure even further. Moreover, ultrasound education and accreditation guidelines have been pivotal in the use of the ALARA principle, with the operators now using less acoustic power and other image optimisation techniques beyond the scope of this chapter, to get optimal diagnostic information.

Ultrasound has been used in clinical practice for more than 40 years, and there has been no evidence of any deleterious effects on the embryo, fetus or neonate [164].

In 1993, results from a randomised controlled study by Newnham's group were published, in which pregnant women were randomly allocated to a protocol of five ultrasound and Doppler scans from 18 to 38 weeks of age, or, to a single scan at 18 weeks with further scans only if clinically indicated [168]. As a post-hoc finding, the results reported a small but statistically significant reduction in birth weight, by 30 grams, in those women who had repeated scanning [76]. The primary hypothesis of Newnham's study was to investigate if a protocol of multiple scans would improve pregnancy and did not address the issue of bio-effects as a primary hypothesis. However, the observation of a small reduction in fetal growth warranted further follow-up. Hence these babies were subsequently followed up at 1, 2, 3, 5 and 8 years of age and the results showed that the differences were no longer apparent in growth and development. There was no difference in the neurological outcome, in terms of speech, language, behaviour and neurological development [169]. This follow-up study therefore provided the reassurance that even if

repeated scans affected the growth minimally, subsequent growth and development in childhood is unaffected. Another potentially uncertain bioeffect is the possibility of non-right handedness, with a speculation of an extra five non right-handed boys in 100 male births [170]. An increase in right handedness was earlier thought to be due to effects on neuronal migration [171], but has since been debated as an occurrence due to pure chance [169]. Recently, non-right-handedness in babies born preterm with severe growth restricted, has also been argued to be a result of redistribution in the fetus, demonstrated by the fetus as a survival mechanism [172]. Currently, the 10 year follow-up is underway, in the same cohort of children described in the Newnham study above, to thoroughly investigate this debate of non right-handedness in babies who have undergone intensive scanning prenatally [169] .

Up until now, “Ultrasound has not been shown to cause harm in the fetus but prudent use should be encouraged; in the hands of a trained and accredited examiner, scans in pregnancy can be safely performed for medical reasons when the benefits outweigh risks, subject to complying with ALARA principle and output display standards”. The table below (Table 4) summarises all the statements and guidelines put forth by the different global accrediting bodies involved in quality assurance of practice in ultrasound.

Table 4 Statements on safety of ultrasound endorsed by accrediting bodies worldwide

ClinicalTask Force	Statements regarding risks and safety of ultrasound.
SOGC 2005 [173]	"Obstetric ultrasound should only be done for medical reasons, and exposure should be kept As Low As Reasonably Achievable because of the potential for tissue heating. Higher energy is of particular concern for Pulsed Doppler, colour flow, first trimester ultrasound with a long transvesical path, second or third trimester exams when bone is in the focal zone, as well as when scanning tissue in minimal perfusion (anembryonic) or in patients who are febrile. Operators can minimise risk by limiting the dwell time, limiting exposure to critical structures, and following equipment generated exposure information."
ISUOG 2000 reconfirmed 2003 [164]	"Based on evidence currently available, routine clinical scanning of every woman during pregnancy using real time B Mode imaging is not contraindicated. Spectral and colour Doppler may produce high intensities and routine examination by this modality is rarely indicated. In addition, because of high acoustic absorption by bone, the potential for heating adjacent tissues must also be kept in mind. Exposure time and acoustic output should be kept to the lowest levels consistent with obtaining diagnostic information and limited to medically indicated procedures, rather than for purely entertainment purposes."
ASUM 2000 [174]	"Care should be taken to ensure that the exams are performed prudently using the ALARA principle of applying the lowest acoustic output and dwell time consistent with that required to obtain the necessary diagnostic information."
BMUS 2000 [166]	"--diagnostic ultrasound can only be considered safe if used prudently".
CPG-Alberta Clinical Practice Guidelines 2008 Update produced by the Canadian Task Force on the Periodic Health Exam and the Society of Obstetricians and Gynaecologists of Canada (1997). [175]	"There is no scientific evidence of a deleterious effect of diagnostic ultrasound on the developing fetus. Low birth weight, dyslexia, increased incidence of cancer such as leukaemia and solid tumours, delayed speech, and delayed reading and writing skills have all been suggested. However, these studies have small sample sizes and all have significant design flaws. Larger, better designed studies refute the suggestion of an immediate or delayed deleterious effect of ultrasound on the fetus as confirmed in a recent review (Salvasen et al, Lancet 1992; Lyons et al Radiology 1988) The biggest risk of ultrasound is over interpretation or missed diagnosis."
ACOG Practice Patterns Evidence-Based Guidelines for Clinical Issues, Obstetrics and Gynaecology 1997 [176]	"There has been no clear evidence about deleterious effects of ultrasound. Ultrasound imaging during pregnancy should be performed only for a specific medical indication and not for routine screening."
AIUM 1994 , reapproved 2002 [162]	"No confirmed biological effects on patients or instrument operators caused by exposure at intensities typical of present diagnostic ultrasound instruments have ever been reported. Although the possibility exists that such biological effects may be identified in the future, current data indicate that the benefits to the patients of the prudent use of diagnostic ultrasound outweigh the risks, if any that may be present."

(2.2) Evaluation of intrauterine fetal shunts

In this section of the chapter, fundamental concepts in fetal cardiophysiology have been summarised as they form a basis for explaining the results of the various studies undertaken for this thesis. In order to be able to explain the abnormalities in haemodynamics of fetal shunts, it is necessary to understand the normal haemodynamics within the fetal shunts with respect to fetal circulation. This section starts with a historical account of fetal circulation and intrauterine shunts. Subsequently, these shunts are discussed in more detail in relation to fetal cardiac haemodynamics, pulmonary and cerebral circulation. Furthermore, this literature review discusses the anatomy, functional morphology, physiology and Doppler studies of central intrauterine shunts foramen ovale and ductus arteriosus, along with a brief overview of the shunt ductus venosus. A comparative anatomy approach evaluating the shunts in different species is included, in order to 'set the scene' for some specific hypotheses on the role of central shunts as facilitators of efficient oxygenation.

Fetal circulation and intrauterine shunts: Historical perspective

The circulation has been a matter of great interest for centuries. Aristotle was the first to describe arteries and veins[177]. Fallopius described the placenta for the first time in 1561, as it resembled a flat cake or pancake [178]. The first account of ductus venosus, ductus arteriosus and placenta, originally described by Galen, as cited by Dunn, [178] was published in 1600 by Fabricius De Formato Foetu (On the formation of the fetus) (Figure 13).

William Harvey, in 1628, described the fetal circulation and introduced the dynamic concept of the fetal heart functioning in parallel.[179].

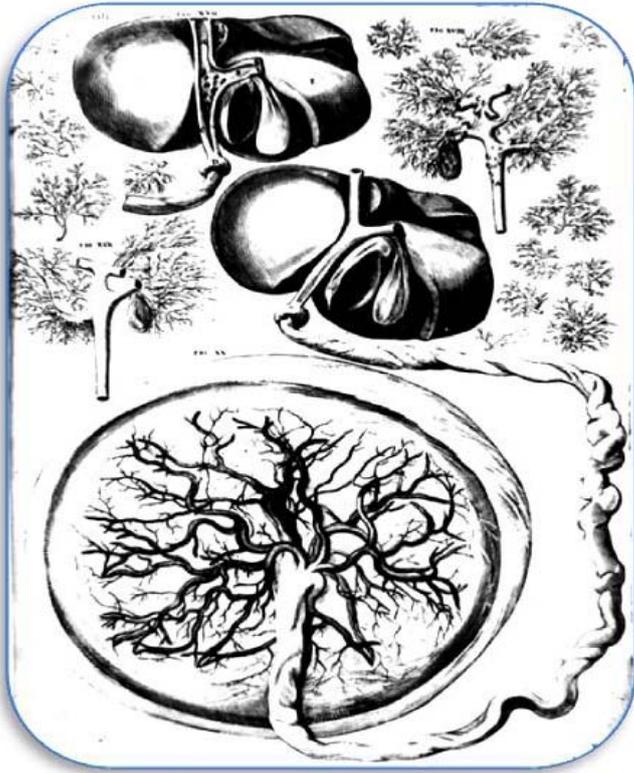


Figure 13 Placenta, umbilical cord, liver, and ductus venosus (from Fabricius' De formato foetu (1600).

© 2003 Archives of Disease in Childhood Fetal and Neonatal Edition [178]

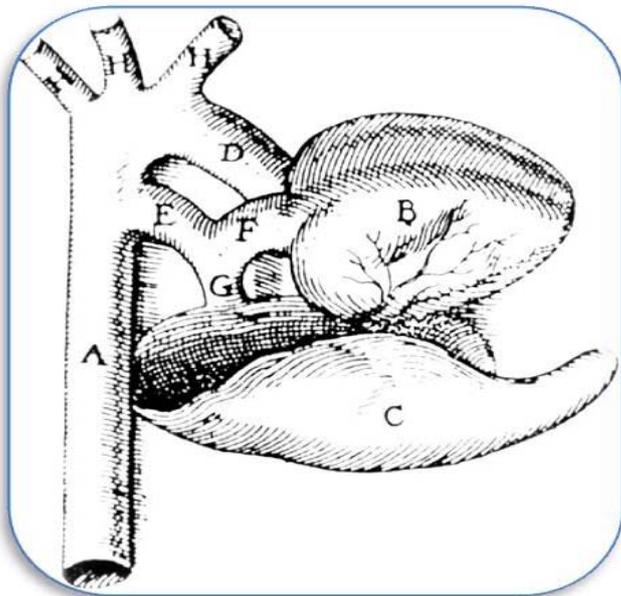


Figure 14 Heart, lung, great vessels, and ductus arteriosus (E) (from Fabricius' De formato foetu (1600)

[178]With permission from Dunn, P M Arch. Dis. Child. Fetal Neonatal Ed. 2003;88:157-F159Archives of Disease in Childhood Fetal and Neonatal Edition 2003;88:F157

© 2003 Archives of Disease in Childhood Fetal and Neonatal Edition accessed on 24th oct 2007

Wolff, in 1776, concluded that the fetal auricles were actually separated from each other by the isthmus of Vieussens, which is a muscular ring surrounding the foramen ovale, in the wall of the right atrium of the heart [178]. He observed that the isthmus was common to both right and left atria, dividing the flow from the IVC into a larger component entering the foramen ovale and left atrium (Figure 14). Wolff observed that the isthmus was nearer the right than the left side, and estimated that near term, two-thirds of the flow through the isthmus would take the right side and one third would take the left side. This view was then proved correct by animal experiments and Doppler studies [180].

James Jeffray, in his monograph titled ‘observations on the heart and on the peculiarities of the fetus, in 1835, proposed that blood from the inferior vena cava passes to the right atrium through the foramen ovale and that blood from the superior vena cava crosses this flow to reach the aorta via the right ventricle, pulmonary trunk and ductus arteriosus [181]. This concept has now been proved with Doppler studies [182].

William Hunter and John Hunter in the 1750s showed that maternal and fetal circulations are separate [183]. William Hunter described the functioning of the placenta and also demonstrated the presence of the spiral arteries in the endometrium, which he called the ‘curling arteries’. In his treatise, “The Anatomy of the Human Gravid Uterus”, in 1774, he described his experiment where, by injecting coloured wax into the uterine arteries and veins, he observed that the wax filled the placenta but did not pass into the umbilical vessels. Similarly, on injecting blue and green coloured wax into the umbilical arteries and veins respectively, and yellow wax into the uterine veins, the blood spaces of placenta were filled with yellow wax [181, 184].

It was only in the early 20th century that experiments on fetal lambs ascertained the exact nature of fetal circulation [68, 185](Figure 15).

NOTE:

This figure is included on page 53 of the print copy of the thesis held in the University of Adelaide Library.

Figure 15 Classic illustration of fetal and neonatal circulation by Dawes (1954).

www.neonatology.org/classics/mj1980/fig14-01.gif accessed on 24th Oct, 2007.

Copyright © 1996-2007 Neonatology on the Web

Comparative anatomy of fetal shunts

Comparative functional anatomy is the study of similarities and differences in the functional anatomy of organisms. A comparative study of the heart in different species reveals an evolutionary transition from a simple two chambered heart, as seen in fish, to a three chambered heart as seen in amphibians to a fully developed 4 chambered heart in mammals. The presence of intrauterine shunts ductus arteriosus, ductus venosus and foramen ovale has been documented in all higher mammals including humans as well as in other species such as the primates, rodents, ruminants, seals, whales, porpoises, elephants as well as carnivores [186]. Several interesting similarities are seen in fetal circulation in higher mammals and the circulation seen in the adults of lower vertebrates such as lungfish, amphibians and reptiles.

Lower vertebrates have to switch between high to low oxygen modes, hence circulatory system in fish is undivided and there is mixing of oxygenated and deoxygenated blood, which enables the fish to vary proportions of oxygenated flow going into the gills or the body [187]. Air-breathing fish (lung fish), amphibians and reptiles have an incompletely separated circulatory system.

Lungfish were the first vertebrates to show a separation of circulation into the right and left circulation. They use gills as well as lungs, and therefore they have an incompletely separated circulation. They have a partial interatrial system and a partial inter-ventricular system. This enables the blood from lungs to go into one side and the blood from the body to another. In the conus arteriosus, which arises from the two chambers, there is a spiral valve, which keeps the flows separate. This apparently enables differential perfusion of the pulmonary circuit with minimal mixing of oxygenated and deoxygenated flows [188]. This incomplete separation allows for changes in flow during gill respiration versus lung respiration. If the lungfish is in well-oxygenated water, it allows more flow to the gills, whereas, in less oxygenated water, it directs more flows to the lungs.

The crocodile heart is another example of an efficient system of oxygenation, depending on whether the animal is on land or in water [189]. The crocodile heart has a shunt named Foramen of Panizza, which is present between the right systemic and

left systemic arch. The right systemic arch arises from the left side of the heart and left systemic arch from the right side of the heart. The Foramen of Panizza shunts flow off flow from right side to left during lung respiration. This enables flow from left side of heart into left systemic arch , with deoxygenated flow going to the lungs [190]. When the crocodile dives and holds its breath, the lungs are useless, and therefore blood is shunted away from pulmonary artery and enters the systemic arch. The Foramen of Panizza allows the crocodile to stay under water for long periods, to allow them to lie in wait for their prey under water[190].

These insights into animal cardiovascular physiology therefore leads one to speculate that the fetal intrauterine central shunts are probably not just passive, embryonic conduits in the fetus which eventually become redundant after the cord is clamped, but are possibly present in the fetus as an evolutionary strategy perfected by vertebrates over millions of years. The presence of intrauterine shunts is possibly a conserved evolutionary mechanism to provide an efficient mechanism to enable adequate oxygenation in the aquatic life, before the fetus transitions to a terrestrial, lung-dependant life [191]

Shunts and fetal heart

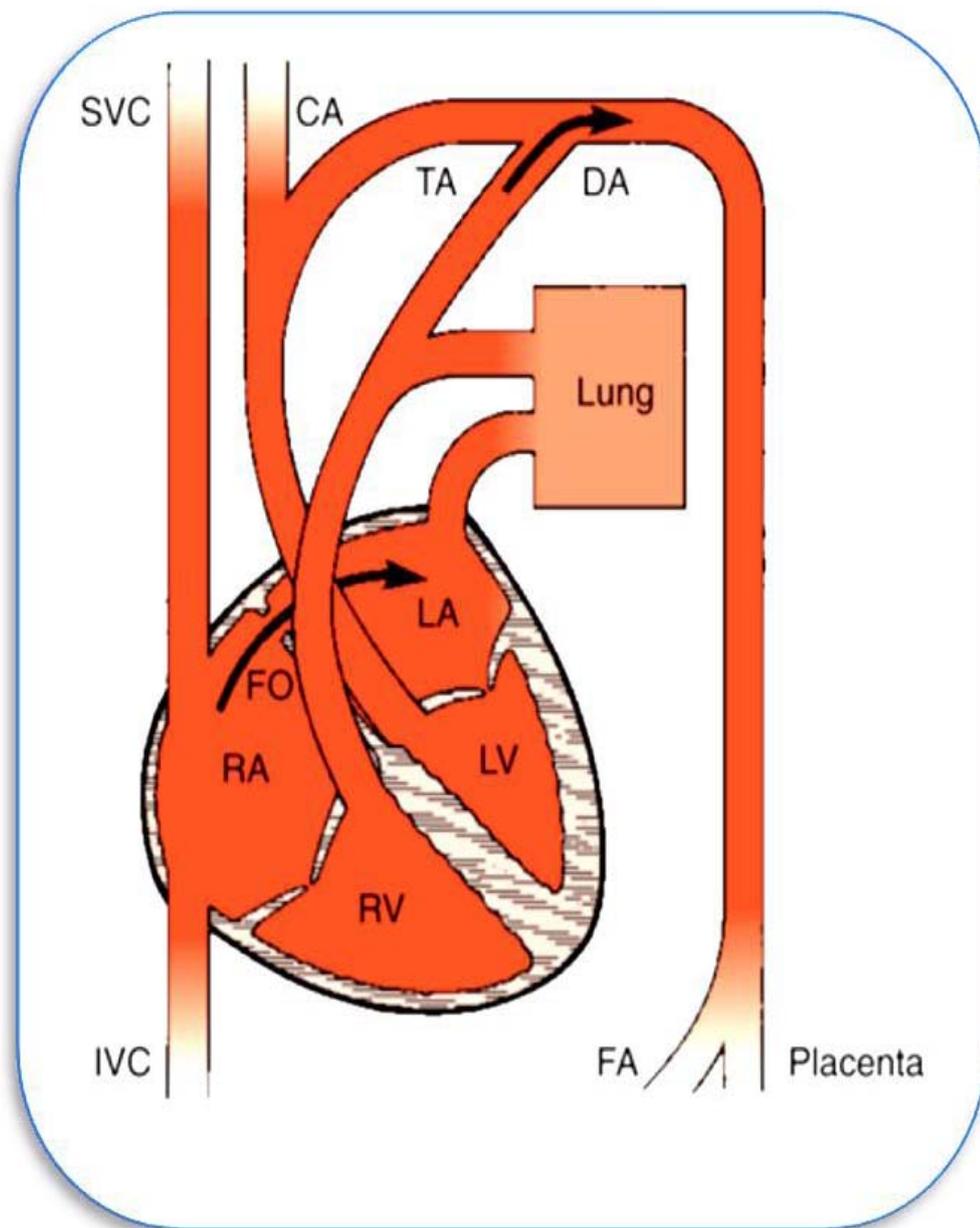


Figure 16 Central shunts and the fetal heart.

Anatomy of fetal heart and central shunts. CA, carotid artery; DA, ductus arteriosus; FA, femoral artery; FO, foramen ovale; IVC, inferior vena cava; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; SVC, superior vena cava; TA, thoracic aorta. [192] with permission Gabbe: Obstetrics: Normal and Problem Pregnancies, 5th ed. Copyright © 2007 Churchill Livingstone, An Imprint of Elsevier

In the human fetus, the heart rate is 110 to 170 beats per minute. The fetal heart works in parallel, with the right ventricle being dominant, ejecting about 60 % of the total cardiac output. The central fetal shunts form important communications between the right and left side of the heart (Figure 16). These shunts may also play an important role in determining ventricular loading. Left ventricular filling preload predominantly depends on the foramen ovale, to allow the highly saturated oxygenated flow returning from placental circulation, via umbilical vein and ductus venosus to stream preferentially towards the right side of the heart.

The left ventricle works against the cerebral and upper body resistance whereas the right ventricle works against the placental and peripheral resistance. Research evidence over the last two decades now supports the theory that the systemic and the umbilical flow is shunted through the ductus venosus to enable blood with high oxygen saturation to reach the heart, and flow through the foramen ovale enables blood with high oxygen saturation to reach the brain through the left ventricle [193] . The right ventricle ejects about 66 % of the combined ventricular output of the fetal heart, 85 to 90 % of which gets diverted from the lungs through the ductus arteriosus. The blood flow through the lungs is quite low during fetal life [194, 195]. This helps conserve precious oxygen from being lost to the lungs, as well as reducing the pulmonary venous return, thereby preventing an unnecessary increase in the volume of work placed on the left ventricle.

With increasing gestation in normal pregnancies, there is a lowering of placental resistance, leading to a low resistance circuit and an increase in fetal cardiac output. Left ventricular preload, with increasing gestation, becomes more dependent on pulmonary venous return, as the proportion of flow through fetal lungs increases [196] and leading to volume loading of the right ventricle and right atrium, which is usually benign and reverses after delivery. The ductus arteriosus (DA) closes at birth [197], to facilitate this process, cushions of tissue develop towards the pulmonary end of DA. This causes an increase in right ventricular afterload towards term, leading to an increased systemic venous pressure in the fetus. Dramatic changes occur in the preload and afterload at birth, when the placental circulation is cut off, leading to a

sixfold increase in pulmonary flow, leading to a rise in left atrial pressure and a cessation of flow in foramen ovale. Ductal closure occurs and ductus venosus closure within a few days after birth [197].

Pulmonary circulation and the heart

The pulmonary circulation, along with volume of flow from the IVC and ductus venosus contributes to redistribution of flow, influencing the LV preload. Pulmonary flows are shunted through the ductus arteriosus to the descending aorta. Groenenberg et al observed a drop in the peak systolic velocity (PSV) in pulmonary artery in 95 percent of fetuses with IUGR, as opposed to a drop in PSV in aortic velocities in just 57 percent of fetuses [123]. Similar results were reported by Rizzo et al. Pulmonary hypoxic vasoconstriction has also been demonstrated in compromised fetuses [198] , suggesting a regional adaptation of the fetal pulmonary circulation as a response to hypoxia. Pulmonary veins are of narrow calibre and drain blood from lungs to the left atrium. The low volume flow from the pulmonary veins to the left atrium occurs throughout the cardiac cycle. The pattern of pulmonary venous flow velocity has been shown to be dependant on cardiac output, absolute pulmonary flow, left atrial pressure and function, mitral valve function and left ventricle pressure and function [199]. An increased pulsatility in pulmonary veins was reported in fetuses with severe IUGR [199].

Shunts and cerebral flows in congenital cardiac disease

Fetal echocardiographic and Doppler studies have demonstrated that fetal central shunt flow pathways are different in congenital heart malformations. When the right side of the heart is underdeveloped, such as hypoplastic right heart disease, fetal survival depends on increased cerebral perfusion through foramen ovale, suggesting a redistribution of fetal cardiac output [200, 201]. When the left side of the heart is underdeveloped, it has been observed that there are reduced flows through FO and FO is smaller than normal. However, it is unclear if the small FO and reduced flows through FO are a cause or consequence of hypoplastic left heart syndrome [201]. Reversal of flows in FO in severe left sided obstructive lesions and reversal of flow in

ductus arteriosus in severe right sided obstructive lesions were associated with more severe forms of CHD with poorer prognosis for survival [202] . These studies suggest that FO plays an important role in redistribution of flows to ensure survival of the fetus in suboptimal oxygen conditions.

Ductus Arteriosus

Embryology

Anatomy

Physiology

Pharmacology

Doppler studies

Embryology

The ductus arteriosus (DA) is embryologically derived from the 6th aortic arch [203]. It originates just beyond the origin of left subclavian artery. It carries about 60 % of the total cardiac output, 90 percent of the blood from the right ventricle. It serves to bypass the lungs, which receive only about 7% of right cardiac output and postpartum. It closes functionally and anatomically to persist as the fibrous ligamentum arteriosum, which connects left pulmonary artery to the aorta.

Anatomy

It is usually a short curved tube, about 1.5 cms in length but can be longer and more tortuous. The curvature has been studied as well, and it has been observed that the curvature increases with gestation; sometimes even kinks have been described [204]. The amount of flow passing through the DA will depend on size, shape, diameter and the difference between systemic and pulmonary resistance.

Morphometric studies of DA done between 13 to 20 weeks of gestation have shown that there is a great variability in the growth of DA with gestation, with the diameter measuring 0.93 to 3.1 mm. Median values were 1.3 mm at 13 weeks and 2.45 mm at 20 weeks. Castillo et al reported that there was not much growth between 16 to 18 weeks, followed by a rapid acceleration in growth from 18 weeks onwards [205] . They also observed that the diameter of DA was smaller than the diameter of aortic isthmus in 91.3 % cases, with a ratio of 0.67 to 0.81.

Histology

Structurally, the mammalian DA is composed of a single endothelial layer, lining the lumen, media and adventitia and also has vasa vasorum [205-209]. The media is made up of smooth muscle and the intima is thicker than the aorta. At birth, the DA closes, and the systemic resistance increases and pulmonary resistance falls. This closure is brought about by a proliferation and migration of the smooth muscle cells, gradually leading to a closure of intima. Recent immunohistochemical studies suggest that an increase in deposition of hyaluronan could be a causal factor of the closure of lumen [210, 211]. DA adventitia and outer media is innervated by sympathetic innervation and branches of the vagus nerves [212, 213]. Ischaemia of the vasa vasorum has also been thought to be an antecedent to closure of DA [214, 215].

Physiology

The DA is a vessel designed to bypass the lung circulation in the fetus until birth, and thereafter close after birth when lung function becomes essential. The closure of the DA is a complex process. The exact mechanism of closure of DA has been a continuing focus of research over the last 40 years, and it has been suggested that the process of closure begins before birth, and is carried on after the baby is born and the cord is clamped, cutting off the placental circulation [216]. This perinatal phase of functional and morphologic closure has been termed as transitional circulation.

Sensitivity to vasomediators

The DA is much more than a passive channel. It has an intrinsic contractile tone. The DA is also extremely sensitive to prostaglandins. Prostanoid receptors have been identified in DA [217]. Prostaglandin E2 is thought to be the naturally occurring relaxant keeping the DA actively patent in utero [218, 219]. Animal experiments seem to suggest that the DA is sensitive to changes in oxygen tension-as seen by studies in chicken embryos [220] and fetal lambs [220, 221]. Fay proposed that with an increase in oxygen in the environment, there is increased oxygen availability to the terminal

cytochrome component, cytochrome a3 [222-224]. This leads to a subsequent increase in oxidative phosphorylation and an increased synthesis of high- energy phosphate compounds, in turn increasing ATP synthesis. This may trigger off DA contraction [225]. ATP depletion is one of the postulated mechanisms for DA closure. Disordered intracellular calcium homeostasis [226], altered potassium channels [227], and oxygen has been thought to control the mitochondrial electron transport chain [228], influencing DA tone.

VEGF has also been implicated in the remodelling of the DA [229]. Nitric oxide has also shown to be dilator of the DA [230, 231], whereas endothelin 1 has also been proposed as a constrictor of the DA [231]. Circulating catecholamines in hypoxic stress may also play a role in ductus constriction [232].

Doppler examination

DA Doppler patterns have been described in normal and compromised fetuses. The normal pattern consists of a predominant systolic peak and a second low velocity diastolic wave of continuous flow until the next beat. Systolic flow is related to the ventricular systolic thrust, and the diastolic flow is related to the combined peripheral and placental resistance [233]. There is a significant change in shape with advancing gestation in the normal fetus [234]. Measurements of DA PI and PSV have been described, and it has been observed that fetuses with ductal constriction as seen with indomethacin administration, are associated with low PI and increased peak systolic as well as diastolic velocities [235].

In view of the shape, structure, innervation and extreme sensitivity to vasomediators, and the presence of prostanoid receptors, it is logical to assume that the DA is an extremely sensitive vessel, very dynamic, and susceptible to changes in oxygen and vasomediators and therefore would reflect any changes as a change in flow velocity waveforms. The effects of indomethacin on the closure of DA have been well documented with Doppler flow examination. However, there is a paucity of data on Doppler waveforms in early stages of fetal compromise in IUGR.

Foramen ovale

Embryology

Anatomy

Physiology

Pharmacology

Doppler studies

Embryology

The foramen ovale is one of the three intrauterine shunts present in the fetal circulation. It is a communication present within the fetal heart atrial septum, between the left and right atrium (LA and RA). It measures about 3 mm to 6mm in width [236]. It allows blood to pass from the right to the left atrium. About 34 % of total cardiac output passes through the FO. The foramen ovale flap is arranged with a flange on the right atrial surface and a flap valve within the left atrium. The flap, which arises from the atrial roof, grows downwards and is called the septum primum. The semilunar flap, which is actually an atrial fold, grows to the right of the septum primum and is called the septum secundum. After birth, the septum primum and septum secundum fuse when there is an increase in the right atrial pressure, leading to the closure of FO.

Histology

Histologically, the interatrial septum is predominantly connective tissue. It is not clear if the FO is innervated or not. It is also not known if the FO has any receptors.

Physiology

The presence of the FO ensures the preferential streaming of highly oxygenated blood to flow from RA to LA, which in turn enters the aorta through the mitral valve, and then is supplied to the upper part of the body, mainly carrying highly oxygenated blood to the myocardium and the brain. This has been observed in animal experiments as well as Doppler studies [180, 237] .

In the adults, a patent foramen has been implicated in several pathologic processes such as ischaemic stroke with thromboembolism, decompression sickness, platypnea–orthodeoxia syndrome (a condition associated with postural hypoxaemia with breathlessness) as well as migraines [238].

Doppler studies

There have been very few ultrasound and Doppler flow studies of the FO in the fetus. The normal FO diameter is similar to the aortic root diameter, and trans-atrial flow velocity patterns in the normal FO suggest that triphasic flow pattern is seen in diastole whereas systolic flows are biphasic[239]. Doppler studies have confirmed the presence of preferential streaming of umbilical blood across the foramen ovale, the oxygenated flows being directed towards the left atrium [240]. Flow changes in the FO have been observed in relation to behavioural changes, with an increase in average flow velocity in active sleep, suggesting redistribution, at the cardiac level [241]. In a cross sectional study of 31 singleton pregnancies complicated by IUGR, FO diameters and right atrial diameters in normal and growth restricted fetuses were examined [242]. It was observed that in growth- restricted fetuses, the right atrial size remained normal, however the FO size in relation to right atrium, reduced. The authors proposed that this finding supported the theory that FO shunting is impaired in severe compromise associated with UPI.

FO flow velocity patterns in early stages of fetal compromise have not been reported so far, however, given its physiological involvement in redistribution of flows in the fetus, as well its role in pathological conditions in adulthood, it is possible that some alteration in Doppler flows in the FO exist, in early stages of fetal compromise.

Ductus venosus

Anatomy

Physiology

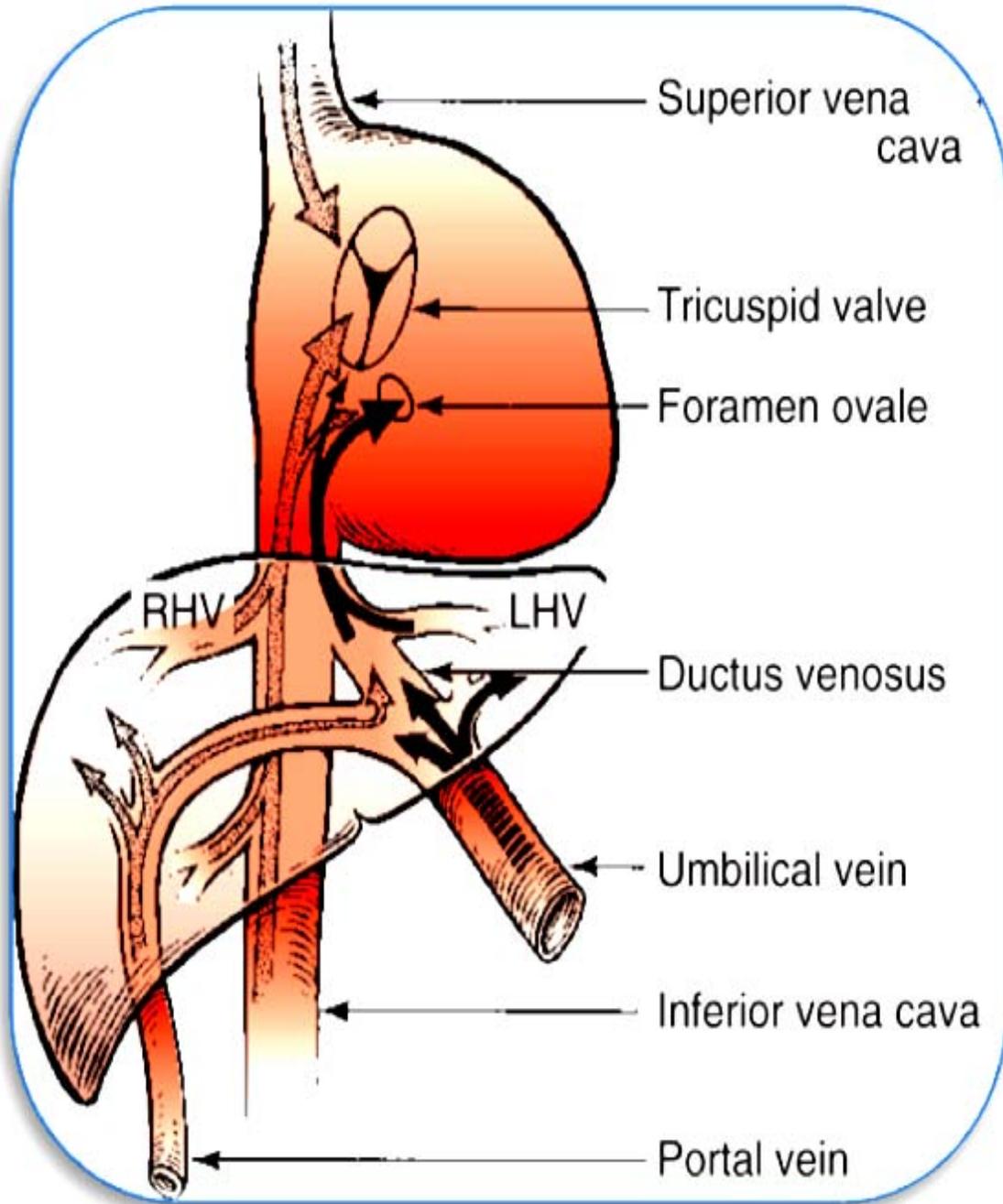


Figure 17 Ductus venosus, foramen ovale and the hepatic circulation.

Anatomy of the umbilical and hepatic circulation. LHV, left hepatic vein; RHV, right hepatic vein.[243] Copyright © 2007 Churchill Livingstone, An Imprint of Elsevier

Anatomy

The ductus venosus (DV) is a continuation of the umbilical vein. Scanning electron microscopic studies after morphometry with immunohistochemistry have confirmed that the DV is a trumpet shaped or hour glass shaped structure, about 3.4 to 6.5 mm in length, narrower at the inlet, inlet diameter being 0.7 to 2.1 mm and wider at the outlet, outlet diameter being 0.8 to 2 mm [244], extending from the umbilical vein to the left hepatic vein, at the confluence of hepatic veins and inferior vena cava (Figure 17).

Histology

Studies in fetal lambs have suggested that the DV has a contractile mechanism like a sphincter, with functional alpha and beta adrenergic nerves, which can therefore cause contraction and relaxation respectively [245, 246]. Morphometric studies have now shown that structurally, it is made up of smooth muscle, nervous tissue, the muscular wall in the inlet is thicker, seems like a shelf, and even though there is no anatomic sphincter, there are elastin rich smooth muscle fibres longitudinally arranged, as well as endothelial corrugations arranged lengthwise and it therefore acts like a sphincter [244]. Vasa vasorum in the DV has also been demonstrated [247].

The presence of the shelf at the inlet, has been speculated to be a mechanism to produce accelerated blood flow when the blood enters the DV from the portal sinus, which is a low pressure-high volume system. The presence of innervation and smooth muscle in its entire length could enable it to regulate its diameter along its entire length in response to different stimuli [247]. In a study of baboon fetuses, an asymmetrical muscular lip at the isthmus portion of the DV was observed, and the intrahepatic branch of portal vein contained more smooth muscle cells than the media of the DV, thus providing structural evidence to support shunting mechanisms [248]. Lamb studies have shown that DV is responsive to prostaglandin mediated ductal relaxation, the active naturally occurring relaxing ingredient being PG12 [249]. The DV sphincter also seems to be relying on the cytochrome P450 mechanism to develop its contractile tone, although the actual constrictor yet remains to be identified [250].

Physiology

The DV provides a bypass of the portal venous flow, which is received through the umbilical vein, and most of the highly saturated oxygenated blood is carried into the foramen ovale through the inferior vena cava. When the cord is clamped postpartum, there is a functional closure of the DV, which then eventually transforms into the fibrotic ligamentum venosum.

Flow distribution patterns in normal and growth-restricted fetuses have been extensively studied in animal experiments and confirmed in human fetuses [119, 160, 251-253]. Studies on distribution of blood flow across the liver have shown that there is unequal distribution of arterial flow to liver. The right lobe gets almost two thirds of flow from the portal vein, while the left lobe gets about one third. Portal venous flow is almost completely distributed through right lobe, a small portion through the DV and almost nothing through the left lobe [243]. These distribution patterns favour more oxygen saturation in the left hepatic vein than the right. The flow from the left hepatic vein combines with DV flows and is preferentially streamed through the inferior vena cava towards the foramen ovale, to be distributed to the upper part of the body. The right hepatic vein joins the inferior vena cava as well, to flow into the tricuspid valve, through the right atrium, and then to the lower part of the body. Research seems to suggest that the two streams remain relatively separate till they enter the heart [254].

Lamb experiments showed that about 50 percent of umbilical venous flow goes into the DV and the remainder to both the lobes of the liver. However, in healthy normal human fetuses, the degree of shunting through the DV is much lower, about 28 to 32 % at 18 to 22 weeks, decreased to 22 % at 25 weeks and decreased even further, around 18 % at 31 weeks [255]. Increased shunting has been observed in the DV as a response to IUGR [256], induced hypoxaemia [257] or acute haemorrhage [258]. Doppler flow waveforms and studies in IUGR have been described in an earlier section of this chapter.

The exact mechanism, which determines an increase in DV shunting, is not known. Mathematical modelling [259] and ultrasound studies seem to suggest that active dilatation along with an increase in the diameter could be responsible for increased shunting as a response to IUGR [259, 260]. This increased shunting, along with an increase in diameter of DV, as demonstrated in pregnant ewes, could also have a second effect in improving placental flows in hypoxia [261]. All these documented changes in the DV have been observed in later stages of fetal compromise, the role of DV in earlier stages of the compromise have not yet been defined.

(2.3) Introduction to fundamental concepts in fetal cardiophysiology

Cardiac output

Cardiac preload

Cardiac contractility (inotropy), Frank Starling law and myocardial stiffness

Cardiac afterload

Cardiac compliance

Distribution of fetal cardiac output

The aim of this section is to provide a review of some fundamental concepts in fetal cardiovascular physiology. An understanding of these concepts enable an understanding of the normal and abnormal function of the fetal heart and will be helpful in evaluating the results of the different studies investigating FO and DA Doppler flow velocity waveforms described in this thesis.

Cardiac output

Cardiac output is the volume of blood pumped by the heart, particularly the ventricle in a minute [262]. Cardiac output is defined as the product of stroke volume and heart rate. Fetal circulation works in parallel. It is influenced by the vascular bed against which the heart is pumping. In the fetus, the pulmonary resistance and placental resistance influence the right cardiac output whereas systemic resistance determines the left cardiac output. Heart rate is the number of times a given volume of blood is ejected per unit of time

Stroke volume

Stroke volume is the volume of blood that the heart can fill with and eject upon contraction. Stroke volume is the difference between end diastolic volume and end systolic volume. The factors influencing stroke volume are summarised below (Figure 18).

NOTE:
This figure is included on page 69 of the print copy of the thesis held in the University of Adelaide Library.

Figure 18 Stroke volume

<http://www.cvphysiology.com/Cardiac%20Function/CF002.htm> accessed on 25th Oct 2007.

Cardiac preload

Preload can be defined as the initial stretching of myocytes before contraction [262].

Preload is the myocardial wall stress at the end of diastole [263, 264] . The factors influencing alterations in preload are depicted in the figure below (Figure 19).

Alterations in preload will cause an alteration in fetal cardiac stroke volume. Increase in preload will cause an increase in stroke volume, whereas decrease in preload will cause a decrease in stroke volume, in line with the Frank Starling mechanism.

NOTE:

This figure is included on page 70 of the print copy of the thesis held in the University of Adelaide Library.

Figure 19 Cardiac preload

<http://www.cvphysiology.com/Cardiac%20Function/CF007.htm> accessed on 25th Oct 2007.

Cardiac contractility (inotropy), Frank Starling law and myocardial stiffness

Cardiac output increases or decreases in response to changes in stroke volume or heart rate. Increased venous return to the heart increases the heart rate. The mechanism by which the heart is able to change its force of contraction and therefore the stroke volume in response to changes in venous return, is called the Frank Starling mechanism or Starling's law of heart, named after pioneering work by physiologists Otto Frank and Ernest Starling. In the fetus, the Frank Starling mechanism has been found to be operating [265], however, it is limited, and functions near the break-point

of the curve, possibly due to immaturity, impaired relaxation [266] and increased stiffness of myocardium [267] in the developing heart. Therefore, the fetal myocardium has very little preload reserve [268]. Contractility, also known as inotropy, is the intrinsic ability of the myocardial fibres to contract at a given fibre length. It is an important determinant of fetal myocardial performance and reflects the strength of contraction. The Frank Starling law and contractility work independently of each other, to achieve optimum myocardial performance.

Cardiac afterload

Afterload is the load the heart must eject against [262]. Afterload is the myocardial wall stress during systolic ejection [264]. Since the aorta arises from the left ventricle, left ventricular afterload will depend on aortic pressure. The fetal circulation is a parallel circulation, where the right ventricle works against the placental resistance. The right ventricular afterload in the fetus, therefore, depends on placental resistance. The ventricular afterload also depends on ventricular pressure itself, which is in turn dependent on ventricular radius, and wall thickness, defined by Laplace law. Laplace law states that wall tension is proportionate to pressure times radius.

Cardiac compliance

Compliance is a term used to describe how easily a blood vessel or the heart is able to expand, when filled with a volume of blood. It is defined as a change in volume divided by change in pressure. Therefore, left ventricular compliance will increase if LV volume increases or LV pressure decreases. LV volume depends on LA volume, which in turn will depend on pulmonary venous return as well as the flow through right atrium and Ductus venosus. LV pressure depends on aortic pressure.

RV compliance will increase with an increase in RV volume or decrease in RV pressure. Thus, RV volume will depend on increasing venous return from the IVC and SVC and RV pressure will depend on PA pressure, DA pressure and descending aortic pressure alterations depending on peripheral resistance.

Distribution of fetal cardiac output

Experimental studies in lambs and Doppler studies in human fetuses have described the distribution of cardiac output with advancing gestation. It has been observed that in normal fetuses, the combined cardiac output increases with gestation. This increase, possibly a maturational or developmental alteration, is associated with an improvement in early diastolic active relaxation of the fetal myocardium as well as an increased efficiency of systolic emptying at any given filling volume and/or pressure [269].

Lamb studies have confirmed the pattern of distribution of cardiac output, and human Doppler studies show similar results. The observations of key human and animal experiments evaluating the distribution of fetal cardiac output, has been summarised in the table below (Table 5).

Table 5 Distribution of Blood Flow Expressed as Percent of Combined (Biventricular) Cardiac Output:

Distribution of cardiac output	Near term fetal lambs N=12 [192]	Human fetuses 13 to 41 wks N= 222 [270]	Human fetuses mid trimester to term n= 63 [271]	Human fetuses (18-37 wks) n=10 [272]
Rt CO %	60	59	53-60	
Lt CO %	40	41	47-40	
DA %	54	46	32-40	47% of PA flow
Pulmonary flow %	6	11	13-25	22 of total CO
FO %	34	76% of LCO and 33% of CCO	34-18	17-31% increased 3 fold from 20 to 35 weeks
Biventricular output ml/kg	462	425	470-503	

Results from lamb experiments and human Doppler studies. [192, 270-272]

In a study on near-term fetal lambs, about 44% of the umbilical venous blood was distributed to the DV. With a 50% reduction in umbilical flow, this proportion increased to 72%, resulting a marked decrease in umbilical blood flow distributed to the fetal liver [273] The preferential distribution of ductus venosus blood flow through the foramen ovale was enhanced (29.4 vs. 47.2%) and the proportion of O₂ delivery to upper body organs derived from the ductus venosus increased (33.2 vs. 49.4%).

Kieserud and his colleagues demonstrated that in normal pregnancies, one third of the fetal combined cardiac output (CCO) is distributed to placenta in most of the second half of pregnancy. This is reduced in placental compromise, although the CCO per kg was maintained within normal levels, suggesting an increased recirculation of umbilical venous flow within the fetus [256].

It has been demonstrated that the ultimate body proportionality of the fetus depends to a great extent on the nature and timing of the impact of the factors causing the growth failure. In hypoxia in asymmetric IUGR fetuses, fetal cerebral redistribution has been thought to be facilitated by a redistribution of intracardiac circulation, although this was not seen in fetuses with symmetric IUGR with normal placental resistance [95]. Cerebral vasodilation leads to decreased left ventricle afterload and systemic vasoconstriction leads to increased right ventricle afterload. These changes cause a preferential shift of cardiac output to the left ventricle, thereby allowing maximum oxygenated blood supply to the brain. This has been speculated to be an adaptive mechanism in early stages of the disease [116], which is hampered when the hypoxia worsens, leading to cardiac decompensation [136, 274] .

Cardiac compliance and myocardial rigidity

Ventricular diastole involves relaxation, compliance, myocardial rigidity, and elastic recoil [275]. Relaxation is an active process occurring with energy consumption in the early ventricular filling, when the myocardial fibres return to their original state after ventricular contraction [275]. Compliance is a passive process occurring during late ventricular filling and atrial contraction, and is related to fibre distensibility [275]. Myocardial rigidity is the opposite of ventricular compliance. Elastic recoil is a continuing decrease in ventricular pressure in early diastole [276].

Summary of chapter 2

A review of the role of Doppler ultrasound in clinical practice, in the first part of this chapter, provides evidence that Doppler ultrasound is useful in decreasing the adverse perinatal outcome in high-risk pregnancies. Serial ultrasound and Doppler monitoring has the potential to identify severe fetal compromise, and several longitudinal studies have documented the sequential changes in circulatory haemodynamics in the compromised fetus by evaluation the fetus for signs of brain- sparing and severity of redistribution of circulation. However, there still remains no marker for identifying the 'high-risk' AGA fetuses. There is also no marker available to identify the fetus at risk before IUGR becomes evident as a reduction in AC.

A review of the anatomy of the shunts and the fetal cardiophysiology has revealed that the main difference between fetal and neonatal circulation is the presence of intrauterine shunts, which work closely with the placenta to ensure appropriate nutrition and oxygenation of the fetus. Since the fetal heart is vital to enable redistribution of flow, and the presence of intrauterine shunts DA, FO and DV has already been documented as a means to efficiently deliver oxygen and nutrition to fetal tissues from the placenta after bypassing the lungs, it can be speculated that the fetal heart and the fetal shunts may be involved in redistribution even before any brain sparing becomes evident by currently established parameters.

The next chapter (Chapter 3) describes the rationale and the specific aims and hypotheses used in the thesis. Chapter 3 also describes the methodology used in this thesis,

1. To explore flows through all shunts-DA, DV, FO to identify flow changes early in disease and document sequential changes in flows through all fetal vessels and shunts irrespective of fetal size.
2. To evaluate the interrelation of flows within the shunts themselves and their relation with fetal growth, fetal cerebral, fetoplacental and uteroplacental circulation, and explore the usefulness of flows through central shunts, i.e., DA and FO, as possible predictors of adverse outcome.

Study design and general methodology

Rationale of the research

The rationale behind the research protocols described in this thesis is based on the hypothesis that intrauterine shunts are pivotal in fetal circulation, in association with the placenta, ensuring appropriate nutrition and oxygenation of critically important fetal organs, and can therefore reflect any early adaptive changes in the fetus as a response to hypoxia.

Fetal hypoxia leads to fetal compromise, and if unrecognised and progressive, may lead to fetal myocardial dysfunction and even death. Previous studies have established that uteroplacental insufficiency (UPI) as a disorder associated with adverse pregnancy outcomes such as intrauterine growth restriction (IUGR), preeclampsia and preterm birth, which still remains a major contributor to fetal morbidity and mortality. In this thesis, we use UPI as an operational term to describe adverse pregnancy outcomes which include preeclampsia, gestational hypertension, placental abruption and SGA < 10th centile.

Based on existing clinical evidence, it is accepted clinical practice to perform serial ultrasound and Doppler monitoring in high risk pregnancies for potentially identifying severe fetal compromise and optimising timing of delivery in an attempt to decrease adverse perinatal outcomes. Doppler ultrasound can be useful in evaluating fetal circulatory derangements associated with progressive fetal compromise in IUGR. Doppler ultrasound is also useful in evaluating uteroplacental and fetoplacental haemodynamics in uteroplacental insufficiency (UPI). Whilst this practice of surveillance is effective in identifying the need for intervention in high-risk fetuses, usually in the third trimester, it does not identify, nor does any other marker or practice, the high-risk 'appropriately grown' fetus. Similarly, there is also no marker that identifies fetuses at risk before IUGR becomes evident as a reduction of abdominal circumference (AC). Furthermore, early changes in fetal hypoxia are poorly defined.

It would be clinically useful to identify the early changes occurring in fetal and maternal vascular haemodynamics before fetal compromise has an adverse effect on the fetus. This research project attempts to address this gap in knowledge and explore early changes in Doppler parameters in both normal and compromised fetuses, as well as test their usefulness as predictors in adverse outcomes.

Hypotheses and specific aims

First Hypothesis: There exists a relationship between flow haemodynamics of fetal central shunts and fetal cerebral resistance in fetuses with adverse pregnancy outcomes.

The specific aims are:

1. To establish reference ranges for the Doppler flows of central shunts i.e. ductus arteriosus (DA) and foramen ovale (FO) by serial measurements, in a longitudinal study (**Study 1**).
2. To prospectively evaluate fetal biometry and Doppler waveforms of maternal uterine arteries, fetal umbilical artery, ductus venosus, middle cerebral artery, FO, DA in high risk and uncomplicated pregnancies (**Study 3**).
3. To compare Doppler flows in central shunts with middle cerebral artery flows and cerebroplacental ratio in normal and adverse pregnancy outcomes (**Study 3**).

Second Hypothesis: Fetal central shunts demonstrate haemodynamic alterations in acute and chronic fetal cardiovascular stress in compromised fetuses.

The specific aims are:

1. To investigate the acute haemodynamic alteration in intracardiac and other fetal shunts as a response to the acute cardiovascular stress of fetal transfusion using Doppler ultrasound (**Study 2**).
2. To examine sequential haemodynamic alterations in intracardiac and other fetal shunts in pregnancies at risk for UPI with a prospective longitudinal

Doppler ultrasound study and compare these values with those values obtained in uncomplicated pregnancies (**Study 3**).

Third Hypothesis: Changes in fetal central shunt flows precede the reduction in fetal biometry and the ‘brain-sparing’ effect in fetal hypoxia or maternal disease in UPI, representing earlier intracardiac redistribution of fetal blood flow in these high-risk pregnancies (**Study 3**).

The specific aims are:

1. To evaluate maternal vascular insufficiency with serial evaluation of uterine artery Doppler flow waveforms (**Study 3**).
2. To identify fetal compromise and reduction of fetal growth with serial evaluation of fetal biometry and umbilical artery and ductus venosus Doppler flow waveforms (**Study 3**).
3. To identify brain sparing in adverse pregnancy outcomes as evidenced by middle cerebral artery Doppler flow waveforms, and Doppler cerebroplacental ratio and to compare foramen ovale and ductus arteriosus Doppler flows in these fetuses with Doppler flow patterns obtained in fetuses in normal pregnancies (**Study 3**).
4. To identify sequence of redistribution of fetal intracardiac and cerebral flows in compromised fetuses (**Study 2 and 3**).

Fourth Hypothesis: The haemodynamic changes in fetal shunts and maternal uteroplacental haemodynamics are associated with abnormal placentation.

The specific aim is to assess the association between Doppler haemodynamics of fetal central shunts, fetal arterial circulation and uteroplacental haemodynamics with placental histopathology and placental bed biopsies. (**Study 4**).

The next part of this chapter details the study design and research methodology for these four studies, in accordance with the guidelines and checklist provided by the STROBE statement [277].

Study Design

Studies 1, 3 and 4 were prospective longitudinal observational studies in a cohort of high risk and low risk pregnancies.

Study 2 was prospective and observational in pregnancies undergoing intrauterine fetal transfusion for fetal anaemia caused by alloimmunisation.

Recruitment strategies

Study population included patients attending the Women's and Children's Hospital, in North Adelaide, an academic tertiary care teaching medical centre.

Ethics approval was obtained from Women's and Children's Hospital Research Ethics Committee. All eligible patients were fully informed about the purpose of the study and the voluntary nature of participation was explained. Informed consent was obtained from all participants.

Many promotional activities were undertaken to encourage appropriate-risk subjects to participate in the trial. Posters were erected throughout the hospital, requesting voluntary participation of patients. Presentations were given in both the high risk and low risk clinics to clinicians and midwives at the hospital, to encourage referral of patients in the study.

The investigators initiated a previously unavailable service and made it accessible at no cost. A clinic named the 'nuchal translucency' clinic was established particularly for high-risk patients. Nuchal translucency measurements were offered and performed between 11 to 14 weeks. During this visit, the research project was introduced and discussed with the eligible patients and patients were invited to participate in the study. Appropriate screening for chromosomal and major structural abnormalities was undertaken and an early dating scan was performed to confirm gestational age. This also encouraged study participation.

Patient classification

The recruited patients were classified into two categories-the high risk and low risk.

Low risk classification

Patients defined as low risk for developing adverse pregnancy outcomes due to UPI, were included as controls.

A '**Control**' was defined as 'low risk' pregnant patients with no previous pregnancy complications or no history of maternal conditions which might be risk factors for UPI in the current pregnancy.

High risk classification

A '**high-risk**' patient was defined as a patient at high-risk for development of UPI.

UPI was defined as preeclampsia, gestational hypertension, placental abruption and SGA < 10th centile due to placental insufficiency.

Hence patients were recruited as high risk if they fulfilled any of the following criteria:

1. Previous history of the following
 - Severe preeclampsia / eclampsia
 - Severe IUGR
 - Previous placental abruption
 - Pre-term birth
 - Greater than or equal to than 3 miscarriages
 - Fetal demise
2. Diabetes / insulin resistance
3. Chronic hypertension / high BMI / PCOS

Study 1: Normograms of fetal central shunts and other parameters

Inclusion criteria: Study 1

Study 1 was a part of an ongoing case-cohort longitudinal study (Study 3). It comprised of a cohort of women considered as ‘controls’, as defined earlier.

Exclusion criteria: Study 1

Participants were excluded from participating in the study in the presence of any of the following:

- Multifetal pregnancies
- Known structural or chromosomal anomalies or fetal demise
- Lack of informed consent.
- Any maternal or fetal condition requiring termination of pregnancy.

60 women fulfilled the criteria of low risk and were included as participants for study 1, of which 54 were included in the final analysis.

Variables for study 1

The variables evaluated for the study 1 is as follows.

Shunts: Doppler pulsatility index (PI) of FO and DA, Doppler PIV, PVIV, S/A ratio and S/D ratio of ductus venosus

Uteroplacental and fetoplacental haemodynamics: Doppler PI and resistance index (RI) for umbilical artery (UA RI), Doppler RI for both uterine arteries, from which mean uterine RI (mean RI) was calculated. Uteroplacental ratio was calculated as a ratio of mean RI to umbilical RI.

Cerebral flow evaluation: Doppler RI of middle cerebral artery (MCA RI) and cerebroplacental ratio (CPR). CPR was defined as a ratio of MCA PI to umbilical RI. All Doppler measurements were made according to techniques previously described. [1-7]. Details of Doppler methodology are provided later in this chapter.

Biometry: The serial measurements for this study also included evaluation of fetal growth trajectory and well being with biometric BPD, HC, AC, FL and amniotic fluid Index. In addition, placental thickness was also measured. Biometric and placental measurements were made as per previously established techniques [8-10]. All recruited control patients underwent serial scans from 16 weeks onwards, and were scanned at regular intervals at 16 weeks, 20 weeks, 24 weeks, 26 weeks, 28 weeks, 30 weeks, 32 weeks, 34 weeks, 36 weeks and 38 weeks, where all the above variables were scanned and stored as digital images.

Figure 20 describes the flow chart regarding study design for study 1 (Normogram study.)

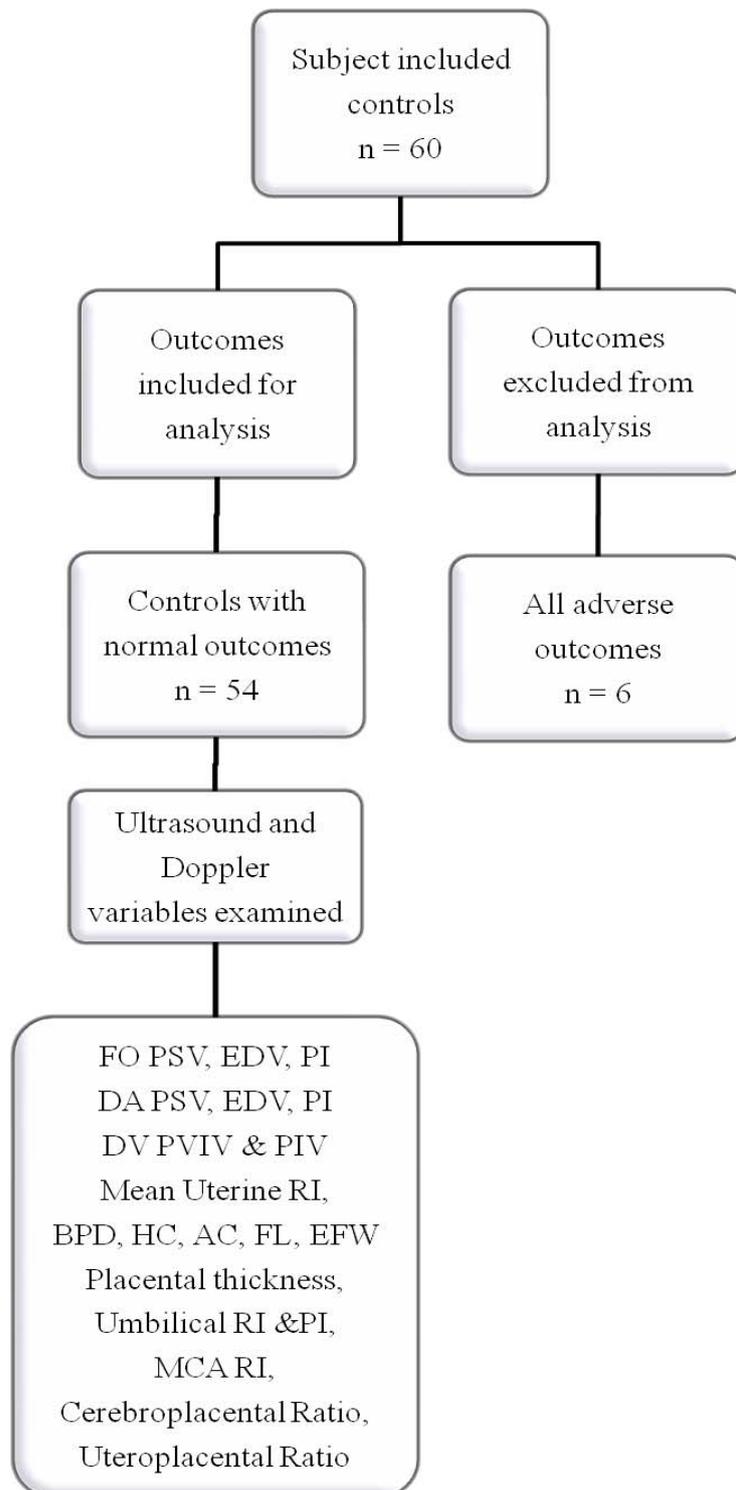


Figure 20 Flow chart describing study design for Study 1

Table 6 describes the number of total observations in study 1. In some circumstances, data was not recorded in the presence of fetal activity or breathing, as it was against the designated scan protocol; previously published data has established that fetal movements and breathing can have an influence on fetal Doppler flow waveforms [278-280]. By default this led to some missing data. Statistical analysis addressing missing data is discussed in a later section in this chapter. Description of the number of observations for each variable at every time point of examination is provided in chapter 4, in the results section for study 1.

Table 6 Number of observations for Ultrasound and Doppler variables in Study 1

	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
umbilical artery - resistance index	496	98.8	6	1.2	502	100
umbilical artery - pulsatility index	497	99.0	5	1.0	502	100
umbilical artery - systolic diastolic ratio	497	99.0	5	1.0	502	100
MCA RI	490	97.6	12	2.4	502	100
MCA PI S-D/Average of Sand D	490	97.6	12	2.4	502	100
cerebroplacental ratio mca ri/ ua ri	487	97.0	15	3.0	502	100
uteroplacental ratio mean ut ri/ umb ri	471	93.8	31	6.2	502	100
ductus venosus preload index (s-a)/s	484	96.4	18	3.6	502	100
ductus venosus peak velocity index(s-a)/d	365	72.7	137	27.3	502	100
ductus venosus - PIV (s-a)/tamx	419	83.5	83	16.5	502	100
ductus venosus s/a ratio	484	96.4	18	3.6	502	100
ductus arteriosus - peak systolic velocity	487	97.0	15	3.0	502	100
ductus arteriosus - end diastolic velocity	487	97.0	15	3.0	502	100
DA PI	487	97.0	15	3.0	502	100
foramen ovale - peak systolic velocity	488	97.2	14	2.8	502	100
foramen ovale - end diastolic velocity	488	97.2	14	2.8	502	100
FO PI	480	95.6	22	4.4	502	100
mean Ut RI	474	94.4	28	5.6	502	100
placental thickness	455	90.6	47	9.4	502	100
Bi Parietal Diameter	497	99.0	5	1.0	502	100
head Circumference	497	99.0	5	1.0	502	100
abdominal Circumference	495	98.6	7	1.4	502	100
Femur Length	497	99.0	5	1.0	502	100

Endpoint for study 1: normal outcome

For statistical analysis, study 1 included controls that had normal clinical outcomes.

‘Normal outcome’ was defined as one where all the three following criteria were satisfied.

1. There was an absence of any antenatal or postnatal maternal complications.
2. There was no abnormality in growth trajectory antenatally on ultrasound.
3. There was an absence of any antenatal or postnatal neonatal complications on the basis of the examination made by the attending paediatrician at the time of discharge.

Statistical methods for study 1

SPSS version 14 software package for Windows (SPSS Inc, Chicago, IL, 2005) was used for statistical analysis

The majority of data was obtained within one week of the nominal gestational ages of 16, 20, 24, 26, 28, 32, 36 and 38 weeks. Gestational age estimation was determined on the basis of the earliest scan, accepting the known 5-6 day variability with even the earliest scan. As exact gestational ages were unobtainable in every case, ages in completed weeks were adjusted to avoid bias by addition of 0.5 weeks [281]. The means and upper and lower limits of 95 % confidence intervals were calculated for all the ultrasound and Doppler variables, using SPSS package, version 14.

Results of Study 1 are described in chapter 4 of this thesis.

Study 2 : Fetal shunts and acute adaptive mechanisms- haemodynamics before and after Intra-Uterine Transfusion (IUT).

Inclusion criteria

All women with fetuses undergoing IUT between March 2006 to August 2006 for fetal anaemia were considered eligible for study 2. Patients were recruited in this part of the study after obtaining informed consent; 12 transfusion procedures were initially recruited for further analysis.

Exclusion criteria

Women were excluded from study 2 if there was no informed consent. Patients with a successful continuation of pregnancy after intrauterine fetal transfusion, were included for statistical analysis

One subject with a fetus, who had to be delivered within 24 hours of the procedure, was excluded from statistical analysis. Thus, data from 11 transfusion procedure were included in the final statistical analysis.

Fetal transfusion procedure

Intrauterine transfusion procedure involved a direct intravascular transfusion of cross-matched, O rhesus negative blood and was performed by the maternal fetal medicine consultant after calculating the volume of blood to be transfused by a formula incorporating fetal haematocrit, donor haematocrit and fetoplacental blood volume.

Fetal heart rate was monitored intermittently throughout transfusions.

Post-procedural Doppler measurements of all the above mentioned parameters were completed with 2 hours of the procedure.

Variables for study 2

The variables evaluated for the study 2 included the following:

Shunts: Doppler PI of FO and DA, Doppler PIV, PVIV, S/A ratio and S/D ratio of ductus venosus

Fetoplacental haemodynamics: Doppler PI and RI for umbilical artery (UA RI) ,

Cerebral flow evaluation : Doppler RI of middle cerebral artery (MCA RI)

Biometry: The serial measurements for the trial also included evaluation of fetal weight using biometric measurements BPD, HC, AC, FL.

Table 7 describes the number of observations of all the variables in Study 2.

Figure 21 is a flow diagram describing the study design for study 2.

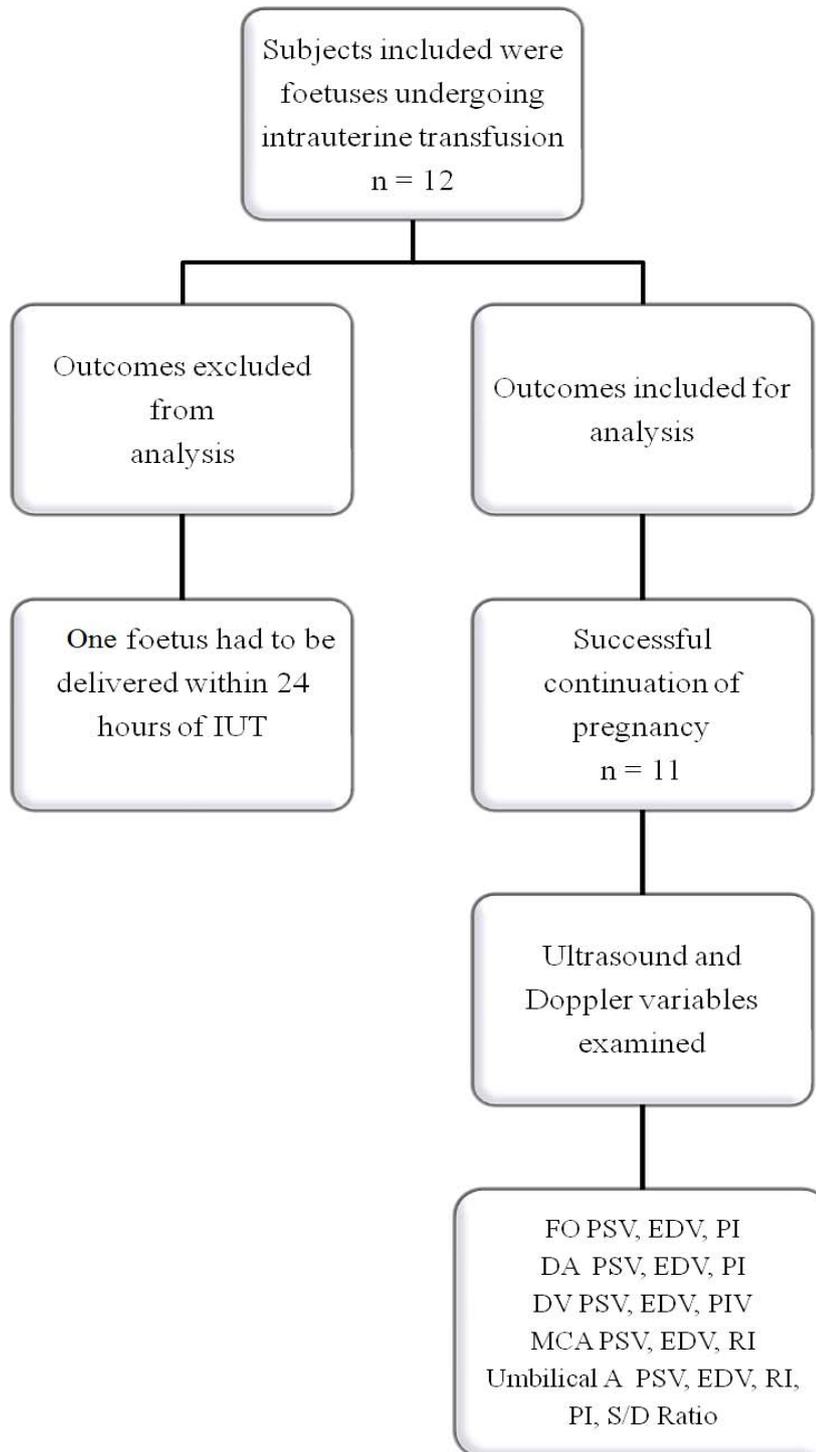


Figure 21 Flow chart describing the study design for Study 2

Table 7 Number of observations for Ultrasound and Doppler variables in Study 2

Ultrasound and Doppler variables	Valid N	Missing
umbilical artery - resistance index	22	2
umbilical artery - pulsatility index	22	2
umbilical artery - systolic diastolic ratio	22	2
middle cerebral artery - peak systolic velocity	24	0
middle cerebral artery - resistance index	23	1
middle cerebral artery - systolic diastolic ratio	24	0
ductus venosus - peak systolic velocity 1	22	2
ductus venosus - peak systolic velocity 2	23	1
ductus venosus - end diastolic velocity	23	1
ductus venosus - PIV (1 - edv)	23	1
ductus venosus - systolic / diastolic ratio	22	2
ductus arteriosus - peak systolic velocity	24	0
ductus arteriosus - end diastolic velocity	24	0
ductus arteriosus - pulsatility index	24	0
foramen ovale - peak systolic velocity	24	0
foramen ovale - end diastolic velocity	24	0
foramen ovale - pulsatility index	24	0

Statistical methods for Study 2

SPSS version 14 software package for Windows (SPSS Inc, Chicago, IL, 2005).

Normality of distribution was tested by Shapiro and Wilk test. Data was assessed with Students paired t test, with each fetus serving as its own control. Wilcoxon signed rank test was used, for non-parametric assumption. $P < 0.05$ was considered to be significant.

Results for study 2 are described in chapter 5 of this thesis.

Study 3 and 4

Study 3 was a study of fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in UPI.

Study 4 was a study of fetal and maternal Doppler flow haemodynamics – a correlation with adverse clinical and placental outcomes.

Studies 3 and 4 were ‘case-control’ studies and therefore included two cohorts each.

These included high risk and low risk pregnancies. They were classified as ‘high risk’ or low risk, based on their obstetric and medical history.

Inclusion criteria for study 3 and study 4

For participation in study 2 and study 3, the ‘low-risk’ and ‘high risk’ cohort of patients was included. Details of the patient classification have been elaborated earlier.

Table 8 describes the classification for allocation of patients into high risk categories.

Table 8 Classification of high risk

History	n
P/H/O Preeclampsia	25
P/H/O IUGR	14
P/H/O Preterm birth	24
P/H/O recurrent first trimester loss	53
P/H/O fetal loss >12 weeks	22
Previous poor pregnancy outcome	5
Prior placental abruption	7
Thrombophilia	11
Insulin Resistance	11
Current H/O oocyte donation	32

Key: P/H/O= past history of, n= total number of cases out of total 170 high risk cases.

Exclusion criteria

Exclusion criteria for participation in study 3 and study 4 were similar to those in study 1.

Variables for study 3 and 4 are the same as for study 1.

Sample size calculations for study 3

To detect a difference of 15%, with a power of 90% and a smaller probability of 0.05, it was estimated that a sample size of 88 women with adverse perinatal outcome would be required for study 3.

Assuming an 80% successful recruitment rate, with an estimate of 25-30% adverse perinatal outcome, 3 years recruitment was estimated to provide around 100 cases with fetal/neonatal and/or maternal morbidity and/or mortality.

For study 3, 240 women, including controls and high-risk pregnancies fulfilled the eligibility criteria and were invited to participate in the study. Five patients declined the invitation to participate. Reasons for non-participation were either lack of time commitments due to full-time work or being primary carer for little children or having to relocate interstate or overseas. Two patients miscarried before 20 weeks and hence had to be excluded. 233 women with singleton pregnancies, comprising of 63 controls and 170 high-risk, consented and were recruited between March 2004 to March 2007.

For statistical analysis, patients with unexplained preterm births and gestational diabetes were excluded from the UPI analysis. Unexplained preterm births were analysed separately. For the final statistical analysis for study 3, a total of 201 patients were included, of which 132 patients had normal outcomes and were compared to 69 patients with adverse outcomes as recruited by the aforementioned UPI definition.

All recruited patients underwent serial scans from 16 weeks onwards, and were scanned at regular intervals at 16 weeks, 20 weeks, 24 weeks, 26 weeks, 28 weeks, 30 weeks, 32 weeks, 34 weeks, 36 weeks and 38 weeks, where all the above variables were scanned and stored as digital images. Variables under consideration were the same as variables for study 1, described above.

Table 9 and Table 10 describe the number of total observations in study 3. Missing data can be attributed to absence of data recording in the presence of fetal movements and breathing because of its influence on fetal Doppler flow waveforms[278]. This led to some missing data as explained in study 1

Figure 22 describes the flow chart regarding study design for study 3 (clinical outcomes).

Statistical analysis addressing missing data is discussed in a later section in this chapter.

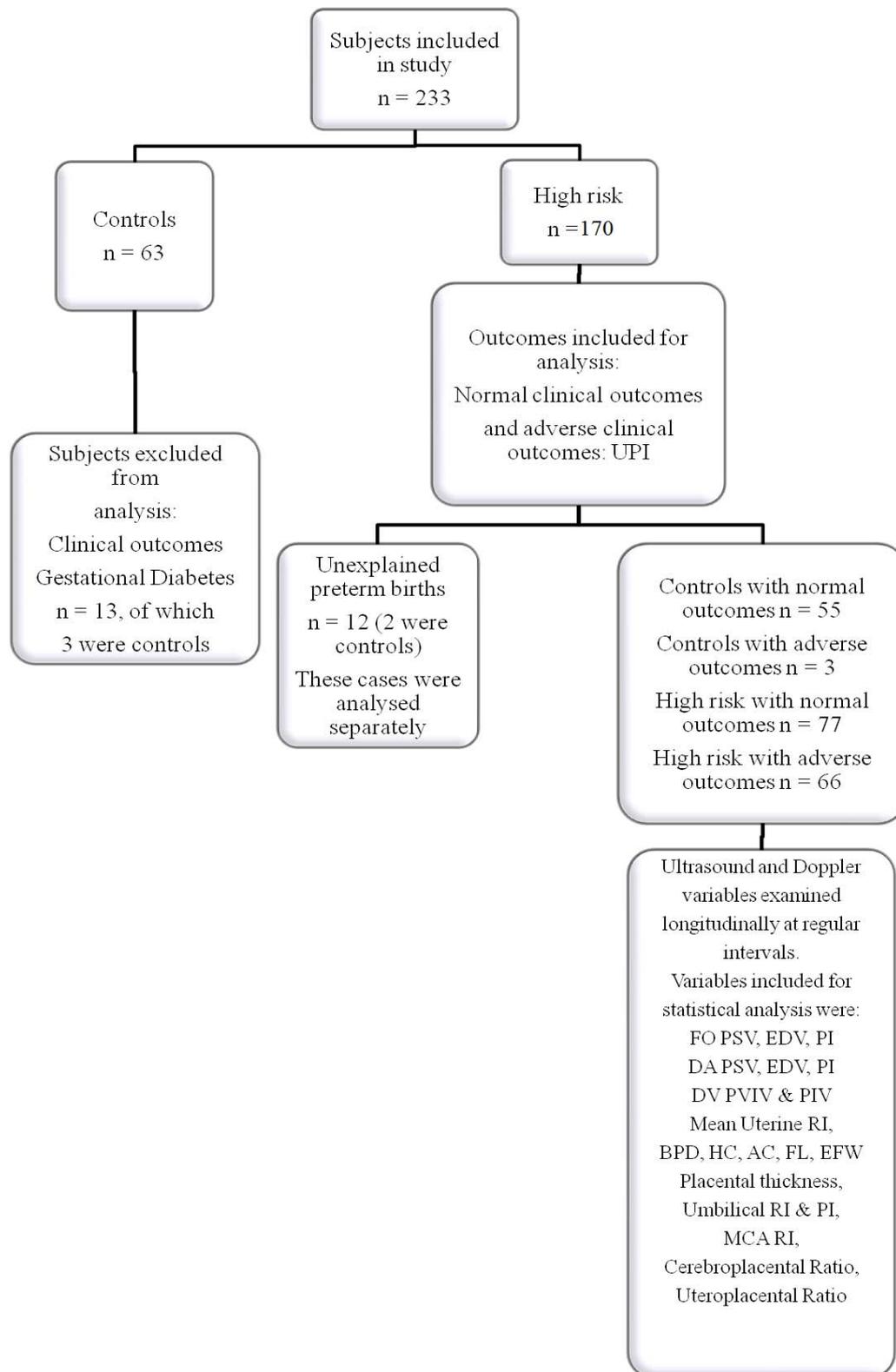


Figure 22 Flow chart describing study design for Study 3

Table 9 Number of observations for Ultrasound and Doppler variables in Study 3

Variable	n = total number of observations
Umbilical artery – RI	1954
Umbilical artery – PI	1919
MCA RI	1930
Cerebroplacental ratio	1905
Uteroplacental ratio	1844
DV Preload index (s-a)/s	1916
DV Peak Velocity index(s-a)/d	1545
DV - PIV (s-a)/tamx	1390
DV s/a ratio	1916
DV systolic / diastolic ratio	1551
DA PI	1839
FO PI	1816
Uterine artery notch	1879
Mean Ut RI	1866
placental thickness	1737
Bi Parietal Diameter	1941
Head Circumference	1942
Abdominal Circumference	1933
Femur Length	1937
Amniotic Fluid index	1201
HC/AC ratio	1933
Estimated fetal weight	1928

Table 10 Number of observations at every time point for study 3.

		Weeks of gestation									
		16	20	24	26	28	30	32	34	36	38
DA PI	n	132	161	200	204	198	202	194	198	168	77
	%	7.2	8.8	10.9	11.1	10.8	11.0	10.6	10.8	9.1	4.2
FO PI	n	136	155	195	203	192	198	195	196	161	82
	%	7.5	8.5	10.7	11.2	10.6	10.9	10.7	10.8	8.9	4.5
DV PIV	n	89	107	146	157	150	151	157	151	139	65
	%	6.4	7.7	10.5	11.3	10.8	10.9	11.3	10.9	10.0	4.7
Umb RI	n	161	174	203	206	207	208	202	202	179	94
	%	8.2	8.9	10.4	10.5	10.6	10.7	10.3	10.3	9.2	4.8
Umb PI	n	151	168	201	209	204	206	200	199	178	93
	%	7.9	8.8	10.5	10.9	10.6	10.7	10.4	10.4	9.3	4.8
Mean RI	n	162	175	206	204	208	205	201	200	165	34
	%	8.6	9.3	11.0	10.9	11.1	10.9	10.7	10.6	8.8	1.8
Pla thi	n	151	163	188	182	180	183	175	182	151	78
	%	8.7	9.4	10.8	10.5	10.4	10.5	10.1	10.5	8.7	4.5
MCA RI	n	159	173	205	214	204	207	200	202	172	77
	%	8.2	9.0	10.6	11.1	10.6	10.7	10.4	10.5	8.9	4.0
CPR	n	159	173	203	206	204	207	200	202	172	77
	%	8.2	9.0	10.4	10.5	10.6	10.7	10.4	10.5	8.9	4.0
BPD	n	162	175	206	203	204	208	200	204	173	88
	%	8.4	9.0	10.6	10.5	10.5	10.7	10.3	10.5	8.9	4.5
HC	n	162	175	206	203	204	208	200	204	174	88
	%	8.4	9.0	10.6	10.5	10.5	10.7	10.3	10.5	9.0	4.5
AC	n	160	174	206	202	204	207	199	204	171	88
	%	8.3	9.0	10.7	10.5	10.6	10.7	10.3	10.6	8.9	4.6
FL	n	160	175	206	203	204	208	200	203	172	88
	%	8.3	9.0	10.6	10.5	10.5	10.7	10.3	10.5	8.9	4.5
EFW	n	160	174	206	202	204	207	199	204	171	88
	%	8.3	9.0	10.7	10.5	10.6	10.7	10.3	10.6	8.9	4.6

Study 4 was a study of comparison of Doppler variables in clinical and placental outcomes. Hence all patients from study 3 (n = 233) were eligible for participation in study 4. At birth, placentas were collected by research midwives; further histopathological analysis was performed by placental pathologists.

Scan protocols and variables under consideration were same as variables for study 1 and 3 as described above.

For statistical analysis of study 4, all subjects in whom placental histopathology and/or placental bed biopsy was available, were considered.

Again, clinical outcomes gestational diabetes and unexplained preterm births were excluded from the analysis for UPI, and unexplained preterm births were analysed separately.

After excluding gestational diabetes and preterm births, 184 placentas were available for histological examination, and therefore these patients were included in the final statistical analysis for study 4.

Figure 23 describes the flow chart regarding study design for study 4.

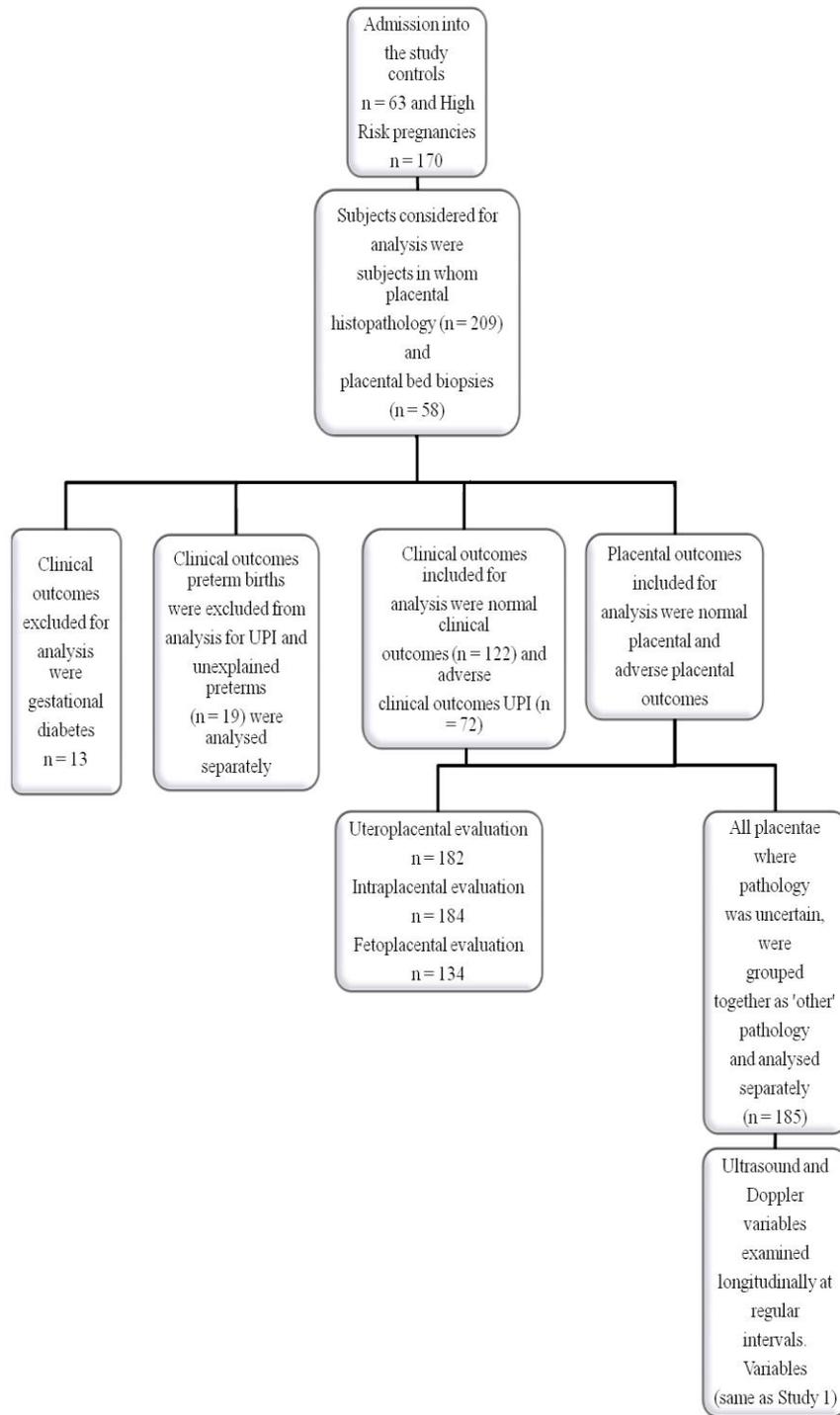


Figure 23 Flow chart describing study design for Study 4

Table 11 describes the number of total observations in study 4. Missing data can be attributed to recruitment of patients after 16 weeks of gestation (recruitment window being 16 to 20 weeks), exclusion of observations from patients with gestational diabetes as well as unexplained preterm births, absence of data recording in the presence of fetal movements and breathing as well as an absence in placental histology. Statistical analysis addressing missing data is discussed in a later section in this chapter.

Table 11 Number of observations for Ultrasound and Doppler variables in Study 4

Ultrasound and Doppler variable	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
Umbilical artery - resistance index	1638	82.0	359	18.0	1997	100
Umbilical artery – Pulsatility index	1604	80.3	393	19.7	1997	100
MCA RI	1615	80.9	382	19.1	1997	100
Cerebroplacental ratio	1597	80.0	400	20.0	1997	100
Ductus venosus preload index (s-a)/s	1603	80.3	394	19.7	1997	100
Ductus venosus peak velocity index(s-a)/d	1289	64.5	708	35.5	1997	100
Ductus venosus - PIV (s-a)/tamx	1134	56.8	863	43.2	1997	100
Ductus venosus s/a ratio	1603	80.3	394	19.7	1997	100
Ductus venosus - systolic / diastolic ratio	1295	64.8	702	35.2	1997	100
Ductus arteriosus - peak systolic velocity	1551	77.7	446	22.3	1997	100
Ductus arteriosus - end diastolic velocity	1549	77.6	448	22.4	1997	100
DA PI	1531	76.7	466	23.3	1997	100
Foramen ovale - peak systolic velocity	1557	78.0	440	22.0	1997	100
Foramen ovale - end diastolic velocity	1549	77.6	448	22.4	1997	100
FO PI	1512	75.7	485	24.3	1997	100
Mean Ut RI	1559	78.1	438	21.9	1997	100
Placental thickness	1455	72.9	542	27.1	1997	100
Bi Parietal Diameter	1628	81.5	369	18.5	1997	100
Head Circumference	1628	81.5	369	18.5	1997	100
Abdominal Circumference	1619	81.1	378	18.9	1997	100
Femur Length	1624	81.3	373	18.7	1997	100
Estimated fetal weight	1615	80.9	382	19.1	1997	100

Endpoint: The primary outcome was UPI.

Outcome measures for statistical analysis

Clinical outcomes for study 3 and 4: UPI and unexplained preterm birth (analysed separately)

Placental outcomes for study 4: Histopathological evidence of UPI.

Definitions for clinical outcomes

Preterm birth: Spontaneous onset of labour and delivery completed before 37 weeks.

Hypertensive disorders in pregnancy were classified following the consensus statement from the Australasian Society for Study of Hypertension in pregnancy (ASSHIP)[282].

Hypertension: Hypertension in pregnancy was diagnosed when Systolic blood pressure is ≥ 140 mmHg and/or Diastolic blood pressure is ≥ 90 mmHg.

Gestational hypertension is hypertension arising in pregnancy after 20 weeks gestation without any other feature of the multisystem disorder preeclampsia and which resolves within 3 months postpartum.

Preeclampsia was defined as hypertension arising after 20 weeks of gestation associated with one or more of;

Proteinuria - > 300 mg/24 h or spot urine protein/ creatinine ratio ≥ 30 mg/mmol

Renal insufficiency - serum/plasma creatinine ≥ 0.09 mmol/L or oliguria

Liver disease - raised serum transaminases and/or severe epigastric or right upper quadrant pain

Neurological problems - convulsions (eclampsia); hyperreflexia with clonus; severe headaches with hyperreflexia; persistent visual disturbances (scotomata)

Haematological disturbances - thrombocytopenia; disseminated intravascular coagulation; haemolysis

Intrauterine Growth Restriction versus SGA

IUGR: Three different endpoints were used to define IUGR and/or SGA. The three methods of classification were as follows:

SGA: < 10th centile by customised centiles as well as by Australian population centiles.

1. Birthweight < 10th centile by previously published Australian population centile charts. [283]
2. Birthweight < 10th centile by customised centiles using customised centile charts.[284] The customised centile calculator used was GROW centile v 5.1.2006., downloaded from the Gestation network, (www.gestation.net)
3. IUGR classification was based on ultrasound.

IUGR by ultrasound: A sonographic criteria of EFW below the 10th centile using appropriate population based growth charts would lead to a diagnosis of approximately 70% of infants identified as IUGR in normal or constitutionally small but normal infants [285]. Therefore, after an early dating scan established the gestational age, sonographic growth curves using ASUM biometric charts were used in the diagnosis of IUGR. Reduction of fetal growth as observed on serial ultrasound scans was used as a criterion for diagnosis.

IUGR by ultrasound was defined as a serial tapering of growth in AC / EFW.

Mild IUGR was defined as serial tapering in growth of AC, associated with a HC to AC disproportion of 2 to 4 weeks for gestation, despite the EFW and umbilical artery Doppler resistance remaining within the normal range.

Moderate IUGR was defined as asymmetric growth restriction, with the following criteria:

- A progressive slowing or flattening in the growth trajectory of AC and EFW with a HC to AC disproportion of more than 4 weeks for gestation’.
- Normal or high resistance flows on umbilical artery Doppler evaluation.

Severe IUGR was defined as

- A severe reduction in ultrasound biometric measurements < 5th centile.

- Associated worsening circulatory haemodynamics such as absent or reverse diastolic flows on umbilical artery Doppler evaluation.

Constitutional small fetus (CSA) was defined as a fetus with small measurements consistently throughout pregnancy on 5th centile, with no abnormalities in umbilical artery Doppler or in the biophysical activity.

Placental outcomes for study 4 were determined by placental histopathology and placental bed biopsies.

Definitions for placental outcomes

Uteroplacental pathology was defined as pathology within maternal vasculature, and manifest as unopened spiral arteries, acute atherosclerosis, placental infarction or intraluminal endovascular trophoblast in the third trimester

Placental pathology was defined as pathology affecting only the placental villi.

Fetoplacental pathology was defined as pathology within the fetal vasculature, and manifest as fetal thrombotic vasculopathy (avascular villi, fetal artery thrombosis)

‘Other’ pathology : was defined as pathology where histological changes seen and considered “possible pathology”, but could not be classified into either of the above three categories : such as amniotic fluid infection, meconium, intervillous thrombus; accelerated maturation, fibrin intimal cushions, acute chorioamnionitis, chorangioma or cord hemangioma .

“Any” pathology was defined as any of the above, including pathology of uncertain significance.

Normal placental outcome was defined as placenta demonstrating normal histopathology, having none of the above features.

Placental bed biopsies (PBB) were classified into “open”, “closed”, “indeterminate” or “non-placental bed”, based on the vasculature seen within the specimen. “Open” was considered normal. PBBs with “indeterminate” and “non-placental bed” were excluded from statistical analysis to prevent “muddying” of data.

Table 12 describes all placental outcomes included for analysis after exclusion of Diabetes, preterm births, “indeterminate PBB” and “non- placental PBB”.

Table 12 Placental outcomes evaluated for histopathology

Placental outcomes	n/N where n= pathology and N= Number of samples included for analysis
Uteroplacental morphology	26/ 182, (12.8%)
Placental morphology	28 / 184, (13.7%)
Fetoplacental morphology	5 / 175, (2.6%)
Other placental morphology	124 /185 (60.2%)
Placental bed biopsies	7 / 22 (12.1%)

Statistical methods for study 3 and 4

SPSS version 14 software package for Windows (SPSS Inc, Chicago, IL, 2005) and GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA), were used for statistical analysis.

Data entry: SPSS version 14 was used to create the database. Patient histories and clinical outcomes were entered as dichotomous outcomes. Categories for histories were high-risk or control, high-risk adverse outcome or high-risk normal outcome etc past H/O Preeclampsia, past H/O IUGR, past H/O Preterm birth, past H/O RMC past H/O fetal loss above 12 weeks, IVF conception with present pregnancy, and pregnancy after oocyte donation .

Data entry

For study 1, 3 and 4, categories for data entry for clinical outcomes were as follows:

- *UPI*
- *Preeclampsia (all)*
- *severe preeclampsia with intervention ≤ 30 weeks*
- *severe preeclampsia with intervention ≤ 37 weeks*
- *preeclampsia of late onset ≥ 37 weeks*
- *SGA by Australian centiles ($< 10^{\text{th}}$ centile)*
- *whether IUGR or no IUGR in present pregnancy based on ultrasound*
- *severity of IUGR- constitutionally small but normal (CSA), mild, moderate or severe based on ultrasound*
- *whether IUGR or no IUGR in present pregnancy based on Australian population centiles.*
- *whether IUGR or no IUGR in present pregnancy based on customized centile charts*
- *gestational age at birth in days*
- *baby customised centiles ($< 10^{\text{th}}$, $> 10^{\text{th}}$ centile)*
- *whether born ≤ 37 weeks, i.e. ≤ 259 days*
- *categories for all “causes” for preterm birth were entered as “UPI” if delivery was undertaken due to UPI, “other known causes” for preterm was associated with other risk factors such as diabetes, congenital anomalies of the uterus, induction of labour was undertaken for other reasons, and “spontaneous unexplained “ if no known causes or risk factors were identified*

Ultrasound biometry and Doppler variables for all the studies were entered as continuous data. They were entered under the following headings, where applicable:

- *umbilical artery peak systolic flow*
- *umbilical artery end diastolic velocity*
- *umbilical artery end diastolic velocity*
- *umbilical artery - resistance index*
- *umbilical artery - pulsatility index*
- *umbilical artery - systolic diastolic ratio*
- *middle cerebral artery - peak systolic velocity*
- *middle cerebral artery - end diastolic velocity*
- *MCA RI*
- *middle cerebral artery - systolic diastolic ratio*
- *middle cerebral artery PI*
- *cerebroplacental ratio of mca PI/ua PI*
- *uteroplacental ratio mean ut PI/ umb RI*
- *ductus venosus - peak systolic velocity*
- *ductus venosus - peak forward velocity early diastole*
- *ductus venosus -lowest forward or peak reversed late diastole during atrial contraction*
- *ductus venosus preload index (s-a)/s*
- *ductus venosus peak velocity index(s-a)/d*
- *ductus venosus - PIV (s-a)/tamx*
- *ductus venosus s/a ratio*
- *ductus venosus - systolic / diastolic ratio*
- *ductus arteriosus - peak systolic velocity*
- *ductus arteriosus - end diastolic velocity*
- *DA PI*
- *FO - peak systolic velocity*
- *FO - end diastolic velocity*
- *FO PI*

- *right uterine artery PSV*
- *right uterine artery EDV*
- *right uterine artery RI*
- *left uterine artery PSV*
- *left uterine artery EDV*
- *left uterine artery RI*
- *uterine artery notch present or absent*
- *uterine artery notch- unilateral, bilateral or absent*
- *Mean Ut RI*
- *placental thickness*
- *Bi Parietal Diameter (BPD)*
- *Head Circumference (HC)*
- *Abdominal Circumference (AC)*
- *Femur Length (FL)*
- *amniotic fluid index (AFI)*
- *HC/AC ratio*
- *FL/AC ratio*

For study 4, placental outcomes were entered as dichotomous outcomes. Categories used were uteroplacental, placental and fetoplacental pathology. In outcomes where there was an uncertainty regarding the presence or absence of pathology, the case was excluded from that particular category and reclassified under 'other' placental pathology. Placental histopathology from patients with unexplained preterm births or diabetes in pregnancy was excluded from analysis. Ultrasound biometry and Doppler variables were entered as continuous data.

Statistical methods for analysis of categorical data

Univariate relations between variables were assessed by Pearson correlation coefficients. Pearson Bivariate Correlation analysis was used to evaluate relationships

between the Doppler Indices and clinical outcomes. All tests were 2-tailed, and probability values were considered significant at the .05 level.

Fisher's exact test was used to detect statistical significance in categorical data, ie to examine the significance of association between clinical and placental outcomes in a 2 x 2 contingency table, with the help of Graphpad version 5; p values <0.05 were considered significant.

Statistical analysis methods for longitudinal data

For each of the ultrasound and Doppler variables, longitudinal analysis by univariate analysis of variance using the linear mixed effects regression model, also known as mixed linear models. Longitudinal analysis of all variables was performed with F test-UNIANOVA, to compare the clinical and Doppler data among the groups of subjects. The test statistic for UNIANOVA is the F statistic, which measures the ratio of between-group variability to within-group variability, p values <0.05 considered significant.

Mixed linear models

Longitudinal analysis for each variable for clinical and placental outcome was performed with univariate as well as multivariate analysis.

Mixed linear models have multiple advantages over generalised linear models, as discussed by Geert and Geert [286].

Mixed linear models can analyze data that exhibit correlation and non-constant variability. It does not assume that an equal number of repeated observations is taken from each individual or that all individuals should be measured at same time points. It works with repeated measures designs, including incomplete repeated measurements in which the number of observations varies across subjects, so that all available data, not only the complete cases, are used in the analysis. It is able to address imbalance in longitudinal data, account for missing data as well as clustering of longitudinal data[287].

In mixed linear models, the measurements can be viewed as being taken at a continuous rather than discrete time scale [286]. It can analyse data that exhibit correlation and non-constant variability. It models not only means but also variances

and covariances in data. Furthermore, the use of random effects allows us to model covariances as continuous functions of time and therefore allowing repeated measures analyses with time-dependent covariates to include time varying covariates in the mean structure, yielding unbiased estimate of parameter variability [286]. The mixed effect models are therefore less restrictive, allow more flexible and efficient use of available data and increase the accuracy of estimation [288]. The mixed linear model was therefore chosen as a suitable model for analysis of the longitudinal observational data in this thesis.

For each of the ultrasound and Doppler variables, longitudinal analysis was performed by univariate analysis of variance using the linear mixed effects regression model. Linear pair wise comparisons of estimated marginal means (EMMEANS), also known as modified population marginal means or predicted means, of the variables were done for clinical outcomes at different gestational ages EMMEANS were preferred over observed means, to account for all factors in the model of the longitudinal data under consideration, details of which are described in Chapter 3. The F test was based on the linearly independent pair wise comparisons of EMMEANS for adverse outcomes as well as normal outcomes. The main effects were compared with least significant difference (LSD) confidence level adjustment; mean difference was significant at the 0.05 level.

Results for study 3 and study 4 are described in chapters 6 and 7 respectively.

Quality assurance and quality control measures

One of the limitations of observational studies is that the results can be distorted by different factors, which include errors, bias, and confounding factors, hence potential sources of errors were identified and several quality assurance and quality control measures were put in place in an effort to minimise them.

Strategies to minimise errors and bias

To ensure adequate *sample size*, good communication was essential with the hospital medical staff and recruitment strategies described above, were put in place.

To minimise *selection bias*, women from diverse education backgrounds and socio-economic status were recruited.

To minimise errors in data collection, a single qualified examiner, experienced in performing advanced level echocardiography, performed all the ultrasound scans. To reduce the “*learning curve effect*”, several pilot scans were performed, to standardise the technique and image views and strict scanning protocols were developed.

A single high-level, high-resolution machine was used to collect all the ultrasound and Doppler data.

Hawthorne effect: The Hawthorne effect refers to the phenomenon that research subjects change their behaviour simply because they know that they are a target of receiving attention, regardless of the type of intervention. This may happen by a modification of diet, lifestyle etc [289, 290]. To address and minimize the Hawthorne effect, similar clinical monitoring and scanning protocols were instituted in controls as well as cases, for study 1, 3 and 4.

Data monitoring: To avoid misclassification, and ensure allocation of patients into appropriate groups for analysis, clinical data and outcomes were confirmed after agreement within a panel discussion. The panel consisting of clinical researchers working with high-risk pregnancies included graduate students, research midwives, obstetricians, a maternal fetal medicine consultant and an obstetric physician.

Ultrasound data collection and entry was monitored by a random selection of ultrasound scans by the co-supervisor of the study.

Customised centile calculations [284] for growth, used in study 3 and study 4, which were based on maternal height, weight, parity and ethnic group and the sex of the baby, were crosschecked by 2 independent graduate students who had used the same cohort for other studies not included in this thesis.

A statistician was consulted in the beginning of the study, to decide on the best method for statistical evaluation, and the *mixed linear model* was chosen. Reasons for choosing mixed linear models have been discussed above.

Strategies to address confounding factors

A possible confounding factor was treatment with thromboprophylaxis in high-risk patients, where some patients were treated with aspirin alone and some were treated with enoxaparin in addition to aspirin. To address this potential confounder, separate

longitudinal analyses with mixed linear models were performed for ultrasound and Doppler variables using enoxaparin and aspirin as covariates.

Fisher's exact test was also performed with thromboprophylaxis with aspirin alone, as well as aspirin in combination with enoxaparin, to test the association between treatment and placental and clinical outcomes.

DOPPLER METHODOLOGY

UMBILICAL ARTERY DOPPLER PROTOCOL

Use B-Mode to identify the umbilical cord at fetal insertion.

Use colour Doppler and spectral Doppler to sample the umbilical artery.

Use a high-pass filter of 50 Hz and a sample volume of 2-3 mm in width.

Use an angle of insonation of zero degrees if possible. If zero angle is not obtainable, use minimal insonation angle (0-45⁰) and obtain a waveform.

Record the signals for a minimum of three to five waveforms of equal shape and amplitude and satisfactory quality.

Measurements are preferably made during general fetal inactivity and apnoea.

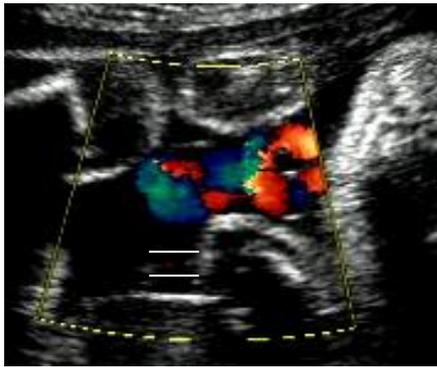
Measure the peak systolic velocity, end-diastolic velocity and measure RI, PI and S/D Ratio with the use of electronic callipers.

Note the type of waveform, i.e., describe if good diastolic flows, high resistance flows, absent end diastolic flows or reverse diastolic flows (**Figure 24**).

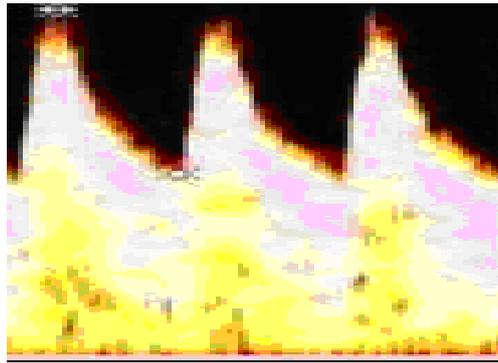
Repeat measurements in other free loops to confirm reduced diastolic flows.

If absent diastolic flows or reverse diastolic flows seen, repeat measurements in other free loops of cord, after decreasing wall filters further and using angle correction.

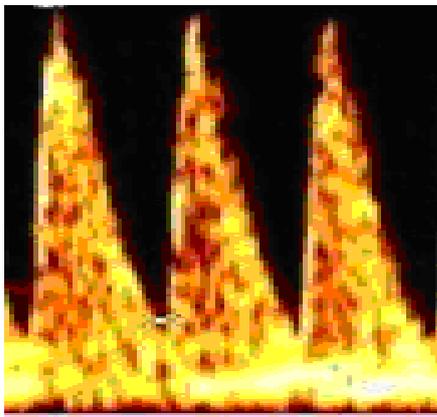
Umbilical Artery Doppler Imaging



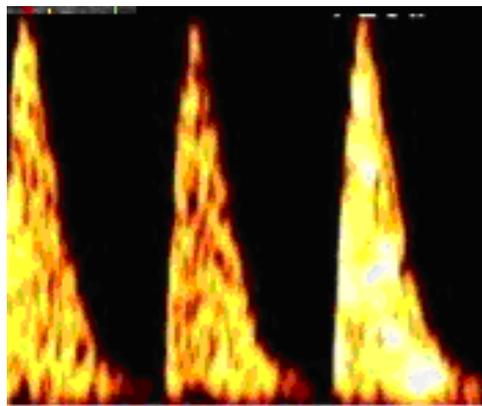
Free Loop of umbilical cord



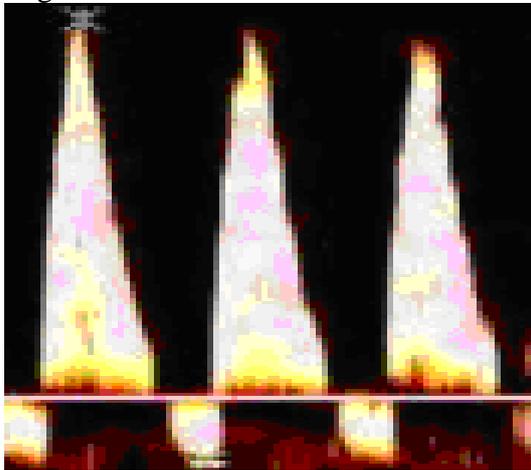
good diastolic flows



High resistance flows



Absent diastolic flows in umbilical artery



Reverse diastolic flows

Figure 24 Umbilical artery Doppler flow patterns

MIDDLE CEREBRAL ARTERY DOPPLER PROTOCOL

Use colour flow to identify the circle of Willis and middle cerebral arteries

Use spectral gate at proximal portion of near fields MCA close to ICA origin.

Be careful not to incorporate any portion of ICA.

Use an angle of insonation of zero degrees if possible. Where zero not obtainable, use angle correction and obtain waveform.

Record the signals for a minimum of three to five waveforms of equal shape and amplitude and satisfactory quality.

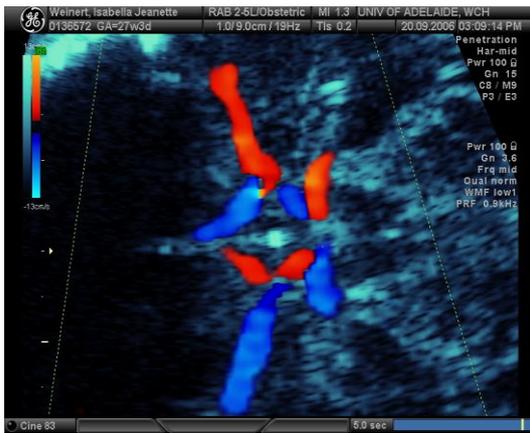
If near field proximal portion cannot be interrogated, use far field portion.

Measure the peak systolic velocity, end-diastolic velocity and measure RI and PI with the use of electronic callipers.

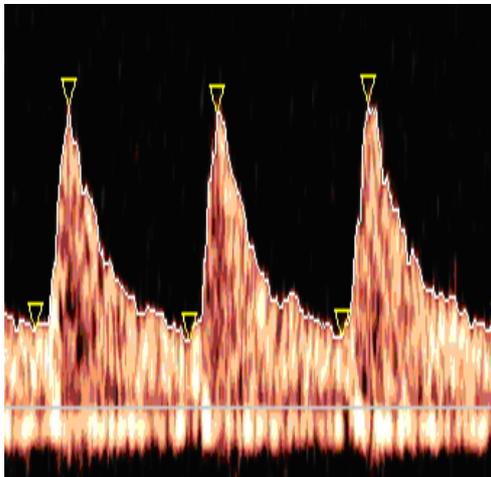
Describe whether normal high resistance flows or increased diastolic flows, i.e., 'brain-sparing', absent diastolic flows or reverse diastolic flows. See

Figure 25.

Middle Cerebral Artery Doppler Imaging



Circle of Willis



Normal Middle cerebral Artery flows



'Brain-sparing' - Increased diastolic flows

Figure 25 Middle cerebral artery Doppler flow patterns

UTERINE ARTERY PROTOCOL

Use a 3.5 to 5 MHz curvilinear array transducer.

Use a high pass filter of 100 Hz.

Place the transducer in the lower lateral quadrant of the maternal abdomen angled medially.

Use B-Mode ultrasound to image the iliac bifurcation and external and internal iliac arteries.

Use colour flow and pulsed Doppler to identify the uterine artery, as it appears to cross the external iliac artery.

Use a sample volume of 2-3 mm and place it over the entire diameter of the uterine artery approximately 1 cm distal to the crossover point.

If the uterine artery is seen branching before the intersection of the external iliac vessels, Doppler sampling is done from the main artery.

Use an angle of insonation of zero degrees if possible. If zero angle is not obtainable, use minimal insonation angle (0-45⁰) and obtain a waveform.

Record the signals for a minimum of three to five waveforms of equal shape and amplitude and satisfactory quality.

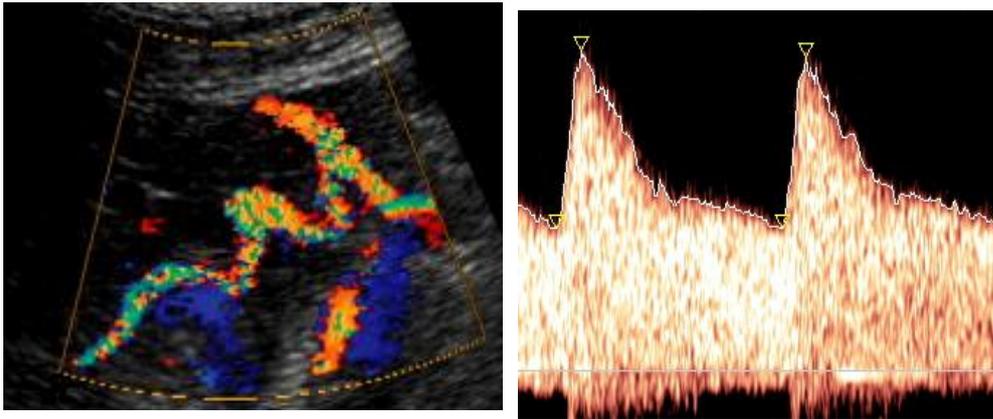
Measure the peak systolic velocity, end-diastolic velocity and measure RI and PI and with the use of electronic callipers.

Note and describe the type of waveform as shown in Figure 26 i.e., whether good diastolic flows or high resistance flows. Note presence or absence of early diastolic notch.

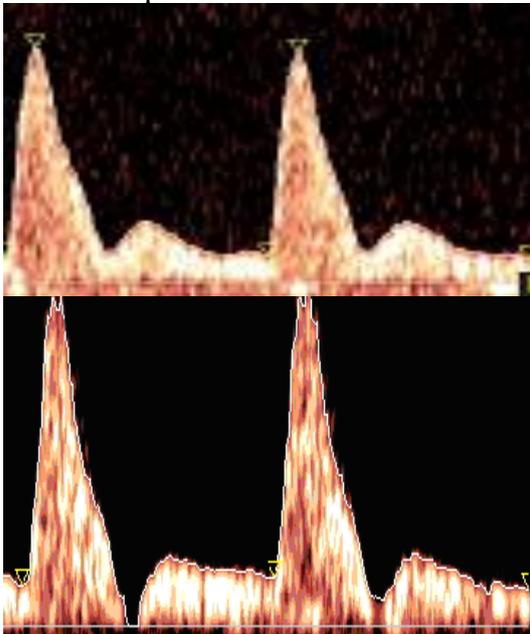
If notch present, then document if unilateral notch or bilateral notch, and type of notch - i.e. steep notch or a 'disappearing' notch.

Document the placental position and label placental side and non-placental side uterine artery.

Uterine artery Doppler Imaging



Normal impedance to flow in the uterine arteries.



Early diastole shows absent flow and steep notch.

Figure 26 Uterine artery Doppler flow patterns

DUCTUS VENOSUS PROTOCOL

Use a 3.5 to 5 MHz curvilinear array transducer.

Use a high pass filter of 100 Hz.

Use real-time B-Mode ultrasound to identify the ductus venosus in a mid-sagittal or oblique section of fetal abdomen.

Use colour flow and pulsed Doppler to identify the colour aliasing due to an increased blood flow velocity at the origin of ductus venosus from the umbilical vein.

Use a sample volume of 2 to 6 mm, place it over the origin of Ductus venosus and adapt the size of sample volume to the diameter.

Use an angle of insonation of zero degrees if possible. If zero angle is not obtainable, use minimal insonation angle ($0-45^{\circ}$) and obtain a waveform.

Using Pulsed Doppler, identify the characteristic biphasic unidirectional flow waveform.

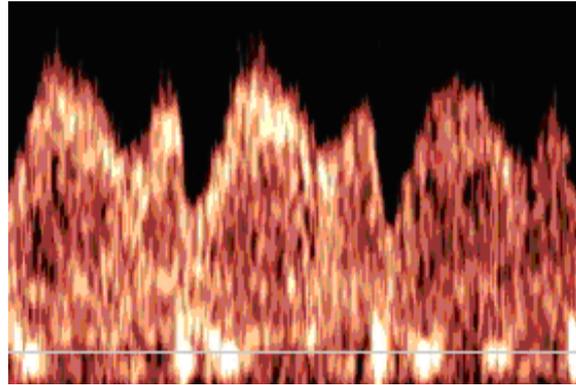
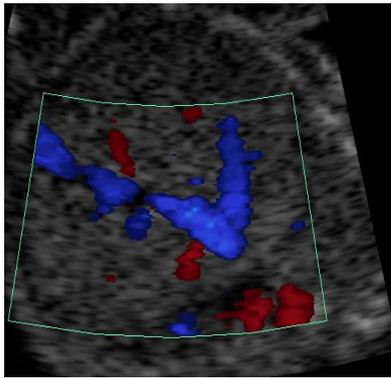
Record the signals for a minimum of three to five waveforms of equal shape and amplitude and satisfactory quality.

Measure the peak velocity (S) and minimum velocity (a) and measure S/a Ratio with the use of electronic callipers.

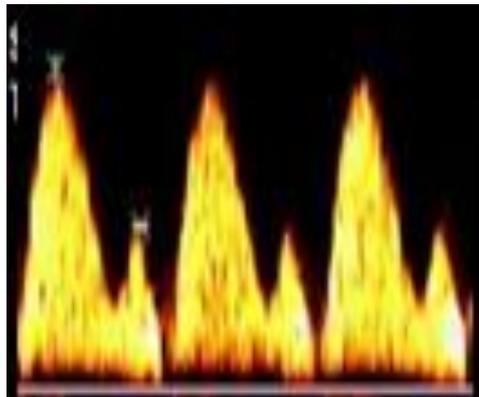
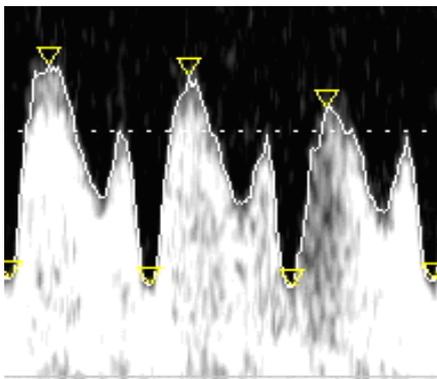
Peak velocity is defined as the maximum velocity during ventricular systole and minimum velocity is defined as the minimum velocity recorded during atrial contraction.

Note and describe the type of waveform, i.e., if good forward flows during atrial contraction, absent flows or reverse flows during atrial contraction (Figure 27).

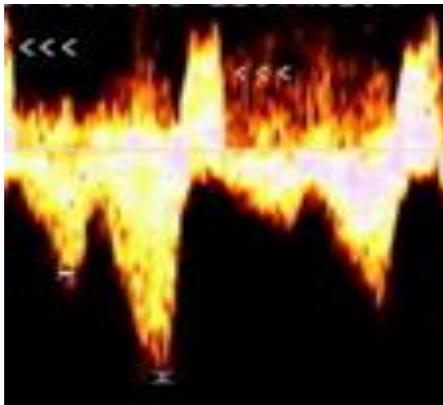
Ductus Venosus Doppler Imaging



Normal Ductus Venosus flows



Reduced 'a wave'.



Reversal of 'a' wave-during atrial contraction

Figure 27 Ductus venosus Doppler flow patterns

DUCTUS ARTERIOSUS PROTOCOL

Use a 3.5 to 5 MHz curvilinear array transducer.

Use a high pass filter of 100 to 200 Hz.

Use real-time B-Mode ultrasound to image a transverse section of the fetal thorax.

Identify the short axis view of the outflow tracts.

Pulmonary artery is seen hooking around the aorta, hence this view is also known as the 'circle-sausage view' (Figure 28). In this view, the long axis of the main pulmonary artery is seen to continue as the ductus arteriosus, which further continues as the descending aorta. Alternately, 3 vessel view or the extended 3 vessel view can be used to visualize the ductus arteriosus.

Use colour flow to identify the colour aliasing due to the increased blood flow velocity present.

Use a sample volume of 1 to 3 mm and place it in the distal ductal part.

Use an angle of insonation of zero degrees as far as possible. If zero degrees not possible, then try to keep a minimum angle less than 20 degrees.

Use pulsed Doppler to evaluate the spectral waveform.

At least three consecutive waveforms with the highest velocities and a narrow band of frequencies, i.e., 'clean' signal, is recorded and one waveform will be analysed.

Measure the Peak systolic velocity and the Pulsatility Index of the ductus arteriosus.

Ductus Arteriosus Doppler Imaging



Short axis view



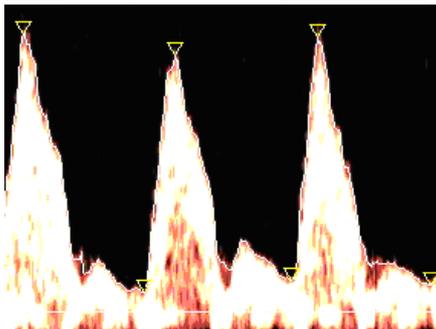
3 Vessel view



Extended 3 vessel view



oblique extended 3 vessel view



Normal flows through Ductus Arteriosus

Figure 28 Ductus arteriosus Doppler flow velocity patterns

FORAMEN OVALE PROTOCOL

Use a 3.5 to 5 MHz curvilinear array transducer.

Use a high pass filter of 100 to 200 Hz.

Use real-time B-Mode ultrasound to image a transverse section of the fetal thorax.

Identify the four-chamber view of the fetal heart. Identify the left and the right atrium.

The foramen ovale is identified between the right and the left atrium.

The flaps of the foramen ovale are seen to be within the left atrium.

Use a sample volume of 2 to 3 mm and position the sample volume within the foramen ovale.

Use pulsed Doppler evaluation with the heart in a four-chamber view, with a zero angle, to allow the orientation of the Doppler sample volume as close to parallel to flow as possible.

Evaluate the nature and pattern of flow, i.e, unidirectional, bi-directional, triphasic or biphasic in systole and diastole (Figure 29).

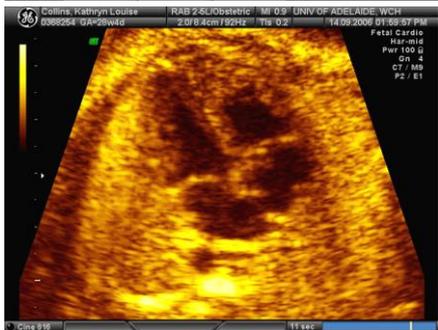
Foramen Ovale Doppler Imaging



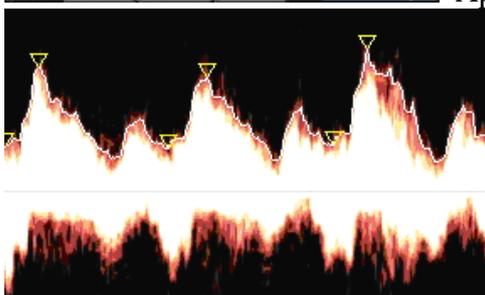
Lateral 4 chamber view



Basal 4 chamber view



Apical 4 chamber view



Normal flows through Foramen Ovale

Figure 29 Foramen ovale Doppler flow patterns

CHAPTER 4

Study 1: Normograms of fetal central shunts and other parameters

Introduction

The objectives of this study were to construct novel normograms for central shunts and placental thickness as well as validate previously published normograms of fetal biometry, uteroplacental and fetoplacental haemodynamics, fetal cerebral haemodynamics, and fetal biometry with serial measurements, based on a longitudinal study in a strictly defined cohort of patients with totally uncomplicated pregnancies. In order to establish normal reference ranges of all data under consideration, stringent and validated methodological guidelines were followed [291, 292] in order to have a reliable reference range for further studies on the same Doppler flow haemodynamics in pregnancies with adverse outcomes in Study 3.

The maternal and fetal circulation has been investigated extensively over the last two decades. Experimental and clinical studies have confirmed that the main difference between fetal and neonatal circulation is the presence of intrauterine shunts. Fetal flow through the heart, follows two pathways, with the right and left sides of the fetal heart working as parallel circuits. Oxygenated flow from the placenta enters the fetus via the ductus venosus via the umbilical vein and is then transferred to the right side of the heart through the inferior vena cava (IVC). It is here that in the IVC that the flow channels diverge into two pathways [240]. Oxygenated flow to the brain and upper part of the body follow the left sided pathway through the ascending aorta via foramen ovale, whereas a less oxygenated flow to the lower part of the body goes into the descending aorta via the ductus arteriosus. It is now possible to non-invasively evaluate these flow haemodynamics with Doppler ultrasound. In this way, Doppler ultrasound has enabled greater insights into fetal physiology.

Doppler evaluates the resistance offered by the circulation within the organ under consideration. Several longitudinal studies in uteroplacental insufficiency (UPI) have documented the sequential changes in circulatory haemodynamics in the compromised fetus by evaluation the fetus for signs of brain sparing and severity of redistribution of circulation. Doppler studies of umbilical artery (UA), middle cerebral artery(MCA) and ductus venosus (DA), have become an integral part of fetal assessment in high risk pregnancies in tertiary centres.

To identify abnormal flow patterns, it is imperative that normal flow patterns are recognised and established. With this aim, a range of fetal arterial and venous vessels have been evaluated Doppler and reference ranges have been described over the last decade [293-295]. However, most of the reference ranges for the different indices in use, have been obtained from cross-sectional studies, which are more useful for single observations [292]. Some longitudinal studies have been published, however, these studies either have fewer number of participants [234], or small number of repeat measurements such as 3 to 5 measurements per fetus [296, 297]. The optimum gestational age for the use of these ultrasound and Doppler indices is also not clear. There is a need for data from longitudinal studies, to allow calculation of reference ranges [298]. Besides, it is likely that in early stages of disease, the changes in circulation are subtle and therefore may not be identified in single artery Doppler studies such as umbilical artery or middle cerebral artery (MCA). Previously published data has shown that an evaluation of multiple vessel evaluation is better at identification of compromised fetuses in a high risk population [299] and is useful in a comprehensive evaluation of fetal compromise, to optimise the timing of delivery [160].

Circulatory patterns in early stages of disease, where adaptations might exist, are still unclear. It would be logical to assume that the fetal heart and the fetal shunts may be involved in redistribution even before any brain sparing becomes evident by currently established parameters. However, there are no longitudinal studies describing the normal flow indices in the FO, a vital central shunt, probably involved in cerebral flow distribution. Furthermore, in addition, there are no studies which have evaluated all the three shunts along with uteroplacental, fetoplacental and cerebral haemodynamics in the same set of fetuses, as this would provide a better reflection of fetal distribution.

This study was therefore undertaken to establish the reference ranges for the three intrauterine shunts DA, DV and FO as well as normal values for uteroplacental, fetoplacental and cerebral haemodynamics as well as fetal biometric measurements and placental thickness in the same set of fetuses, by serial measurements, in a longitudinal study.

Hypothesis

Fetal circulation is a shunt – dependant circulation. There exists a relationship between flow haemodynamics of fetal central shunts and fetal cerebral resistance.

Aim

To establish reference ranges for the Doppler flow waveforms of (DA) and (FO) as well as evaluate fetal biometry and Doppler waveforms of maternal uterine arteries, fetal umbilical artery, ductus venosus, middle cerebral artery with serial measurements, in the same set of control pregnancies, in a longitudinal study.

Methods and study design

This study was part of a large ongoing prospective observational longitudinal cohort study to evaluate uteroplacental and fetal circulation in healthy pregnancies and pregnancies complicated by placental insufficiency, to identify the relationship between Doppler of fetal and maternal circulation and prediction of adverse perinatal outcome. Ethics approval was obtained from the Institutional Research Ethics Committee.

Inclusion criteria included a singleton structurally and chromosomally normal fetus, at risk for developing IUGR, preeclampsia or preterm labour. Women carrying structurally and chromosomally normal singleton fetuses with past history of healthy pregnancies as well as low-risk primi gravidas were recruited to serve as controls.

Informed consent was taken and after an early dating scan to confirm gestational age. All pregnancies were scanned serially from 16 weeks onwards at 4 weekly intervals until 24 weeks and two weekly intervals thereafter, until 38 weeks.

Doppler studies were performed by Advanced Technology Laboratories HDI 5000 ultrasound machine (Australia) on the first thirty patients. The rest of the patients were serially scanned with Voluson 730 Expert (Korea). Echocardiographic studies of FO and DA flow velocity waveforms were performed. Other Doppler studies included descriptive waveform analysis of both umbilical arteries (reversed, absent or positive end diastolic flows), as well as peak systolic velocities, end diastolic velocities and pulsatility Index (PI). Similar observations were also made in middle

cerebral artery, ductus venosus and uterine arteries. The serial measurements for the trial also included evaluation of fetal growth trajectory and well being with biometric measurements BPD, HC, AC, FL and amniotic fluid Index. Details of methodology for biometric and Doppler measurements have been described in chapter 3.

All angles of insonation were 0 degrees, for echocardiographic examinations, and for the other vessels, as close to 0 degrees as possible, and always less than 30 degrees. All scans were performed by a single investigator, with extensive experience in fetal arteriovenous sonography and fetal echocardiography. Doppler methodology is described in detail in Chapter 3.

A team of physicians comprising of a maternal fetal medicine expert, high- risk pregnancy expert and an obstetric physician evaluated all clinical outcomes independently. Patients with adverse outcomes were excluded and 54 patients with normal outcomes were included for data analysis.

Normal outcome was defined as one where all the three following criteria were satisfied.

- No maternal antenatal or postnatal maternal complications.
- No abnormality in growth trajectory antenatally on ultrasound.
- No antenatal fetal or postnatal neonatal complications on the basis of the examination made by the attending paediatrician at the time of discharge.

Statistical analysis

SPSS version 14 software package for Windows (SPSS Inc, Chicago, IL, 2005) was used for statistical analysis

The majority of data occurred within one week of the nominal gestational ages of 16, 20, 24, 26, 28, 32, 36 and 38 weeks. Gestational age estimation was determined on the basis of earliest scan acknowledging the known 5-6 day variability associated with even the earliest scan. As exact gestational ages were unobtainable in every case, ages in completed weeks were adjusted to avoid bias by addition of 0.5 weeks [292].

The means and upper and lower limits of 95 % confidence intervals were calculated for all the ultrasound and Doppler variables, using SPSS package, version 14.

Figure 30 describes the study design for evaluation of the different variables in study 1, the Normogram study, and Table 13 describes the total number of observations used to generate the normograms.

.

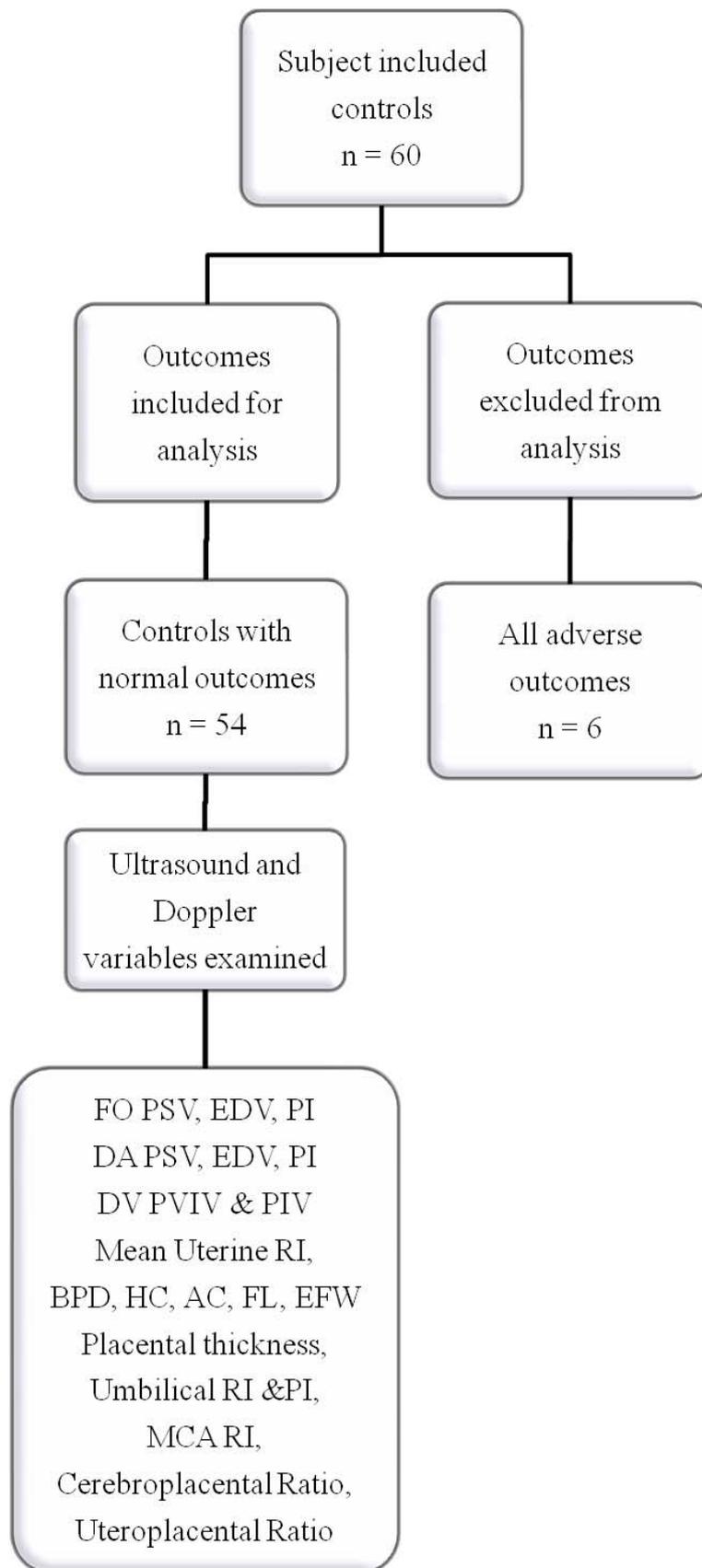


Figure 30 Flow chart describing the study design for Study 1

Table 13 Number of observations for Ultrasound and Doppler variables in Study 1

	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Umbilical artery (UA) RI	496	98.8	6	1.2	502	100
UA PI	497	99.0	5	1.0	502	100
UA S/D ratio	497	99.0	5	1.0	502	100
MCA RI	490	97.6	12	2.4	502	100
MCA PI	490	97.6	12	2.4	502	100
CPR	487	97.0	15	3.0	502	100
uteroplacental ratio	471	93.8	31	6.2	502	100
DV preload index (s-a)/s	484	96.4	18	3.6	502	100
DV PVIV (s-a)/d	365	72.7	137	27.3	502	100
DV PIV (s-a)/tamx	419	83.5	83	16.5	502	100
DV s/a ratio	484	96.4	18	3.6	502	100
DA PSV	487	97.0	15	3.0	502	100
DA EDV	487	97.0	15	3.0	502	100
DA PI	487	97.0	15	3.0	502	100
FO PSV	488	97.2	14	2.8	502	100
FO EDV	488	97.2	14	2.8	502	100
FO PI	480	95.6	22	4.4	502	100
Mean Ut RI	474	94.4	28	5.6	502	100
Placental thickness	455	90.6	47	9.4	502	100
BPD	497	99.0	5	1.0	502	100
HC	497	99.0	5	1.0	502	100
AC	495	98.6	7	1.4	502	100
Femur Length	497	99.0	5	1.0	502	100

Explanation for missing data is provided in chapter 3.

Results

This study established the first known published gestation – specific reference ranges of intrauterine fetal central shunt FO. The reference ranges for all the other fetal vessels evaluated were also constructed using longitudinal data in the same cohort of controls for the first time.

This section describes the results in the following format. Initially, there is a brief description of the Doppler methodology, followed by a description of the number of observations used to generate the reference ranges defined in this study. Subsequently, a graph is shown, depicting the reference ranges throughout gestation. The actual values of the reference ranges along with the 95 % Confidence Intervals are described in Appendix 1. This is then followed by a comparison of the results from this study with previously published data.

NORMOGRAMS OF CENTRAL SHUNTS

Foramen Ovale

Figure 31 briefly describes the Doppler methodology for imaging Foramen Ovale (FO).

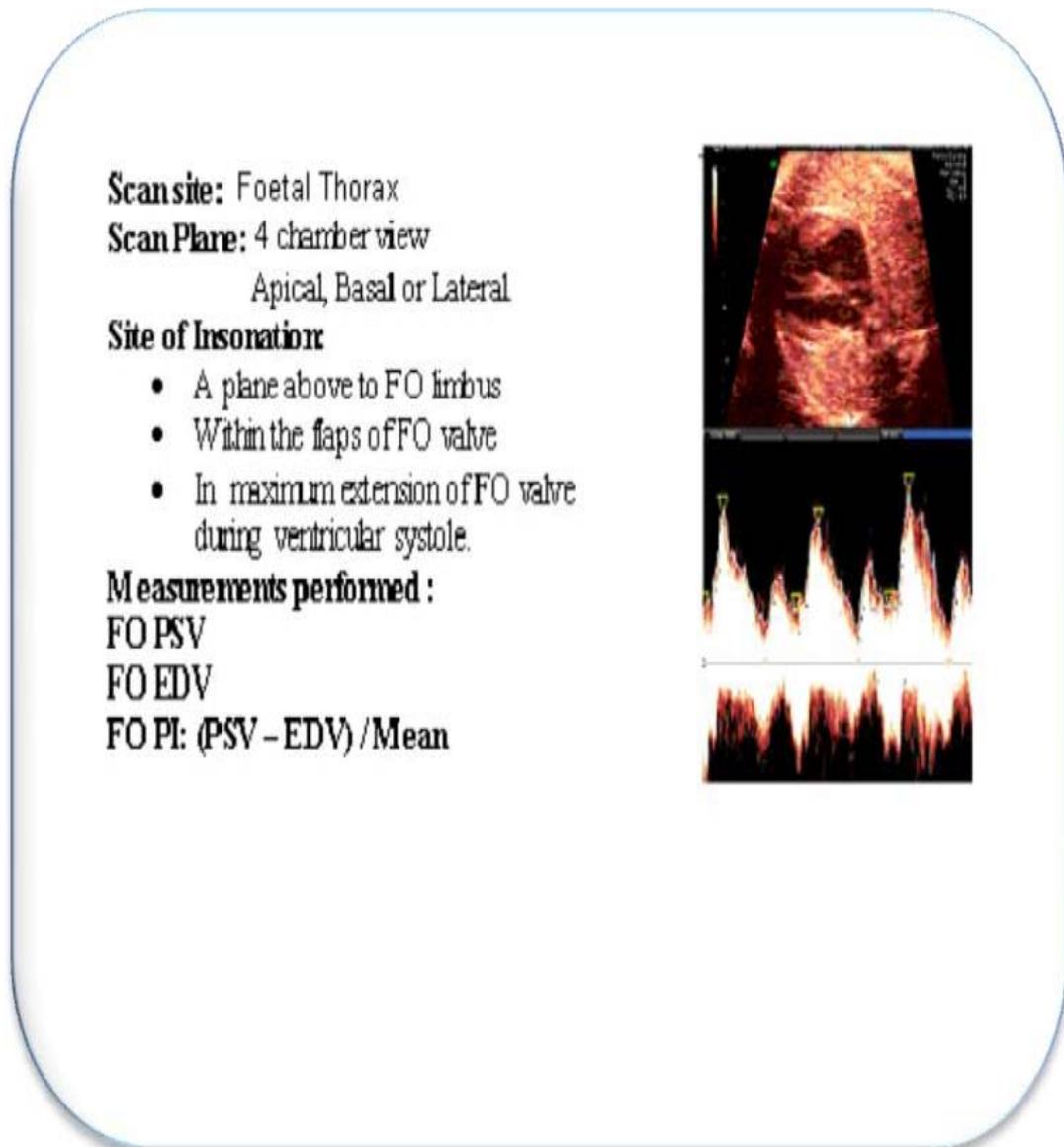


Figure 31 Foramen Ovale Doppler (FO) Methodology

Foramen Ovale (FO) demonstrated ‘To and fro flows’, the flow direction being from right to left (Figure 31).

FO PSV

In normal pregnancies, the PSV of FO increased linearly with gestational age, mean FO PSV from 26.8 cm/s at 16 weeks to 43.3 cm/s at 38 weeks (Figure 32). Table 14 describes the gestation-specific reference ranges for FO PSV with 95 % Confidence Intervals.

Table 14 Reference ranges for Foramen Ovale Peak Systolic Velocity

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	31	91.2	3	8.8	26.8	23.2	30.4
20	41	97.6	1	2.4	30.2	26.7	33.7
24	51	100	0	0	33.9	30.4	37.4
26	47	95.9	2	4.1	35.4	32.1	38.7
28	49	94.2	3	5.8	38.8	35.3	42.3
30	54	100	0	0	36.2	33.1	39.4
32	52	100	0	0	38.3	35.6	41.1
34	47	100	0	0	39.9	35.2	44.7
36	51	100	0	0	41.5	37.6	45.3
38	34	91.9	3	8.1	43.4	39.0	47.7

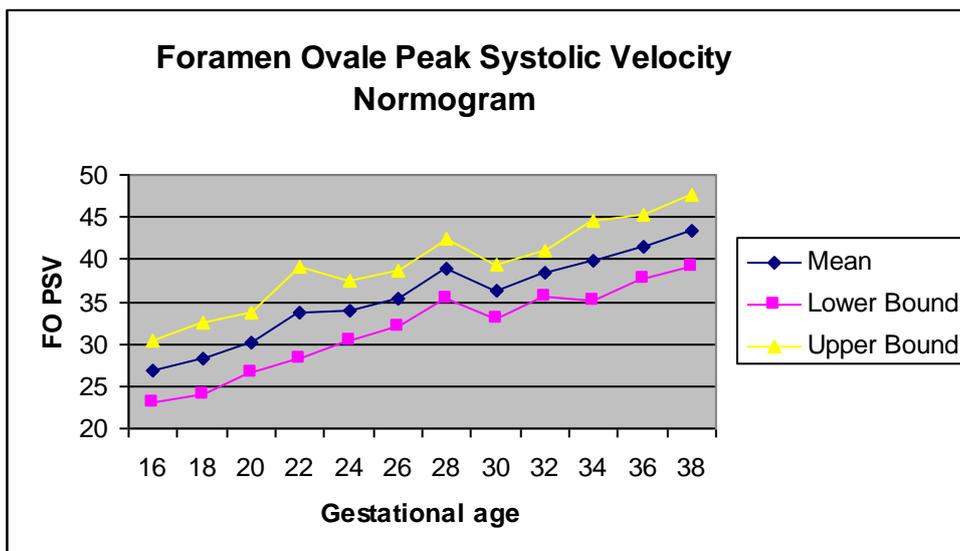


Figure 32 Reference ranges for Foramen Ovale Peak Systolic Velocity.

FO EDV

In normal pregnancies, the EDV of FO increased linearly with gestational age, mean FO EDV from 9.4 cm/s at 16 weeks to 17.7 cm/s at 36 weeks (Figure 33).

Table 15 describes the gestation-specific reference ranges for FO EDV with 95 % Confidence Intervals.

Table 15 Reference ranges for Foramen Ovale End Diastolic Velocity

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	31	91.2	3	8.8	9.4	7.5	11.2
20	41	97.6	1	2.4	10.6	9.3	11.8
24	51	100	0	0	13.1	11.4	14.8
26	47	95.9	2	4.1	14.1	12.7	15.5
28	49	94.2	3	5.8	13.1	11.9	14.4
30	54	100	0	0	15.6	13.9	17.4
32	52	100	0	0	15.9	13.8	18.1
34	47	100	0	0	15.9	14.2	17.6
36	51	100	0	0	17.7	15.3	20.2

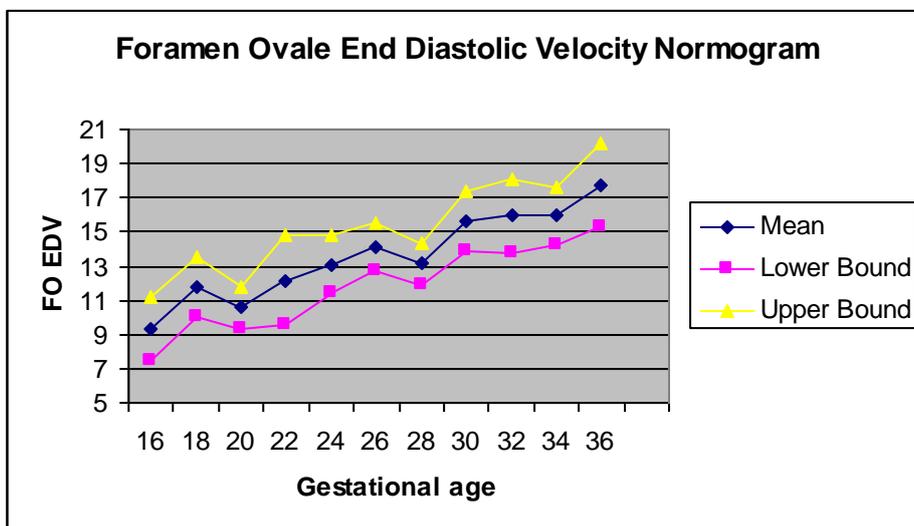


Figure 33 Reference ranges for Foramen Ovale End- diastolic Velocity.

FO PI

In normal pregnancies, the PI of FO decreased linearly with gestational age, mean PI from 1.7 at 16 weeks to 1.3 at 36 weeks (Figure 34).

Table 16 describes the gestation-specific reference ranges for FO PI with 95 % Confidence Intervals.

Table 16 Reference ranges for Foramen Ovale Pulsatility Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	29	85.3	5	14.7	1.7	1.3	2.2
20	40	95.2	2	4.8	2.1	1.4	2.7
24	50	98.0	1	2.0	1.6	1.2	2.1
26	47	95.9	2	4.1	1.4	1.0	1.8
28	49	94.2	3	5.8	1.6	1.3	1.9
30	54	100	0	0	1.6	1.2	2.0
32	52	100	0	0	1.4	1.1	1.8
34	47	100	0	0	1.7	1.1	2.3
36	49	96.1	2	3.9	1.3	1.0	1.7

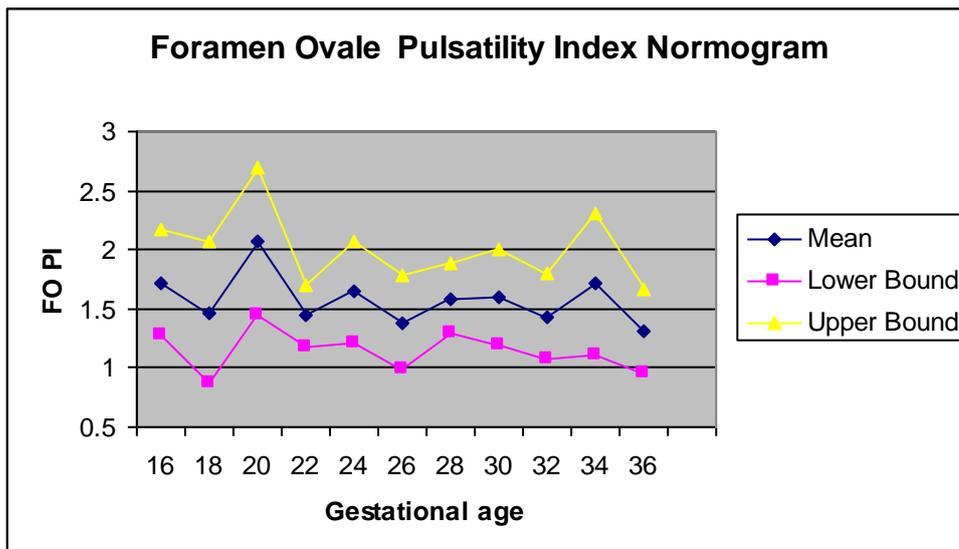


Figure 34 Reference ranges for Foramen Ovale Pulsatility Index

Ductus Arteriosus

Figure 35 briefly describes the Doppler methodology for imaging ductus arteriosus (DA).

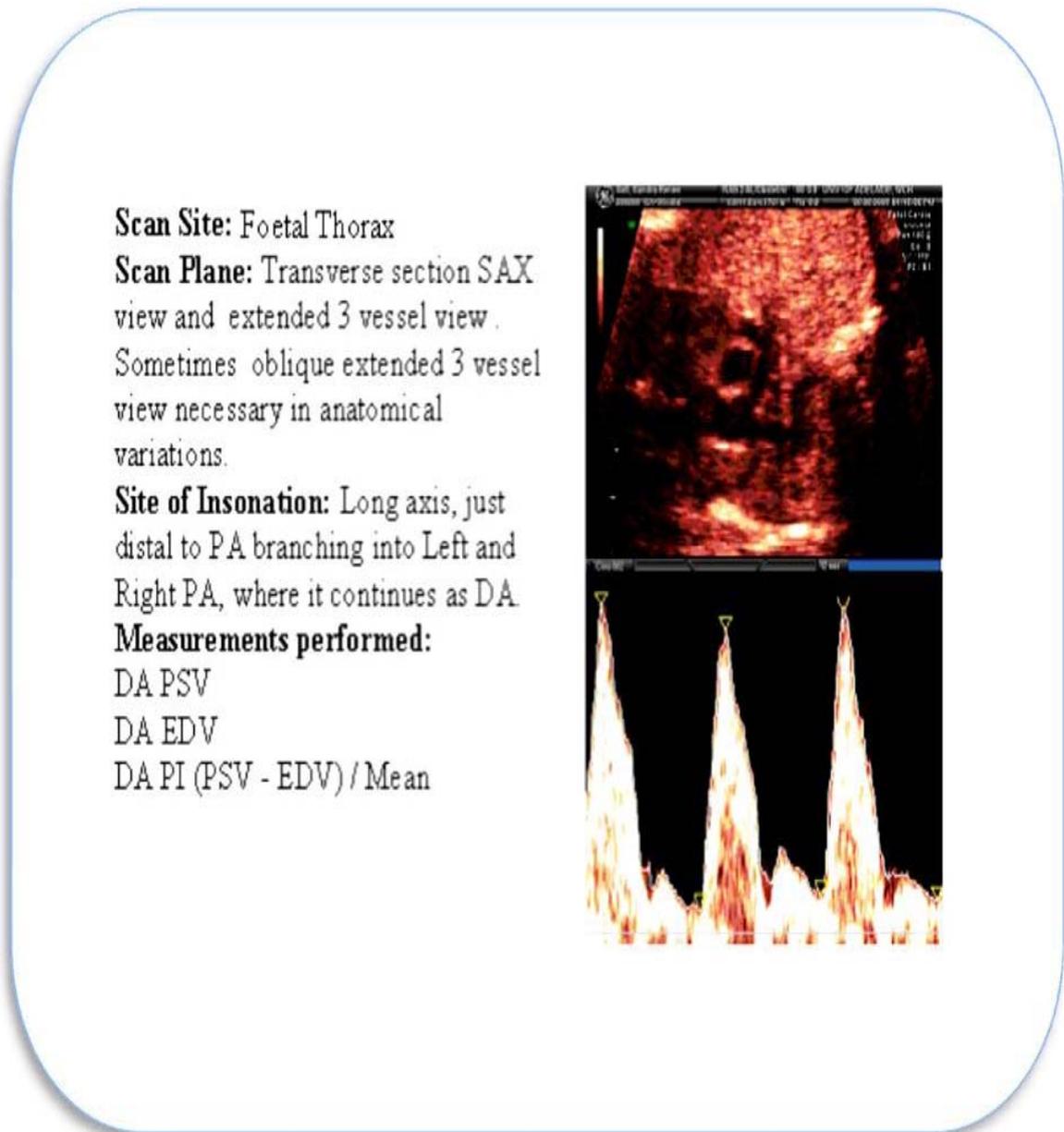


Figure 35 Ductus Arteriosus Doppler (DA) Methodology

DA demonstrated high resistance biphasic waveform with forward diastolic flows throughout throughout pregnancy (

Figure 35).

DA PSV

In normal pregnancies, the PSV of DA increased linearly with gestational age, mean PSV from 43.4 cm/s at 16 weeks to 95.6 cm/s at 38 weeks (Figure 36).

Table 17 describes the gestation-specific reference ranges for DA PSV with 95 % Confidence Intervals.

Table 17 Reference ranges for Ductus Arteriosus Peak Systolic Velocity

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	28	82.4	6	17.6	43.5	38.9	48.1
20	41	97.6	1	2.4	49.9	44.9	55.0
24	51	100	0	0	61.7	56.5	66.9
26	48	98.0	1	2.0	68.1	61.8	74.5
28	50	96.2	2	3.8	71.1	64.2	78.0
30	54	100	0	0	81.6	75.5	87.8
32	52	100	0	0	80.1	72.8	87.4
34	47	100	0	0	91.2	81.6	100.8
36	51	100	0	0	94.8	86.8	102.9
38	33	89.2	4	10.8	95.6	85.2	106.0

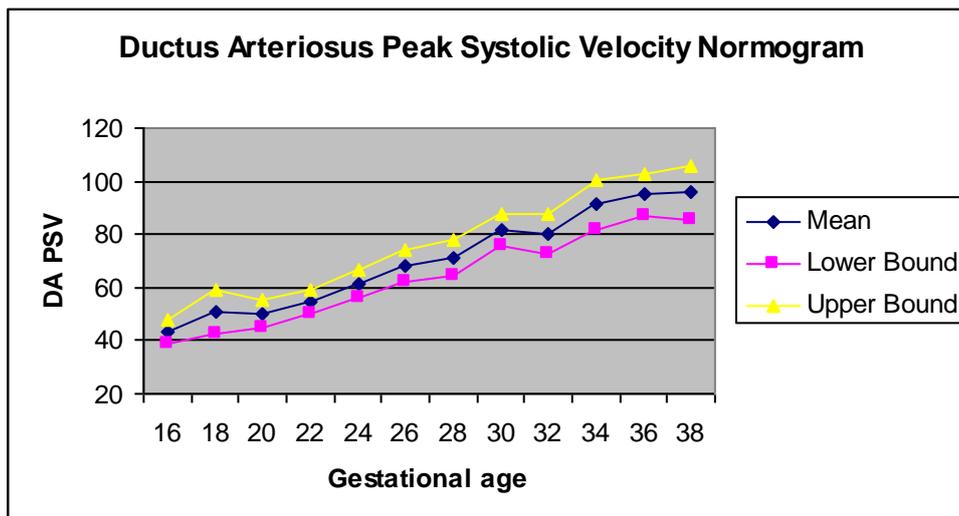


Figure 36 Reference ranges for Ductus Arteriosus Peak Systolic Velocity

DA EDV

In normal pregnancies, the EDV of DA also increased linearly with gestational age, mean DA EDV from 6.6 cm/s at 16 weeks to 10.4 cm/s at 38 weeks (Figure 37). Table 18 describes the gestation-specific reference ranges for DV EDV with 95 % Confidence Intervals.

Table 18 Reference ranges for Ductus Arteriosus End Diastolic Velocity

valid observations	N	Percent	95% Confidence Interval for Mean				
			N	Percent	Mean	Lower Bound	Upper Bound
16	28	82.4	6	17.6	6.6	4.5	8.7
20	41	97.6	1	2.4	6.5	5.6	7.3
24	51	100	0	0	7.3	6.3	8.4
26	48	98.0	1	2.0	7.6	7.0	8.3
28	50	96.2	2	3.8	7.8	7.1	8.6
30	54	100	0	0	8.9	7.9	9.9
32	52	100	0	0	8.6	7.6	9.5
34	47	100	0	0	11.3	8.4	14.3
36	51	100	0	0	11.3	9.6	12.9
38	33	89.2	4	10.8	10.4	8.9	11.9

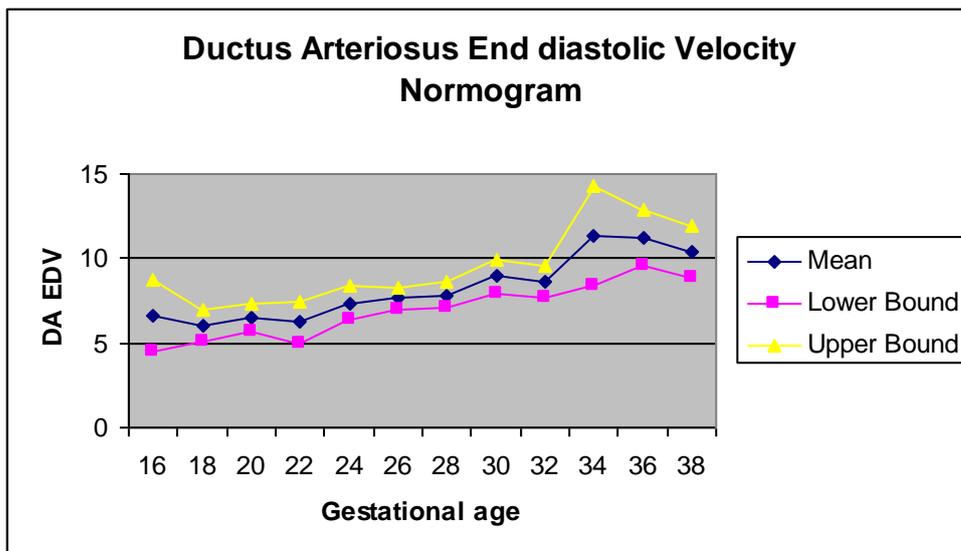


Figure 37 Reference ranges for Ductus Arteriosus End-diastolic Velocity

DA PI

In normal pregnancies, PI of DA was relatively constant throughout gestation, mean values ranging between 2.4 to 2.8 (Figure 38). Table 19 describes the gestation-specific reference ranges for DA PI with 95 % Confidence Intervals.

Table 19 Reference ranges for Ductus Arteriosus Pulsatility Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	28	82.4	6	17.6	2.58	2.14	3.01
20	41	97.6	1	2.4	2.84	2.40	3.29
24	51	100	0	0	2.52	2.29	2.75
26	48	98.0	1	2.0	2.43	2.27	2.60
28	50	96.2	2	3.8	2.52	2.31	2.72
30	54	100	0	0	2.56	2.26	2.86
32	52	100	0	0	2.56	2.35	2.77
34	47	100	0	0	2.76	2.37	3.16
36	51	100	0	0	2.82	2.40	3.25
38	33	89.2	4	10.8	2.83	2.54	3.12

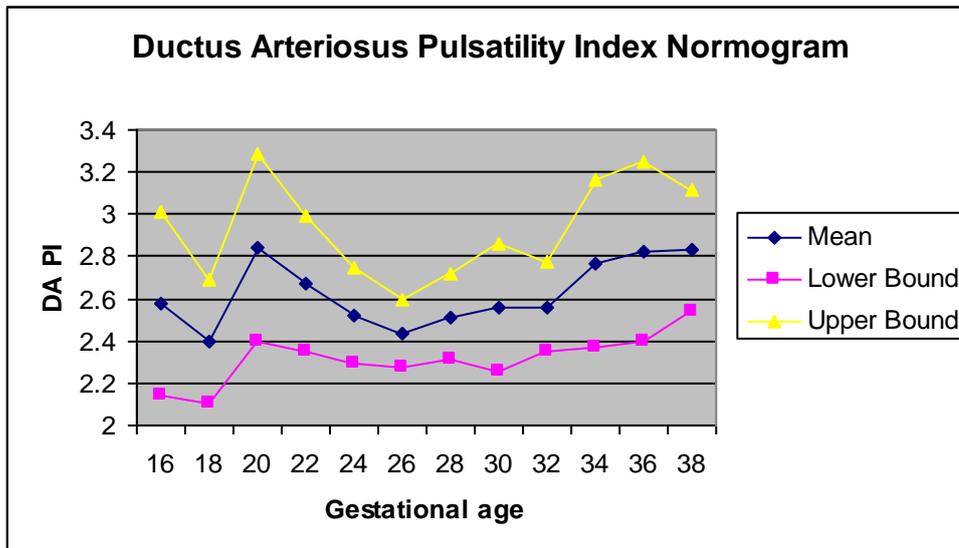


Figure 38 Reference ranges for Ductus Arteriosus Pulsatility Index

DA PSV, EDV and PI normogram results were similar to the cross sectional study of 222 fetuses by Mielke and Benda [294] and a continuous wave Doppler longitudinal study of 41 fetuses by Tulzer et al [300].

NORMOGRAMS OF VENOUS INTRAUTERINE SHUNT: DUCTUS VENOSUS

Figure 39 briefly describes the Doppler methodology for imaging ductus venosus (DV).

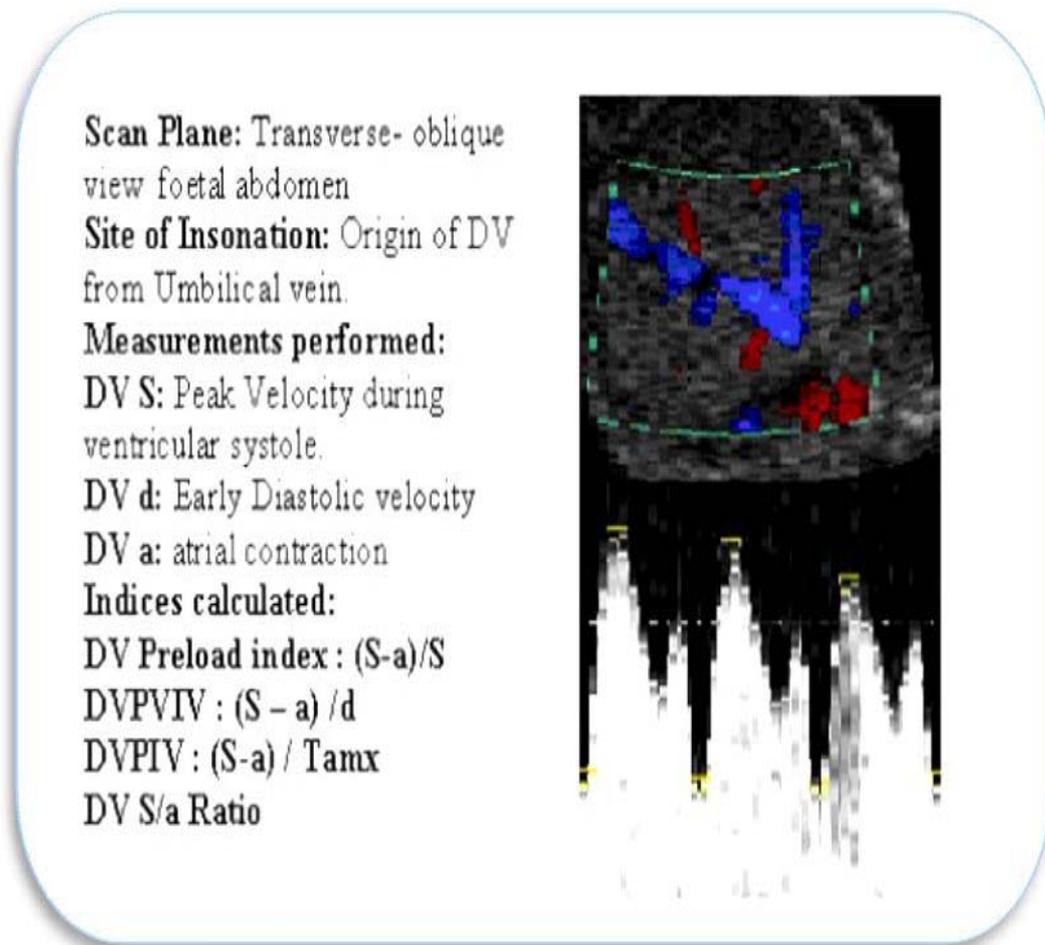


Figure 39 Ductus venosus Doppler (DV) Methodology

DV demonstrated forward flows throughout pregnancy. The normal DV flow pattern has two forward peaks, the first seen in systole (s) and the second seen in early diastole (d), the trough between two cycles representing atrial contraction (‘ a’ wave).

DV Preload Index

Table 20 describes the number of observations used to generate the reference ranges for DV Preload index. In normal pregnancies, the DV Preload Index decreased linearly with gestational age, mean DV Preload Index from 0.57 at 16 weeks to 0.43 at 36 weeks (Figure 40). Table 20 describes the reference ranges for DV Preload index.

Table 20 Reference ranges for Ductus venosus Preload Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	31	91.2	3	8.8	0.57	0.51	0.63
20	42	100	0	0	0.49	0.44	0.55
24	50	98.0	1	2.0	0.45	0.41	0.49
26	48	98.0	1	2.0	0.45	0.41	0.49
28	51	98.1	1	1.9	0.42	0.38	0.46
30	53	98.1	1	1.9	0.44	0.39	0.49
32	51	98.1	1	1.9	0.42	0.37	0.47
34	45	95.7	2	4.3	0.43	0.37	0.48
36	50	98.0	1	2.0	0.43	0.38	0.47

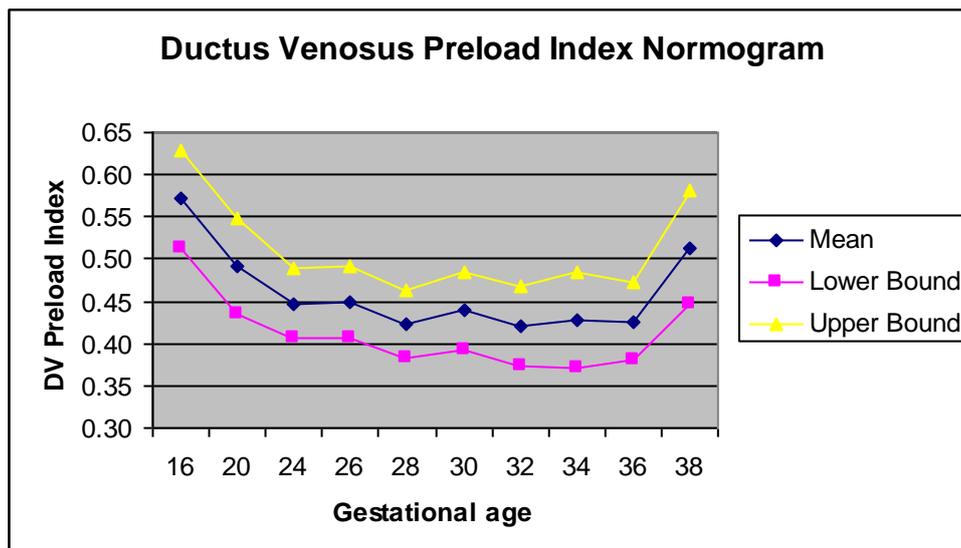


Figure 40 Reference ranges for Ductus venosus Preload Index

DV S/a ratio

Table 21 describes the number of observations used to generate the reference ranges for DV S/a ratio. In normal pregnancies, the DV S/a ratio decreased linearly with gestational age, mean DV S/a ratio from 2.74 at 16 weeks to 2.52 at 38 weeks (Figure 41). Table 21 describes the reference ranges for DV S/a ratio.

Table 21 Reference ranges for Ductus Venosus S/a ratio

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	31	91.2	3	8.8	2.74	2.26	3.22
20	42	100	0	0	2.10	1.87	2.32
24	50	98.0	1	2.0	1.99	1.77	2.20
26	48	98.0	1	2.0	1.98	1.78	2.18
28	51	98.1	1	1.9	1.87	1.69	2.04
30	53	98.1	1	1.9	1.95	1.79	2.11
32	51	98.1	1	1.9	1.89	1.72	2.05
34	45	95.7	2	4.3	2.04	1.73	2.35
36	50	98.0	1	2.0	1.93	1.72	2.15
38	30	81.1	7	18.9	2.27	1.92	2.62

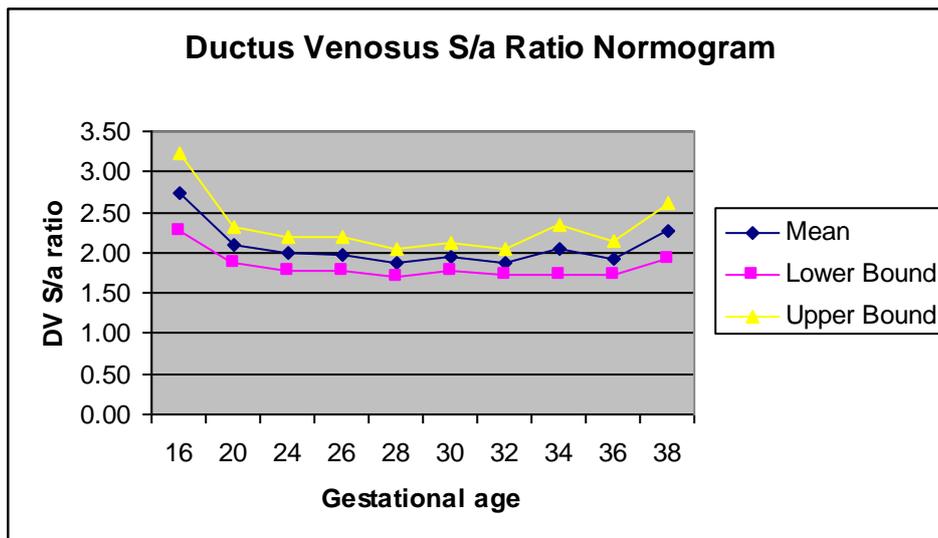


Figure 41 Reference ranges for Ductus Venosus S/a ratio.

DV Peak velocity Index

Table 22 describes the reference ranges as well as the number of observations used to generate the reference ranges for DV Peak velocity index. In normal pregnancies, the DV Peak velocity index decreased linearly with gestational age, mean DV Preload Index from 0.68 at 16 weeks to 0.51 at 36 weeks (Figure 42).

Table 22 Reference ranges for Ductus Venosus Peak velocity Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	23	67.6	11	32.4	0.68	0.59	0.77
20	33	78.6	9	21.4	0.62	0.50	0.73
24	31	60.8	20	39.2	0.55	0.46	0.63
26	34	69.4	15	30.6	0.50	0.44	0.57
28	38	73.1	14	26.9	0.52	0.46	0.59
30	40	74.1	14	25.9	0.54	0.47	0.61
32	39	75	13	25	0.55	0.46	0.64
34	36	76.6	11	23.4	0.51	0.42	0.61
36	44	86.3	7	13.7	0.51	0.43	0.60

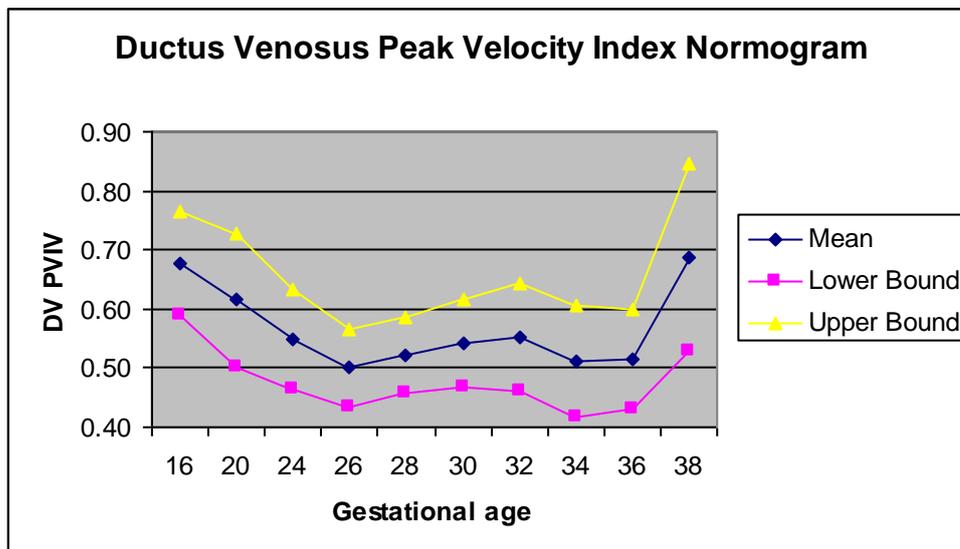


Figure 42 Reference ranges for Ductus venosus Peak velocity Index

DV Pulsatility Index

Table 23 describes the reference ranges as well as the number of observations used to generate the reference ranges for DV Pulsatility index. In normal pregnancies, the DV pulsatility index decreased linearly with gestational age, mean DV Pulsatility Index from 0.74 at 16 weeks to 0.80 at 36 weeks (Figure 43).

Table 23 Reference ranges for Ductus Venosus Pulsatility Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	23	67.6	11	32.4	0.74	0.56	0.92
20	30	71.4	12	28.6	0.76	0.41	1.11
24	46	90.2	5	9.8	0.55	0.46	0.64
26	43	87.8	6	12.2	0.54	0.45	0.64
28	46	88.5	6	11.5	0.49	0.43	0.56
30	44	81.5	10	18.5	0.52	0.43	0.61
32	47	90.4	5	9.6	0.50	0.42	0.59
34	40	85.1	7	14.9	0.61	0.46	0.77
36	46	90.2	5	9.8	0.56	0.45	0.67
38	27	73.0	10	27.0	0.80	0.63	0.96

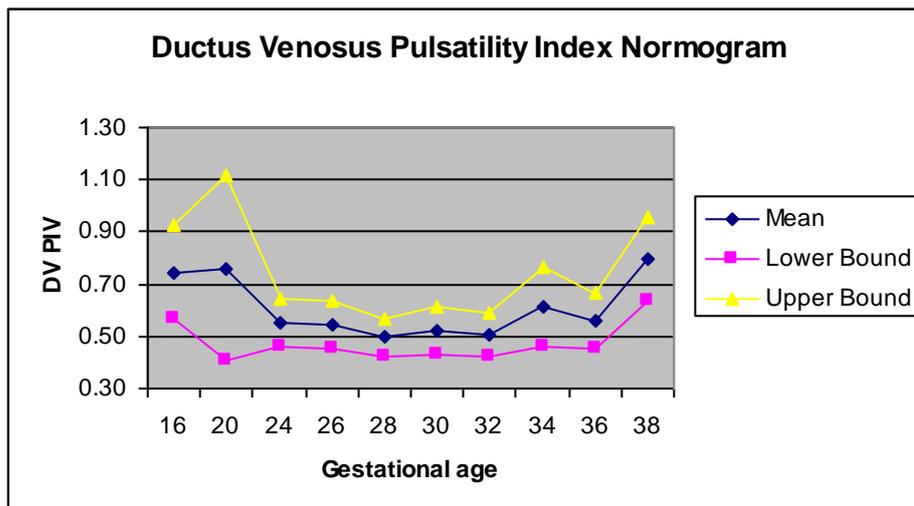


Figure 43 Reference ranges for Ductus Venosus Pulsatility Index

DV pulsatility index normograms results in our study were slightly higher than those in a recently published longitudinal study of 160 fetuses by Kessler et al

(Kessler 2006), the mean pulsatility index was 0.76 at 20 weeks and 0.80 at 38 weeks in our study, as compared to 0.57 at 20 weeks and 0.46 at 38 weeks in the Kessler study [297], although similar to the values obtained in Bahlmann study (0.77 to 0.47), which had included 696 observations [293].

NORMOGRAMS FOR UTEROPLACENTAL AND FETOPLACENTAL CIRCULATION

Uterine artery Doppler Mean RI

Figure 30 Figure 44 briefly describes the Doppler methodology for imaging uterine artery.

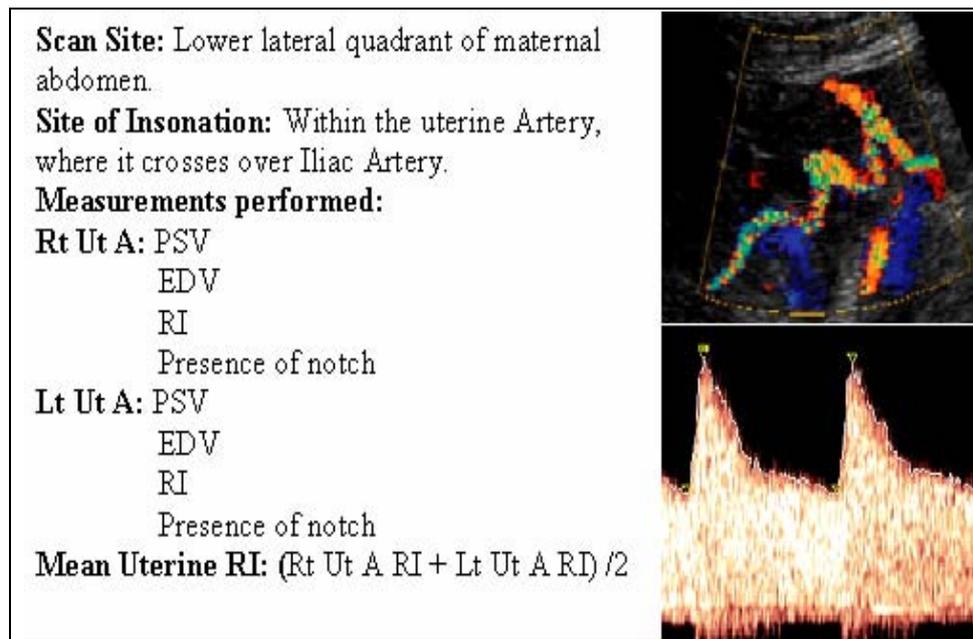


Figure 44 Uterine arteries Doppler (Ut RI) Methodology

Uterine artery demonstrated progressively decreasing resistance waveform with forward diastolic flows throughout pregnancy (Figure 44).

The presence or absence of uterine notches was documented throughout the study. However, there have been inconsistencies in the definition of uterine notches in published literature. Most of the published data regarding uterine notches has come from cross sectional analysis [301].

On sequential examination, it was observed that when the notches were present in earlier gestation, the steepness of the notch gradually reduced and eventually disappeared over a period of time with advancing gestation. Longitudinal analysis of data regarding the notches was therefore difficult to analyse and not meaningful.

As a result, data regarding uterine notches was not included in the final data analysis.

Mean Uterine RI

Table 24 describes the number of observations used to generate the reference ranges for Mean uterine RI. In normal pregnancies, the Mean uterine RI decreased linearly with gestational age, mean RI from 0.57 at 16 weeks to 0.49 at 38 weeks (Figure 45). Table 24 describes the reference ranges for Mean RI.

Table 24 Reference ranges for Mean Uterine RI

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	0.57	0.54	0.60
20	42	100	0	0	0.55	0.52	0.58
24	51	100	0	0	0.53	0.50	0.55
26	47	95.9	2	4.1	0.52	0.49	0.54
28	51	98.1	1	1.9	0.48	0.46	0.50
30	54	100	0	0	0.48	0.46	0.51
32	52	100	0	0	0.47	0.45	0.50
34	47	100	0	0	0.47	0.45	0.50
36	48	94.1	3	5.9	0.49	0.46	0.52
38	16	43.2	21	56.8	0.49	0.44	0.53

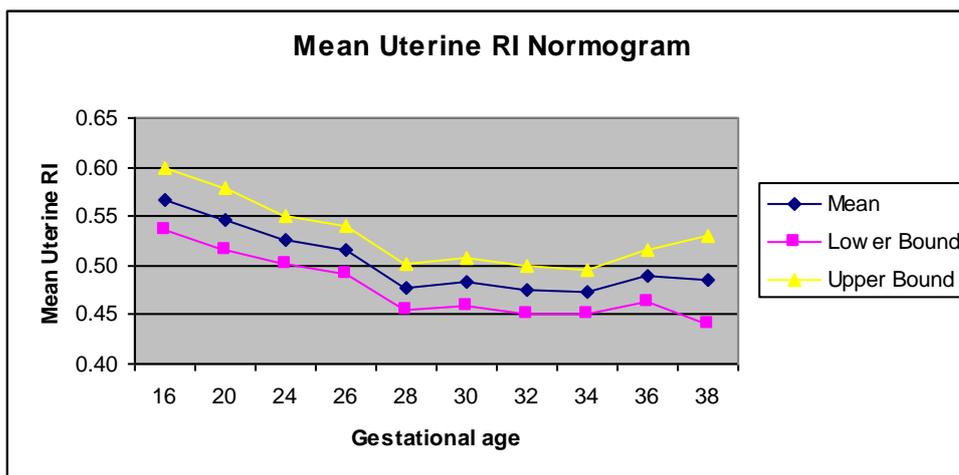


Figure 45 Reference ranges for Mean Uterine RI

These results were slightly higher than the centile charts in a previously published large cross-sectional study by Kurmanavicius et al [302]; our Mean RI values

showed 0.53 at 24 weeks and 0.49 at 38 weeks, whereas the fitted values in Kurmanavicius study showed 0.45 at 24 weeks and 0.43 at 38 weeks.

Umbilical artery

Figure 46 briefly describes the Doppler methodology for imaging umbilical artery.

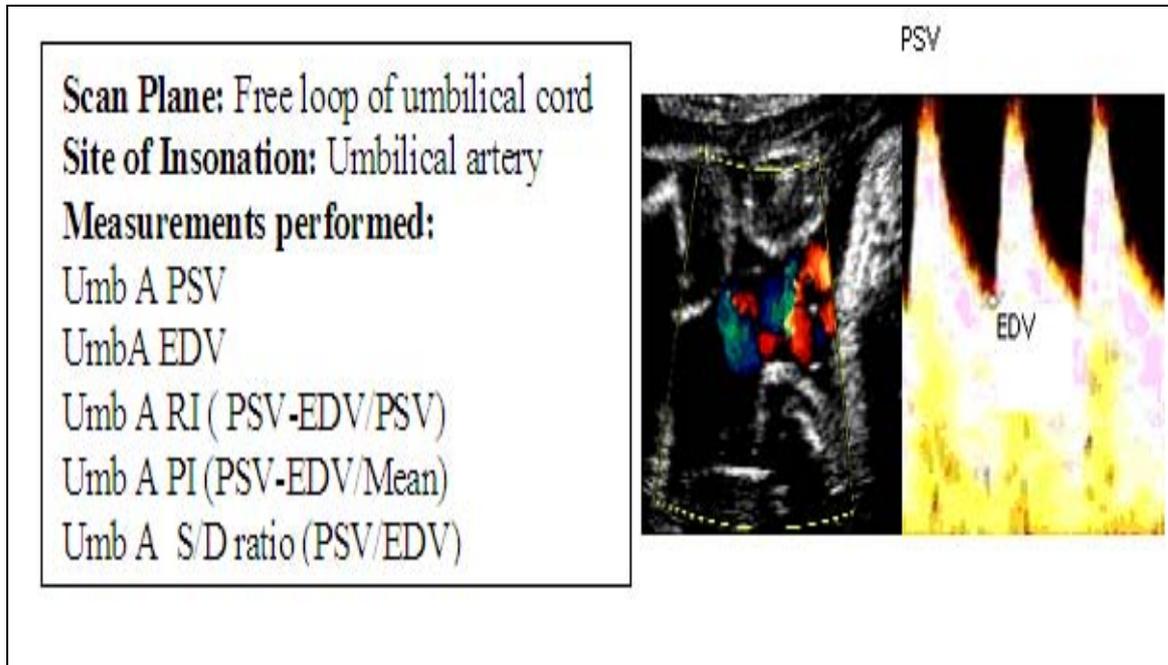


Figure 46 Umbilical artery Doppler (Umb A) Methodology

Umbilical artery demonstrated progressively decreasing resistance waveform with increasing forward diastolic flows with advancing gestation throughout pregnancy (Figure 46). Umbilical S/D ratio and RI were recorded as candidate variables and umbilical RI values were utilised to design uteroplacental ratio. Umbilical PI was the variable selected as the variable to represent uteroplacental circulation as it has been shown to be useful in monitoring high risk pregnancies [13].

Umbilical artery PI

Table 25 describes the reference ranges number of observations used to generate the reference ranges for umbilical artery PI. In normal pregnancies, the mean umbilical artery RI decreased linearly with gestational age, mean PI from 1.52 at 16 weeks to 0.84 at 38 weeks (Figure 47).

Table 25 Reference ranges for Mean Umbilical Artery Pulsatility Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	1.52	1.31	1.72
20	42	100.0	0	0.0	1.28	1.22	1.34
24	50	98.0	1	2.0	1.13	1.06	1.19
26	47	95.9	2	4.1	1.13	1.07	1.20
28	51	98.1	1	1.9	1.09	1.03	1.15
30	54	100.0	0	0.0	1.04	0.98	1.10
32	52	100.0	0	0.0	0.95	0.90	1.00
34	47	100.0	0	0.0	0.94	0.89	0.99
36	51	100.0	0	0.0	0.88	0.83	0.93
38	37	100.0	0	0.0	0.84	0.77	0.91

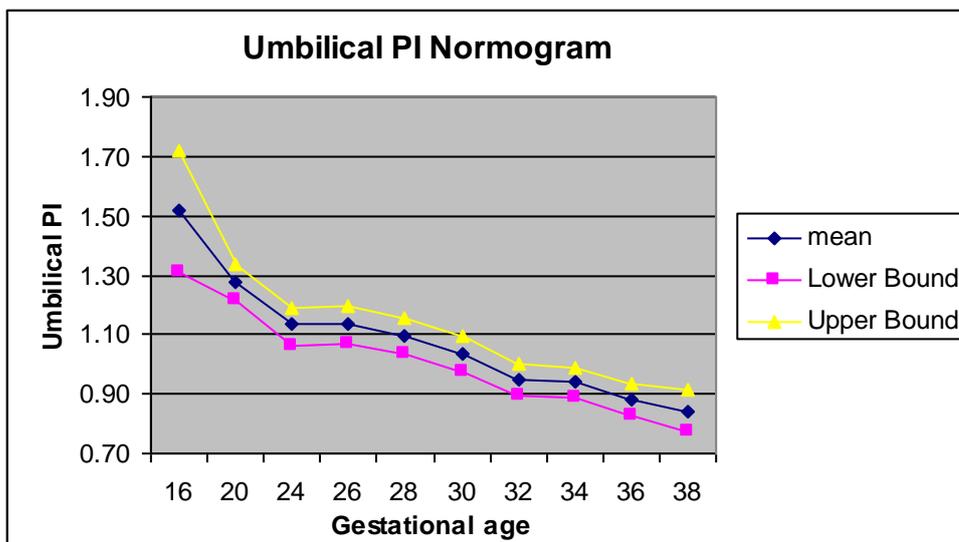


Figure 47 Reference ranges for Umbilical Artery Pulsatility Index

Uteroplacental ratio

Uteroplacental ratio was defined as the ratio of Mean RI to umbilical RI. Table 26 describes the reference ranges for uteroplacental ratio as well as the number of observations used to generate the reference ranges for uteroplacental ratio.

In normal pregnancies, the mean uteroplacental ratio increased slightly linearly with gestational age, mean uteroplacental ratio from 0.76 at 16 weeks to 0.75 at 38 weeks (Figure 48).

Table 26 Reference ranges for uteroplacental ratio

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	0.76	0.69	0.83
20	42	100	0	0	0.74	0.69	0.79
24	49	96.1	2	3.9	0.78	0.74	0.83
26	46	93.9	3	6.1	0.76	0.71	0.80
28	51	98.1	1	1.9	0.71	0.66	0.76
30	54	100	0	0	0.77	0.71	0.82
32	52	100	0	0	0.79	0.74	0.85
34	47	100	0	0	0.79	0.75	0.84
36	48	94.1	3	5.9	0.87	0.81	0.93
38	16	43.2	21	56.8	0.87	0.75	0.98

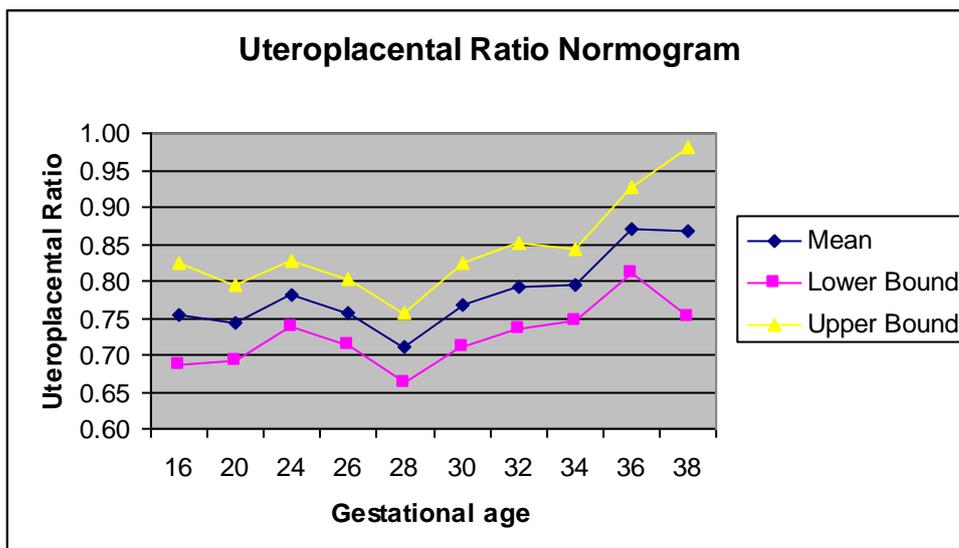


Figure 48 Reference ranges for uteroplacental ratio

CEREBRAL FLOW WAVEFORMS NORMOGRAMS

Middle cerebral artery RI

Figure 49 briefly describes the Doppler methodology for imaging middle cerebral artery.

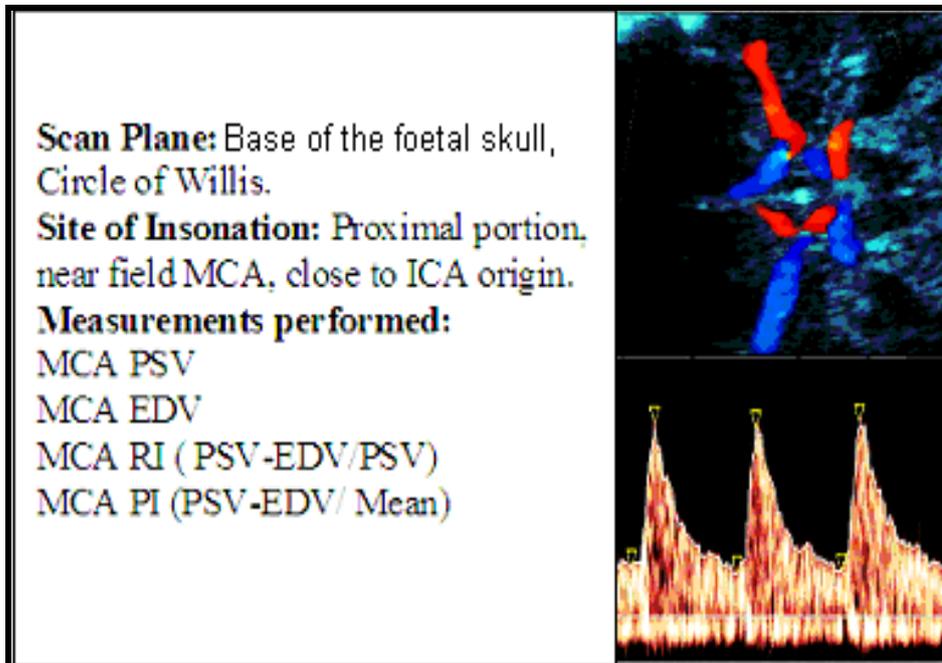


Figure 49 Middle Cerebral Artery Doppler (MCA) Methodology

MCA showed high resistance waveforms throughout pregnancy. The MCA RI pattern followed an inverted parabolic curve and demonstrated progressively increasing resistance waveform till early third trimester after which there was a steady decrease in resistance till term.

MCA RI

Table 27 describes the reference ranges for MCA RI as well as the number of observations used to generate the reference ranges for MCA RI. In normal pregnancies, the mean value for MCA RI rose from 0.79 at 16 weeks to at 16 weeks to 0.85 at 32 weeks, after which it declined to 0.74 at 38 weeks (Figure 50).

Table 27 Reference ranges for Middle Cerebral Artery Resistance Index

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	32	94.1	2	5.9	0.79	0.76	0.82
20	42	100	0	0	0.77	0.75	0.79
24	51	100	0	0	0.82	0.80	0.83
26	48	98.0	1	2	0.84	0.81	0.86
28	51	98.1	1	1.9	0.83	0.80	0.85
30	53	98.1	1	1.9	0.85	0.84	0.87
32	52	100	0	0	0.85	0.82	0.87
34	47	100	0	0	0.80	0.77	0.84
36	50	98.0	1	2	0.80	0.77	0.82
38	32	86.5	5	13.5	0.74	0.71	0.77

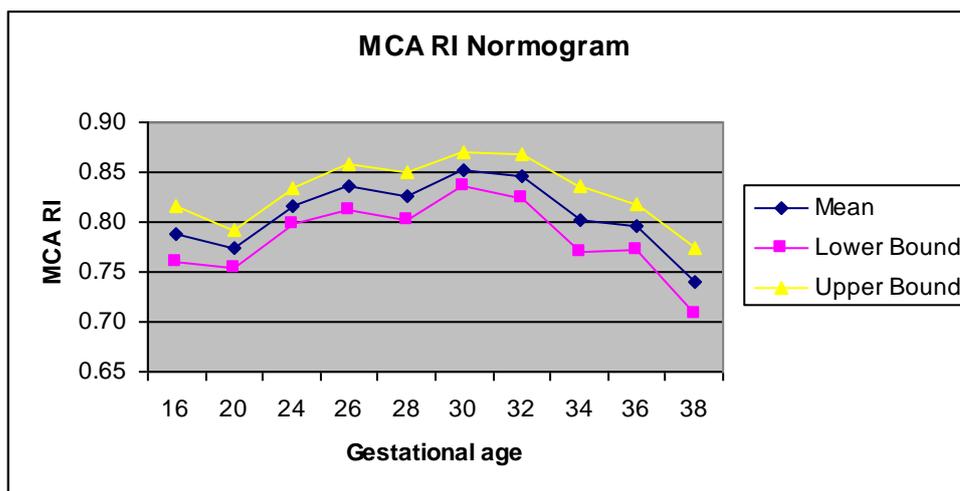


Figure 50 Reference ranges for Middle Cerebral Artery Resistance Index

The mean values obtained in this study were in line with the centile charts from a previously published large cross-sectional study by Kurmanavicius [302].

Cerebroplacental ratio

Cerebroplacental ratio was defined as the ratio of MCA to Umbilical artery PI.

Table 28 describes the reference ranges for cerebroplacental ratio as well as the number of observations used to generate the reference ranges for uteroplacental ratio.

In normal pregnancies, the mean cerebroplacental ratio increased slightly linearly with gestational age, mean cerebroplacental ratio from 1.17 at 16 weeks to 1.72 at 38 weeks (Figure 51).

Table 28 Reference ranges for Cerebroplacental ratio

gestational age	valid observations		missing data		Mean	95% Confidence Interval for Mean	
	N	Percent	N	Percent		Lower Bound	Upper Bound
16	32	94.1	2	5.9	1.17	0.98	1.36
20	42	100	0	0	1.10	1.04	1.16
24	50	98.0	1	2.0	1.39	1.31	1.47
26	47	95.9	2	4.1	1.41	1.33	1.49
28	51	98.1	1	1.9	1.43	1.34	1.52
30	53	98.1	1	1.9	1.60	1.48	1.72
32	52	100	0	0	1.72	1.60	1.84
34	47	100	0	0	1.60	1.48	1.71
36	50	98.0	1	2.0	1.67	1.55	1.79
38	32	86.5	5	13.5	1.72	1.32	2.12

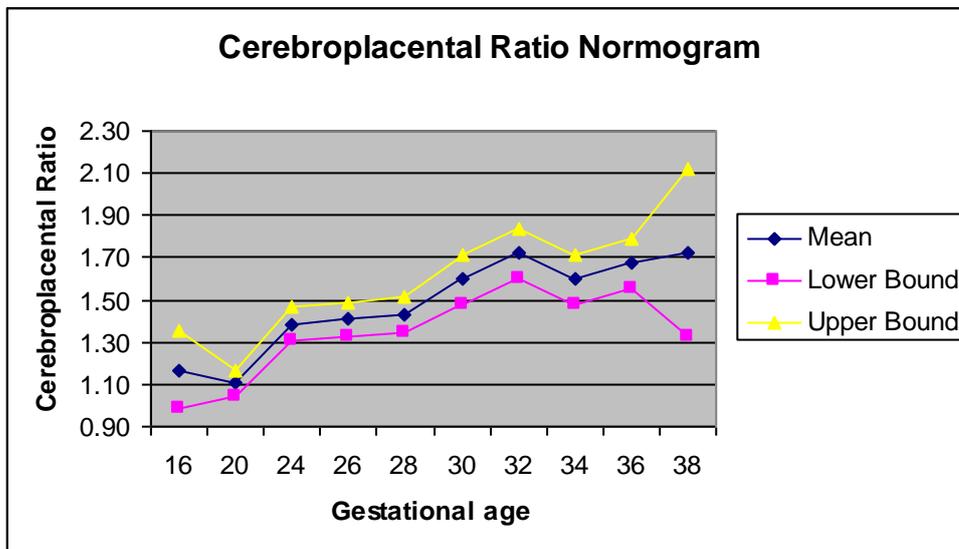


Figure 51 Reference ranges for Cerebroplacental ratio

The mean values obtained in the present study were slightly lower than those from the conditional centiles in a recently published longitudinal study by Ebbing. et al [296] and those from unconditional centiles in a cross-sectional study by Baschat [303]. The mean CPR ratios were 1.39 at 24 week and 1.72 at 38 weeks in the present study, versus 1.74 at 24 week and 2.09 at 38 weeks in the Ebbing study and 1.53 at 24 weeks and 1.90 at 38 weeks in Baschat's study. All the above mentioned studies were slightly different from the present study in terms of study methodology, with different Doppler sampling sites (mid region of MCA in Baschat study versus proximal region at origin of MCA in the present study) and different statistical methods for analysis

(conditional centiles in Ebbing study versus unconditional in the present study).

PLACENTAL THICKNESS AND BIOMETRY NORMOGRAMS

Placental thickness

Figure 52 briefly describes the ultrasound methodology for imaging placental thickness.

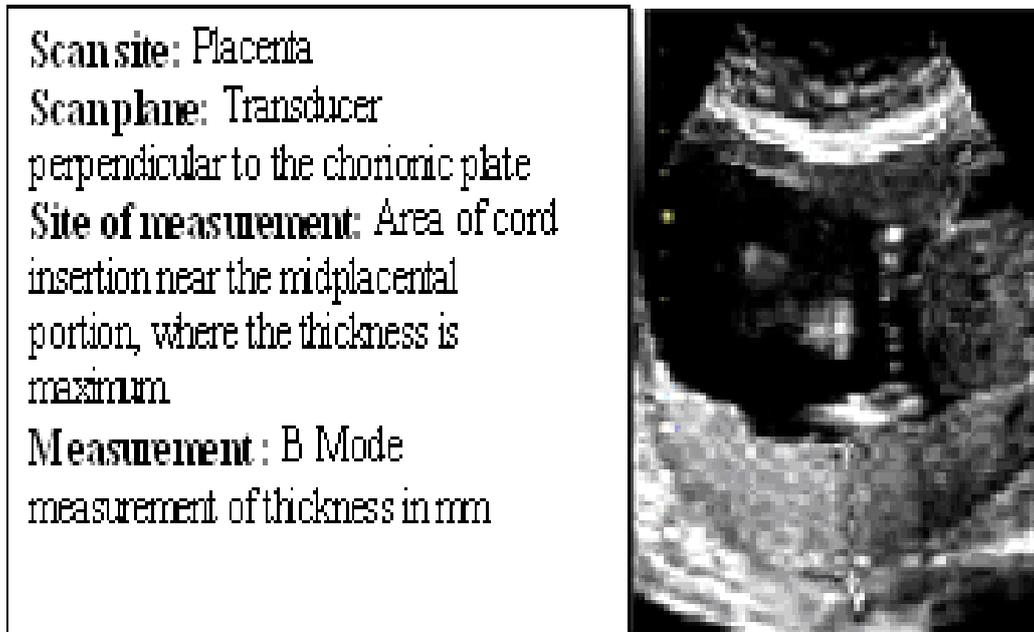


Figure 52 Placental thickness Methodology

Placental thickness increased linearly with gestational age.

Table 29 describes the reference ranges for placental thickness as well as the number of observations used to generate the reference ranges for placental thickness.

In normal pregnancies, the mean placental increased linearly with gestational age, mean placental thickness from 19.4 mm 16 weeks to 36. 8 at 38 weeks (Figure 53).

Table 29 Reference ranges for placental thickness

gestational age	valid observations		missing data		Mean	95% Confidence Interval for Mean	
	N	Percent	N	Percent		Lower Bound	Upper Bound
16	32	94.1	2	5.9	19.4	18.1	20.6
20	40	95.2	2	4.8	24.0	22.8	25.3
24	49	96.1	2	3.9	27.0	25.5	28.5
26	41	83.7	8	16.3	28.2	27.0	29.4
28	46	88.5	6	11.5	31.3	29.4	33.3
30	48	88.9	6	11.1	31.8	29.9	33.7
32	46	88.5	6	11.5	33.8	31.9	35.7
34	43	91.5	4	8.5	35.2	32.9	37.6
36	48	94.1	3	5.9	34.5	32.8	36.1
38	32	86.5	5	13.5	36.8	34.9	38.7

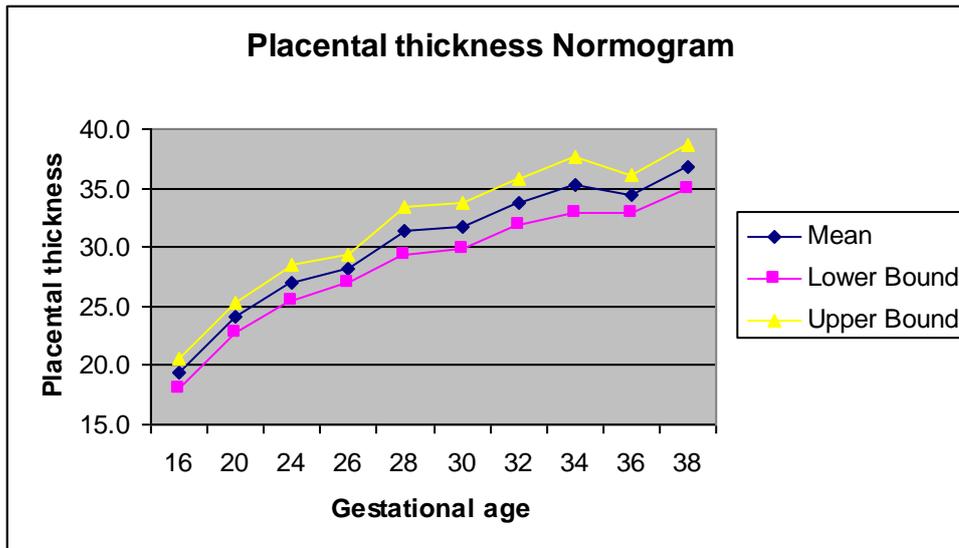


Figure 53 Reference ranges for placental thickness.

These reference ranges were similar to the results of a previously published cross-sectional study [304].

Fetal biometric measurements

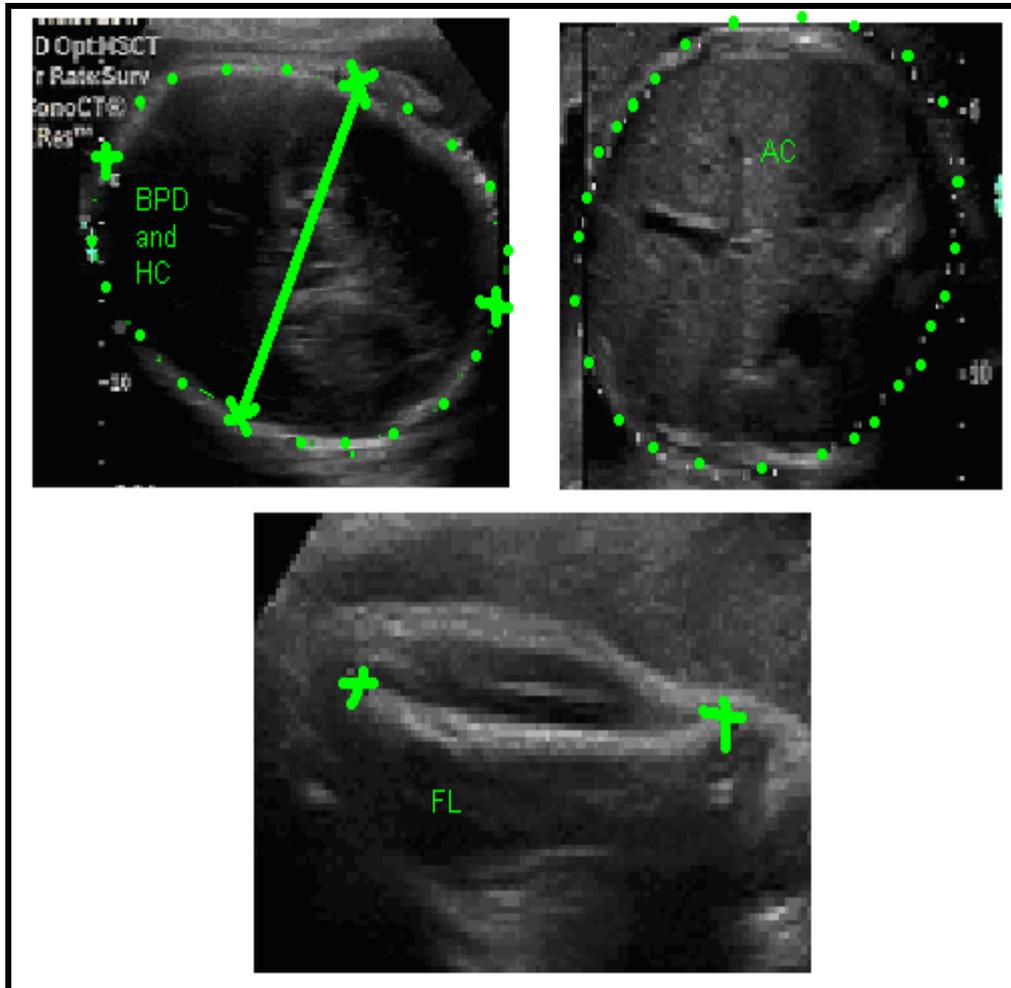


Figure 54 Sonographic planes for biometric measurements.

This figure depicts the sonographic planes for measurements of fetal biometry i.e., the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL).

BPD

Figure 54 describes the sonographic plane for measurement of the BPD. Table 30 describes the reference ranges for BPD and the number of observations used to generate the reference ranges for BPD. In normal pregnancies, the mean BPD increased from 3.6 mm at 16 weeks to 8.9 mm at 38 weeks (Figure 55).

Table 30 Reference ranges for biparietal diameter

gestational age	valid observations		missing data		Mean	95% Confidence Interval for Mean	
	N	Percent	N	Percent		Lower Bound	Upper Bound
16	33	97.1	1	2.9	3.6	3.5	3.7
20	42	100	0	0	4.8	4.7	4.9
24	51	100	0	0	6.2	6.1	6.3
26	48	98	1	2.0	6.8	6.5	7.1
28	51	98.1	1	1.9	7.5	7.4	7.6
30	54	100	0	0	8.1	8.0	8.2
32	52	100	0	0	8.4	8.1	8.7
34	47	100	0	0	8.9	8.8	9.0
36	51	100	0	0	9.3	9.2	9.4
38	35	94.6	2	5.4	8.9	8.2	9.6

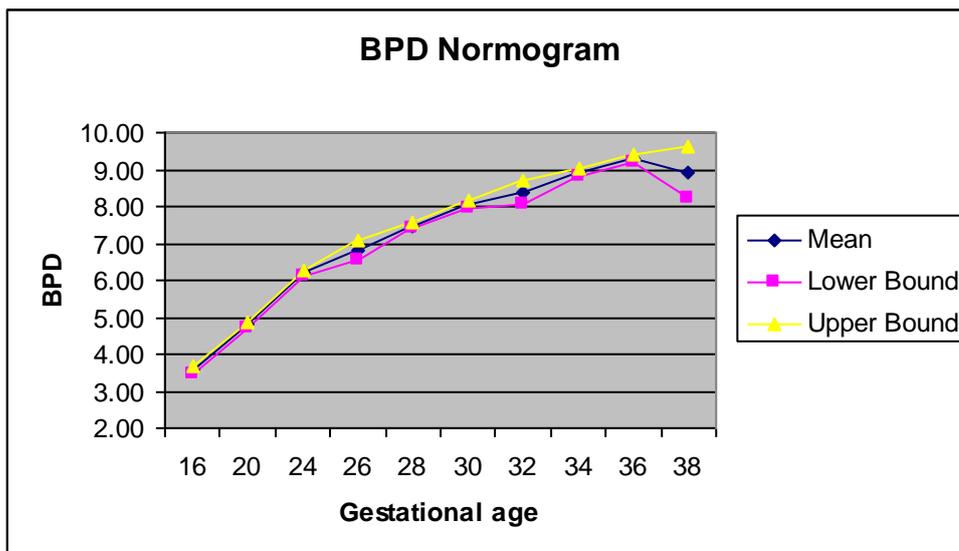


Figure 55 Reference ranges for biparietal diameter

HC

Figure 54 describes the sonographic plane for measurement of HC. Table 31 describes the reference ranges for HC and the number of observations used to generate the reference ranges for HC. In normal pregnancies, the mean HC increased from 13 cm at 16 weeks to 32.2 cm at 38 weeks (Figure 56).

Table 31 Reference ranges for head circumference

gestational age	valid observations		missing data		Mean	95% Confidence Interval for Mean	
	N	Percent	N	Percent		Lower Bound	Upper Bound
16	33	97.1	1	2.9	13.0	12.6	13.4
20	42	100	0	0	17.5	16.6	18.3
24	51	100	0	0	22.8	22.5	23.1
26	48	98.0	1	2	24.8	23.8	25.8
28	51	98.1	1	1.9	25.0	23.1	27.0
30	54	100	0	0	29.2	28.9	29.4
32	52	100	0	0	30.2	29.0	31.3
34	47	100	0	0	32.1	31.8	32.5
36	51	100	0	0	33.5	33.1	33.8
38	35	94.6	2	5.4	32.2	29.7	34.8

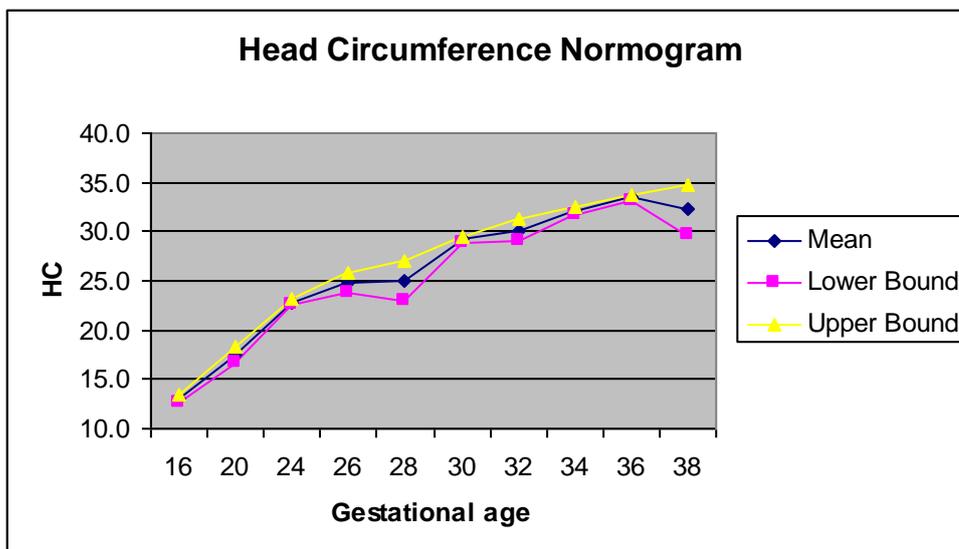


Figure 56 Reference ranges for head circumference

AC

Figure 54 describes the sonographic plane for measurement of AC. Table 32 describes the reference ranges for AC as well as the number of observations used to generate the reference ranges for AC. In normal pregnancies, the mean AC increased from 11 cm at 16 weeks to 34.5 cm at 38 weeks (Figure 57).

Table 32 Reference ranges for abdominal circumference

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	11.0	10.8	11.3
20	41	97.6	1	2.4	15.7	15.6	15.9
24	51	100	0	0	20.5	20.3	20.7
26	47	95.9	2	4.1	22.7	22.5	22.9
28	51	98.1	1	1.9	24.8	24.6	25.0
30	54	100	0	0	27.2	27.0	27.4
32	52	100	0	0	29.3	29.1	29.5
34	47	100	0	0	31.1	30.8	31.5
36	51	100	0	0	32.9	32.6	33.1
38	35	94.6	2	5.4	34.5	34.0	35.0

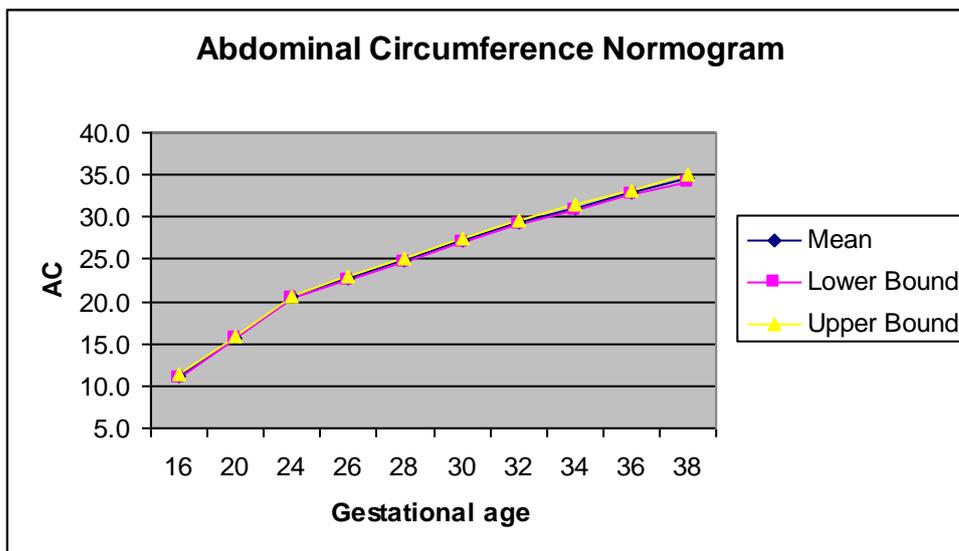


Figure 57 Reference ranges for abdominal circumference

FL

Figure 54 describes the sonographic plane for measurement of FL. Table 33 describes the the reference ranges for FL and number of observations used to generate the reference ranges for FL. In normal pregnancies, the mean FL increased from 2.1 cm at 16 weeks to 7.3 cm at 38 weeks (Figure 58).

Table 33 Reference ranges for femur length

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	2.1	2.0	2.1
20	42	100	0	0	3.3	3.3	3.4
24	51	100	0	0	4.4	4.4	4.5
26	48	98	1	2	4.9	4.9	5.0
28	51	98.1	1	1.9	5.4	5.3	5.4
30	54	100	0	0	5.9	5.8	5.9
32	52	100	0	0	6.3	6.2	6.3
34	47	100	0	0	6.7	6.6	6.7
36	51	100	0	0	7.0	7.0	7.1
38	35	94.6	2	5.4	7.3	7.3	7.4

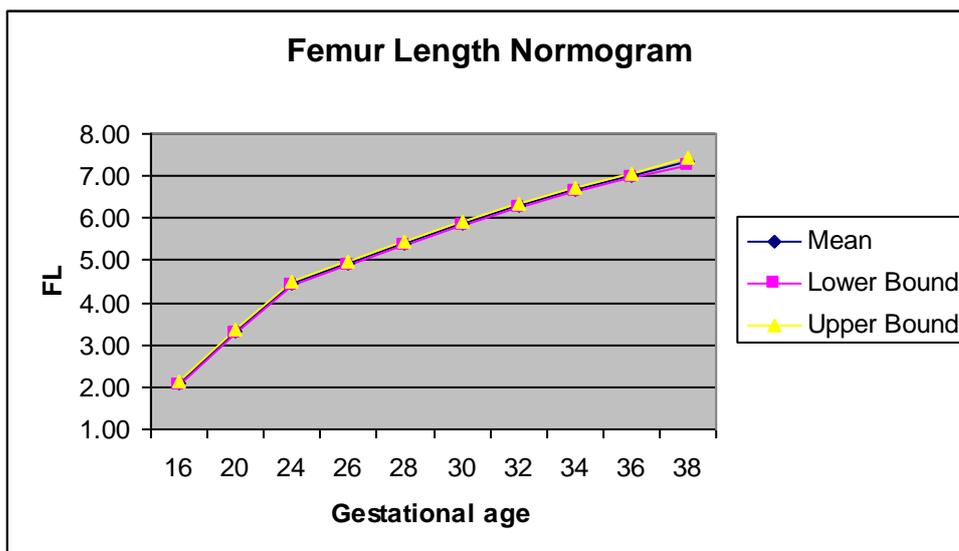


Figure 58 Reference ranges for femur length

EFW

Estimated weight was calculated using BPD, HC, AC and FL, with the following formula by Hadlock [305]. The formula was as follows:

$$EFW =: \text{Log}_{10}EFW = 1.3596 + 0.0064 (HC) + 0.0424 (AC) + 0.174 (FL) + 0.00061 (BPD) (AC) - 0.00386 (AC) (FL)$$

Table 34 describes the reference ranges for EFW as well as the number of observations used to generate the reference ranges for EFW. In normal pregnancies, the mean EFW increased from 165 gms at 16 weeks to 3422 gms at 38 weeks (Figure 59).

Table 34 Reference ranges for estimated fetal weight

gestational age	valid observations		missing data		95% Confidence Interval for Mean		
	N	Percent	N	Percent	Mean	Lower Bound	Upper Bound
16	33	97.1	1	2.9	165	159	170
20	41	97.6	1	2.4	371	362	379
24	51	100	0	0	755	739	771
26	47	95.9	2	4.1	1013	991	1035
28	51	98.1	1	1.9	1320	1292	1348
30	54	100	0	0	1741	1709	1773
32	52	100	0	0	2162	2121	2203
34	47	100	0	0	2608	2559	2658
36	51	100	0	0	3037	2977	3097
38	35	94.6	2	5.4	3422	3303	3541

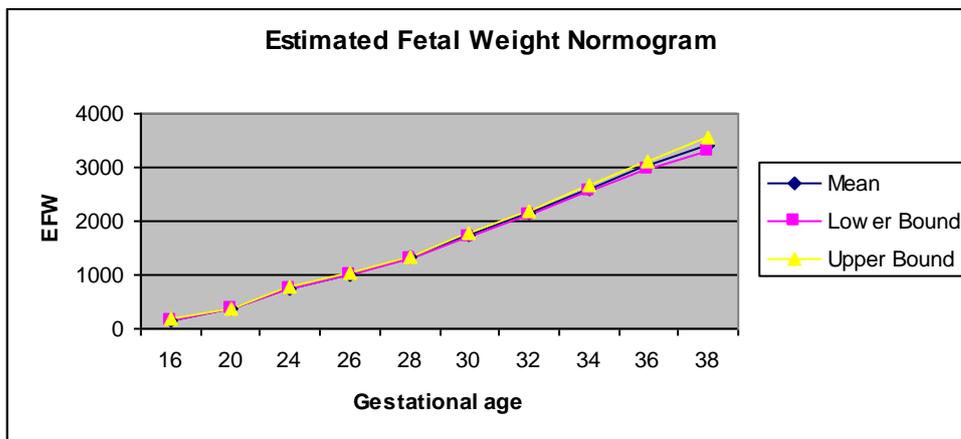


Figure 59 Reference ranges for estimated fetal weight

All the biometric parameters BPD, HC, FL, AC and EFW were similar to the ASUM charts, used regularly in clinical practice [44, 306].

Key findings

Study 1 was performed to establish reference ranges for the Doppler flows of central shunts i.e. DA and FO as well as evaluate fetal Biometry and Doppler waveforms of maternal uterine arteries, fetal umbilical artery, ductus venosus, middle cerebral artery with serial measurements, in the same set of control pregnancies, in a longitudinal study . The key findings of Study 1 have been summarised below.

Shunts: * Standard deviations and 95% Confidence Intervals (CI) have been provided earlier, in Table 14 to Table 23.

FO: FO PSV and EDV increased linearly and FO PI decreased with gestational age.

- mean FO PSV increased from 26.8 cm/s at 16 weeks to 43.3 cm/s at 38 weeks
- mean FO EDV increased from 9.4 cm/s at 16 weeks to 17.7 cm/s at 36 weeks
- the mean PI of FO decreased from 1.7 at 16 weeks to 1.3 at 36 weeks

DA: DA PSV and EDV increased linearly, DA PI was constant with gestational age.

- mean DA PSV increased from 43.4 cm/s at 16 weeks to 95.6 cm/s at 38 weeks
- mean DA EDV increased from 6.6 cm/s at 16 weeks to 10.4 cm/s at 38 weeks
- PI of DA was relatively constant, mean values ranging between 2.4 to 2.8.

DV: DV indices preload index, S/a ratio, Peak velocity index and pulsatility index reduced linearly with gestational age.

- mean DV Preload Index decreased from 0.57 at 16 weeks to 0.43 at 36 weeks.
- mean DV S/a ratio decreased from 2.74 at 16 weeks to 2.52 at 38 weeks
- mean DV Preload Index decreased from 0.68 at 16 weeks to 0.51 at 36 weeks.

Uteroplacental and fetoplacental haemodynamics

* Standard deviations and 95% Confidence Intervals (CI) have been provided in Table 24, Table 25 and Table 26.

Uterine artery mean RI: the mean uterine RI decreased linearly with gestational age, mean RI from 0.57 at 16 weeks to 0.49 at 38 weeks.

Umbilical artery PI: the mean umbilical artery resistance decreased linearly with gestational age, mean umbilical artery mean PI decreased from 1.52 at 16 weeks to 0.84 at 38 weeks.

Uteroplacental ratio: the mean uteroplacental ratio increased slightly linearly with gestational age, from 0.76 at 16 weeks to 0.87 at 38 weeks.

Fetal cerebral flow dynamics

* Standard deviations and 95% Confidence Intervals (CI) have been provided in Table 27 and Table 28.

Mean MCA RI: the mean values for MCA RI rose from 0.79 at 16 weeks to at 16 weeks to 0.85 at 32 weeks, after which it declined to 0.74 at 38 weeks.

Cerebroplacental ratio: the mean cerebroplacental ratio increased slightly linearly with gestational age, mean cerebroplacental ratio from 1.17 at 16 weeks to 1.72 at 38 weeks.

Biometry and placental thickness

* Standard deviations and 95% Confidence Intervals (CI) has been provided in Table 29 to Table 34.

Biometric parameters and placental thickness increased linearly with gestational age

- mean placental thickness increased from 19.4 mm 16 weeks to 36. 8 at 38 weeks
- mean BPD increased from 3.6 mm at 16 weeks to 8.9 mm at 38 weeks
- mean HC increased from 13 cm at 16 weeks to 32.2 cm at 38 weeks.
- mean AC increased from 11 cm at 16 weeks to 34.5 cm at 38 weeks
- mean FL increased from 2.1 cm at 16 weeks to 7.3 cm at 38 weeks
- mean EFW increased from 165 gms at 16 weeks to 3422 gms at 38 weeks.

CHAPTER 5

Study 2: Fetal shunts and acute adaptive mechanisms: haemodynamics before and after intrauterine transfusion (IUT)

Introduction

The objective of this study was to evaluate fetal response to acute cardiovascular stress. Fetal anaemia is a condition that can cause cardiac decompensation and death and can be treated successfully with intrauterine transfusions in most cases. Intrauterine transfusion and fetal needling, however, are invasive procedures which lead to acute stress response, by means of the needling procedure causing a noxious response [307], as well as the sudden increase in intravascular volume leading to increased cardiac workload causing cardiovascular stress [308]. As a result, the fetus mounts independent hormonal responses, such as an activation of the hypothalamo-pituitary axis [309, 310].

A response to intrauterine transfusions leading to improvement in circulatory dynamics might hence represent fetal circulatory adaptation to acute cardiovascular stress of anaemia. Circulatory Doppler flow responses to fetal intrauterine transfusion in fetal anaemia may thus represent a model to evaluate fetal circulatory responses to acute cardiovascular stress.

Studies of fetal haemodynamics have demonstrated that as a consequence of fetal anaemia, there is a redistribution of flow to major organs such as brain and the heart which may minimise vital organ hypoxia [311]. With the advent of Doppler ultrasound, it is now possible to non-invasively assess for fetal anaemia by evaluating middle cerebral artery peak systolic flow using Doppler ultrasound [312]. This is because middle cerebral artery peak flow measurement strongly correlates with the degree of fetal anaemia [104]. Anaemic fetuses demonstrate an increase in peak systolic velocity, which dramatically resolves after intrauterine fetal transfusion [313].

Fetal arterial and venous systems have been studied in anaemic fetuses before and after transfusions, including parameters such as ductus venosus and portal venous systems. A study of the portal venous system has shown a pulsatile pattern after transfusion, which may suggest a transient portal hypertension [314]. Ductus venosus flows show increased velocities [315] and ventricular shortening fraction in the fetus has been demonstrated to decrease immediately after transfusion [316]. PI of MCA, ICA, thoracic aorta, renal, internal iliac arteries, femoral, umbilical

arteries have all been studied before and after fetal transfusions, and the relevant PIs did not show any significant differences a day later [313].

All these studies seem to suggest that effective adaptive mechanisms exist in the fetus, to cope with the acute cardiovascular stress of fetal transfusion.

Hypothesis

We hypothesised that there is an adaptive redistribution seen as changes in the flows within the fetal heart via the central intrauterine shunts in addition to blood flow changes in the brain as an acute response to fetal intrauterine transfusion.

Aim

The aim of this study was to prospectively investigate the haemodynamic alteration in intracardiac and other fetal shunts as a response to the acute cardiovascular stress of fetal transfusion in fetal anaemia.

Methods

A prospective observational study was undertaken at the Womens' and Children's Hospital, North Adelaide. The study recruited women undergoing intrauterine transfusions for fetal anaemia. The Pulsatility Index (PI) of the fetal ductus arteriosus, PI of the foramen ovale, PIV (Pulsatility Index of vein) of the ductus venosus and Resistance Index (RI) of the middle cerebral artery, and RI of the umbilical artery were measured by Doppler ultrasonography immediately before and after intrauterine transfusions (Figure 60).

Study data from 12 transfusion procedures were included in the study, with the mean gestational age at the time of transfusion being 28 weeks

Doppler methodology has been described in detail in Chapter 3 of the thesis.

Fetal heart rate was monitored intermittently throughout transfusions.

Post-procedural Doppler measurements of all the above-mentioned parameters were completed within 2 hours of the procedure.

Statistical analysis

Statistical analysis was performed using SPSS 14 (SPSS, Chicago, IL, USA). All values are medians \pm SD.

Normality of distribution was tested by Shapiro and Wilk test. Data was assessed with Students paired t test, with each fetus serving as its own control.

Wilcoxon signed rank test was used, for non parametric assumption.

P < 0.05 was considered to be significant.

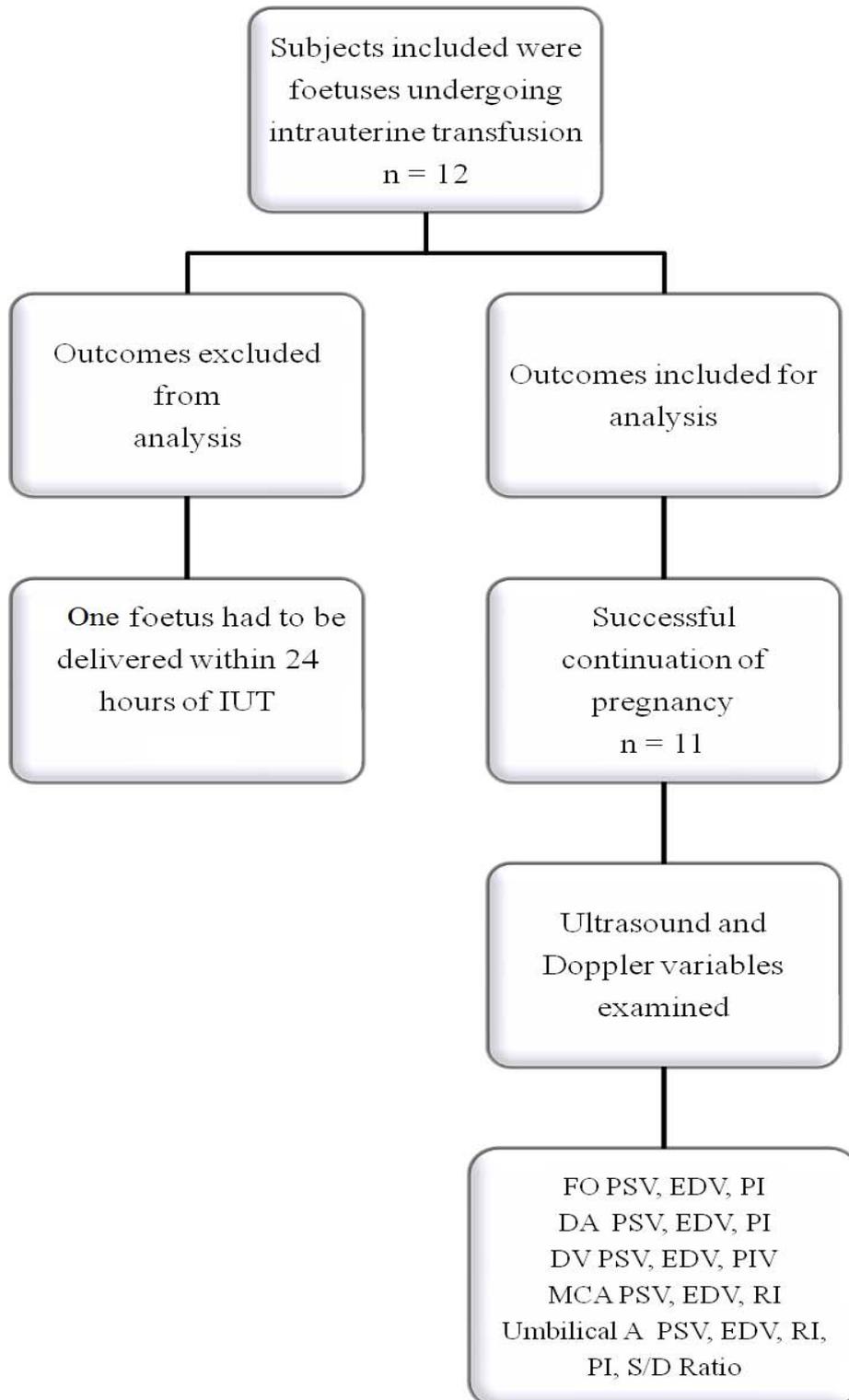


Figure 60 Flow diagram for study design for Study 2

Results

12 fetuses, of which 2 fetuses belonged to a twin pregnancy, underwent IUT, their characteristics are described below (Table 35).

Table 35 Transfusion details of fetuses undergoing transfusion

Fetus undergoing transfusion	Estimated fetal weight	Volume transfused	Hb before transfusion	Hb after transfusion
1 PH 24/02/2005	1040g	40mls	8.2	15.4 (calc)
2 GM 10/03/2005 23 + 5	840g	45mls	4.8	14.8
3 GM 24/03/2005 25 + 5	1128g	60mls	7.9	17.8
4 NT 31/03/2005	923g	55mls	5.7	17.9
5 GM 15/04/2005 28 + 6	1667g	85mls	7.6	17.1
6 NT 21/04/2005	1624g	80mls	5.8	16.3 (calc)
7 GM 12/05/2005 32 + 5	2730g	105mls	6.9	14.3 (calc)
8 NT 13/05/2005	1927g	40mls	10.6	15.3
9 KF 11/05/2005	2,300g	25mls	10.3	12.3
10 NT 06/07/2005 (T1)	820g	70mls	3.8	18.7
11 NT 20/07/2005 (T2)	1,000g	60mls	4.9	17.0
12 NT 20/07/2005 (T1)	1,100g	15mls	8.6	11.3

Key: (calc) = calculated Hb as satisfactory sample was unable to be obtained after transfusion. in 3 cases we could not get a satisfactory post transfusion sample, and the Hb value is calculated.

Table 36 describes the Doppler results and p values before and after transfusion.

Table 36 Doppler results and p values before and after IUT

Doppler Variable	Paired Samples T Test Sig. (2-tailed) p value	Before T/F or after		Before T/F or after T/F	
		T/F		Std. Deviation	
		Means		before	after
		Before	after	before	after
umbilical artery PSV	0.037	42.8	34.9	11.0	7.3
umbilical artery EDV	0.738	14.0	13.8	3.9	4.7
umbilical artery - RI	0.016	0.7	0.6	0.1	0.1
umbilical artery - PI	0.048	1.1	0.9	0.2	0.2
umbilical artery - S/D ratio	0.022	3.3	2.7	0.6	0.6
middle cerebral artery - PSV	0.002	46.1	33.3	11.3	11.9
middle cerebral artery - EDV	0.545	9.0	8.3	2.8	3.6
middle middle cerebral artery - RI	0.022	0.8	0.7	0.0	0.1
ductus venosus - PSV	0.996	49.0	50.3	19.8	21.0
ductus venosus -EDV	0.181	26.2	36.0	13.2	19.4
ductus venosus - PIV	0.053	0.7	0.4	0.3	0.2
ductus arteriosus - PSV	0.788	76.0	78.1	18.0	19.0
ductus arteriosus - EDV	0.955	11.7	11.6	7.0	8.8
ductus arteriosus - PI	0.059	3.2	3.8	1.4	1.7
foramen ovale - PSV	0.850	37.6	36.9	14.4	8.9
foramen ovale - EDV	0.971	13.0	12.9	9.1	6.0
foramen ovale - PI	0.087	3.5	8.3	2.9	10.9

Umbilical artery PSV, RI, PI and S/D ratio reduced significantly after the procedure. (Figure 61, Table 36).

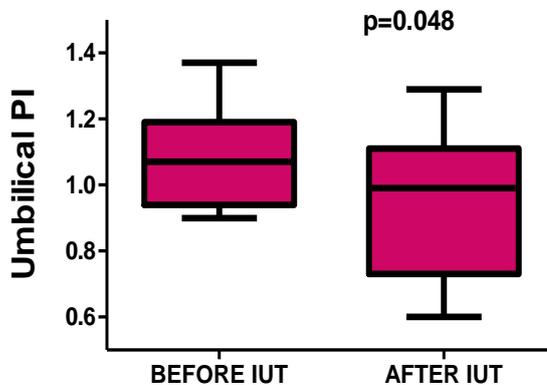


Figure 61 Umbilical artery PI before and after fetal intrauterine transfusion

MCA PSV and RI also showed a significant reduction after transfusion (Figure 62, Table 36).

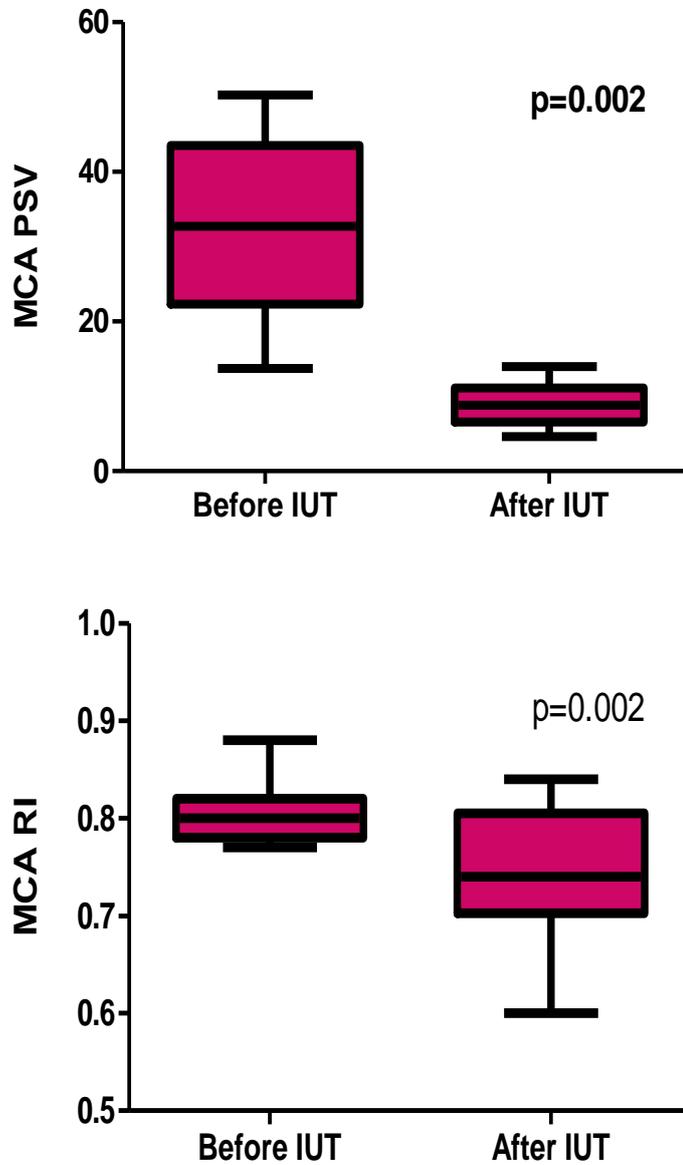


Figure 62 MCA PSV and RI before and after fetal intrauterine transfusion

The PIV of ductus venosus showed a significant decrease after transfusion. Ductus venosus EDV showed a slight increase, although not significant. DV PSV was unchanged (Figure 63, Table 36).

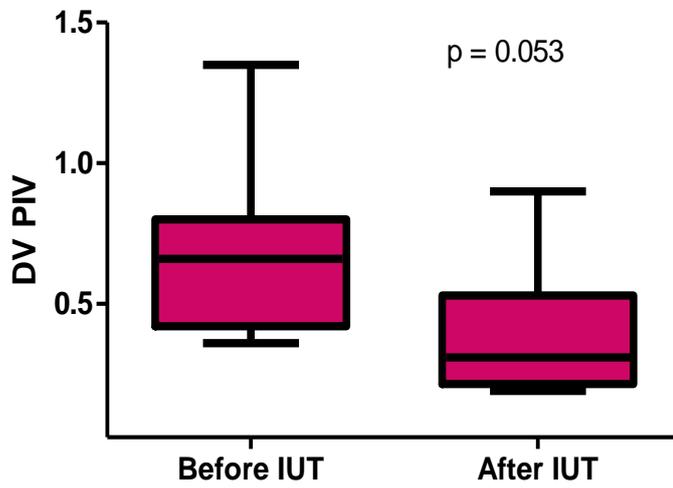


Figure 63 DV PIV before and after fetal intrauterine transfusion.

DA PSV and EDV did not alter with transfusion however, the PI of ductus arteriosus showed a rising trend after transfusion which was not statistically significant (Figure 64).

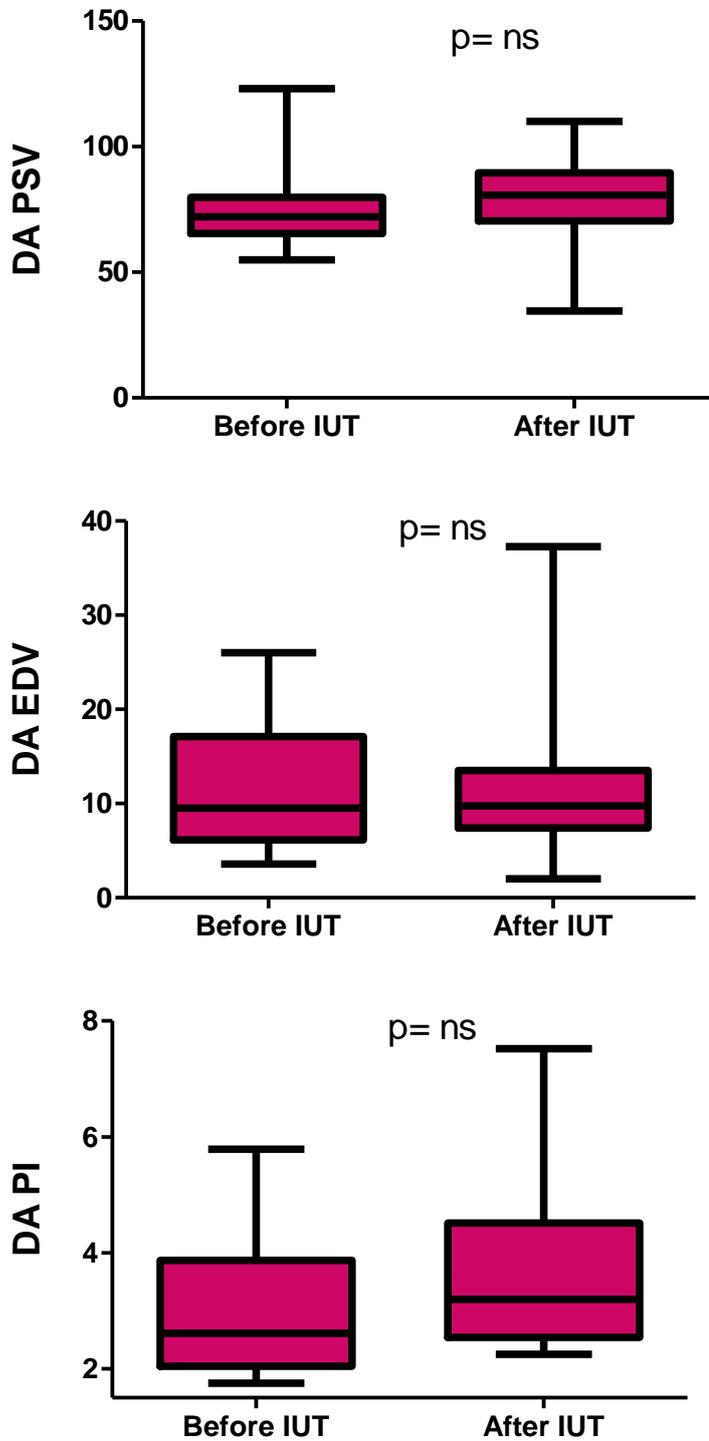


Figure 64 DA PSV, EDV, and PI before and after fetal intrauterine transfusion

FO PSV and EDV did not alter with transfusion (Figure 65), however, the PI of foramen ovale showed a rising trend after transfusion which was not statistically significant. (Figure 65, Table 36).

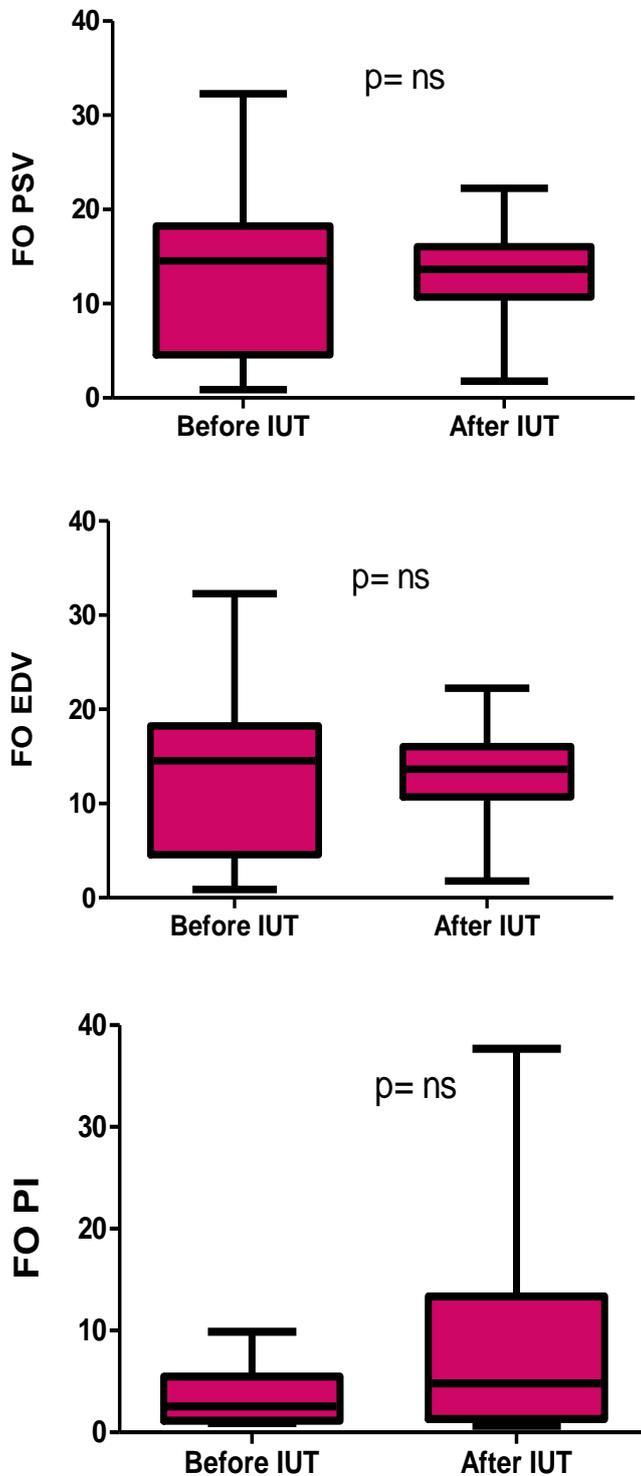


Figure 65 FO PSV, EDV and PI before and after fetal intrauterine transfusion

Key findings

Study 2 was undertaken to test the hypothesis that there is an adaptive redistribution through all fetal intrauterine shunts in addition to blood flow changes in the brain as an acute response to fetal intrauterine transfusion.

The results showed that

- The peak systolic velocity of middle cerebral artery reduced significantly following IUT, in accordance with previously published data. The PI, RI and S/D ratio of umbilical artery and PIV of ductus venosus also reduced immediately after the procedure as observed in previously published studies.
- The PI of fetal foramen ovale and fetal ductus arteriosus did show a consistent increase, even though this increase was not statistically significant. However, there was no statistically significant difference in PSV, EDV or PI of the central shunts DA and FO after transfusion.

CHAPTER 6

Study 3: Fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in uteroplacental insufficiency

Introduction

The objective of this study was to explore the correlation between Doppler of maternal and fetal circulation as well as placental thickness in adverse placental outcomes in pregnancies complicated by Uteroplacental Insufficiency (UPI) and explore the usefulness of flows through central shunts, i.e. DA and FO, as possible predictors of adverse outcome.

UPI is associated with preeclampsia and intrauterine growth restriction (IUGR) and leads to an increased proportion of adverse pregnancy outcomes, with the fetuses at high risk for compromise due to hypoxia. Several tests have therefore been employed in clinical practice to identify the mother with a fetus at- risk for hypoxia. One of the tests widely used in clinical practice is ultrasound. There is good evidence indicating that ultrasound, especially Doppler ultrasound, is useful as a monitoring tool as well as in decreasing the risk for adverse perinatal outcome in high-risk pregnancies [13].

Several longitudinal studies in UPI have documented the sequential changes in circulatory haemodynamics in the compromised fetus by evaluation the fetus for signs of brain sparing and severity of redistribution of circulation [2-8]. Data from these studies suggest that IUGR is associated with a reduction in fetal size, which can be identified with a reduction in fetal abdominal circumference (AC). IUGR is also associated with an increasing resistance in umbilical artery Doppler and a reduction in middle cerebral artery (MCA) resistance, and a reduction in cerebroplacental ratio (CPR).

Further worsening of Doppler parameters in an IUGR fetus include deterioration in cardiac function, which manifests as increased pulsatility in fetal venous waveforms leading to absent or even reversal of flows in the Ductus venosus [154]. These signs maybe associated with CTG changes as well as reduction in peak velocities through the AV valves, pulmonary valves and aortic valves. If these signs are present and there is no intervention, tricuspid regurgitation (TR) followed by fetal death, may occur [137]. The limitation of these markers of fetal compromise is that they only

manifest themselves and are observed late in pregnancy, after the disease is already established. Currently, there are no early markers for identifying either the “high-risk” IUGR fetus before a sonographic reduction in fetal AC or to recognize the high risk ‘appropriate for gestational age (AGA) fetus in earlier stages of pregnancy, when earlier intervention may result in minimizing adverse maternal and fetal outcomes.

It is well accepted that in regard to the anatomy of shunts, circulatory haemodynamics and fetal cardiovascular physiology, the main difference between fetal and neonatal circulation is the presence of intrauterine shunts. In the fetus, blood flows via two pathways, one through the right side and one through the left, both working as parallel circuits. Oxygenated flow from the placenta enters the fetus via the umbilical vein into the ductus venosus (DV). The DV is pivotal in diverting the flow channels into two pathways [317]. Oxygenated blood flows to the brain and upper part of the body via the left sided pathway through the ascending aorta via foramen ovale, whereas blood flow to the lower part of the body traverses through the descending aorta via the ductus arteriosus.

Thus, it could be reasoned that the intrauterine shunts foramen ovale (FO), ductus arteriosus (DA) and DV work closely with the placenta to ensure appropriate nutrition and oxygenation of the critically important fetal organs and are vital to enable redistribution of flow. It was therefore hypothesised that the fetal heart and the fetal shunts are primary structures involved in redistribution and may therefore reflect any early adaptive changes in the fetus as a response to hypoxia. These changes might probably be recognised prior to currently established parameters such as brain sparing or a reduction in fetal size as a response to increased placental resistance. A longitudinal study was therefore designed to evaluate the parameters measuring these changes in the central shunts, FO and DA.

Hypotheses and aims

The specific hypotheses and aims have been described below.

First Hypothesis: The fetal circulation is a shunt – dependant circulation. There exists a relationship between flow haemodynamics of fetal central shunts and fetal cerebral resistance.

Aims:

1. To prospectively evaluate fetal biometry and Doppler waveforms of maternal uterine arteries, fetal umbilical artery, ductus venosus, middle cerebral artery, and the central shunts foramen ovale and ductus arteriosus in high-risk and control pregnancies.
2. To compare Doppler flows in central shunts with middle cerebral artery flows and cerebroplacental ratio in normal and adverse pregnancy outcomes.

Second Hypothesis: Fetal central shunts are involved in adaptive mechanisms in acute and chronic fetal stress.

Aim:

To examine sequential haemodynamic alterations in intracardiac and other fetal shunts in pregnancies at risk for UPI with a prospective longitudinal Doppler ultrasound study and compare these values with those values obtained in uncomplicated pregnancies.

Third hypothesis: Changes in fetal central shunt flows precede the reduction in fetal biometry and the ‘brain-sparing’ effect in fetal hypoxia or maternal disease in UPI representing earlier intracardiac redistribution of fetal blood flow in these high-risk pregnancies.

Aims:

1. To evaluate uteroplacental circulation with serial evaluation of uterine artery Doppler.
2. To identify fetal compromise and reduction of fetal growth with serial evaluation of fetal biometry and umbilical artery and ductus venosus Doppler flow waveforms.
3. To identify brain-sparing in adverse pregnancy outcomes as evidenced by middle cerebral artery Doppler and Doppler cerebroplacental ratio and compare foramen

ovale and ductus arteriosus Doppler flows in these fetuses with Doppler flow patterns obtained in fetuses with normal pregnancy outcomes.

4. To identify sequence of redistribution of fetal intracardiac and cerebral flows in compromised fetuses.

Methods and Study design

To address the aims and hypotheses mentioned above, a prospective longitudinal observational study was undertaken in a cohort of high-risk and low risk pregnancies.

Sample size

To demonstrate a statistical difference between the two groups (adverse outcomes and normal outcomes) of 15 %, with a power of 90 % and a smaller probability of 0.05, it was estimated that a sample size of 88 women would be required.

Assuming an 80% rate of acceptance, with an estimate of 25-30% adverse perinatal outcome, 3 years recruitment would provide around 100 cases with fetal/neonatal and/or maternal morbidity and/or mortality.

Study population

240 women, including controls and high-risk pregnancies fulfilled the eligibility criteria and were invited to participate in the study.

The eligibility criteria have been described in Chapter 3. The distribution of high-risk pregnancies has been described in Table 37.

233 women with singleton pregnancies, comprising of 63 controls and 170 high-risk, consented and were recruited between March 2004 to March 2007 by obstetricians in the High-risk Pregnancy clinic at Women's and Children's Hospital, Adelaide, which is an academic tertiary care teaching medical centre. Informed consent was obtained from all the participants in the study.

Reasons for non-participation were lack of time commitments due to full-time work or being primary carer for little children or having to relocate interstate or overseas.

Data collection

At the first visit, demographic data, medical and obstetric histories were recorded. Figure 66 and Table 37 describe the clinical history of the high-risk patients.

Table 37 Classification of clinical history of high-risk patients at first visit

History	n
P/H/O Preeclampsia	25
P/H/O IUGR	14
P/H/O Preterm birth	24
P/H/O recurrent first trimester loss	53
P/H/O fetal loss >12 weeks	22
Previous poor pregnancy outcome	5
Prior placental abruption	7
Thrombophilia	11
Insulin Resistance	11
Current H/O oocyte donation	32

Key: P/H/O= past history of, n= total number of cases out of total 170 high risk cases.

All participants were scanned serially at 10 time points (n = 1954 scans) from 16 weeks onwards at regular intervals, at 16, 20, 24, 26, 28, 30, 32, 34, 36 and 38 weeks till delivery and clinical and placental outcomes compared. Figure 6a describes the study design of the participants.

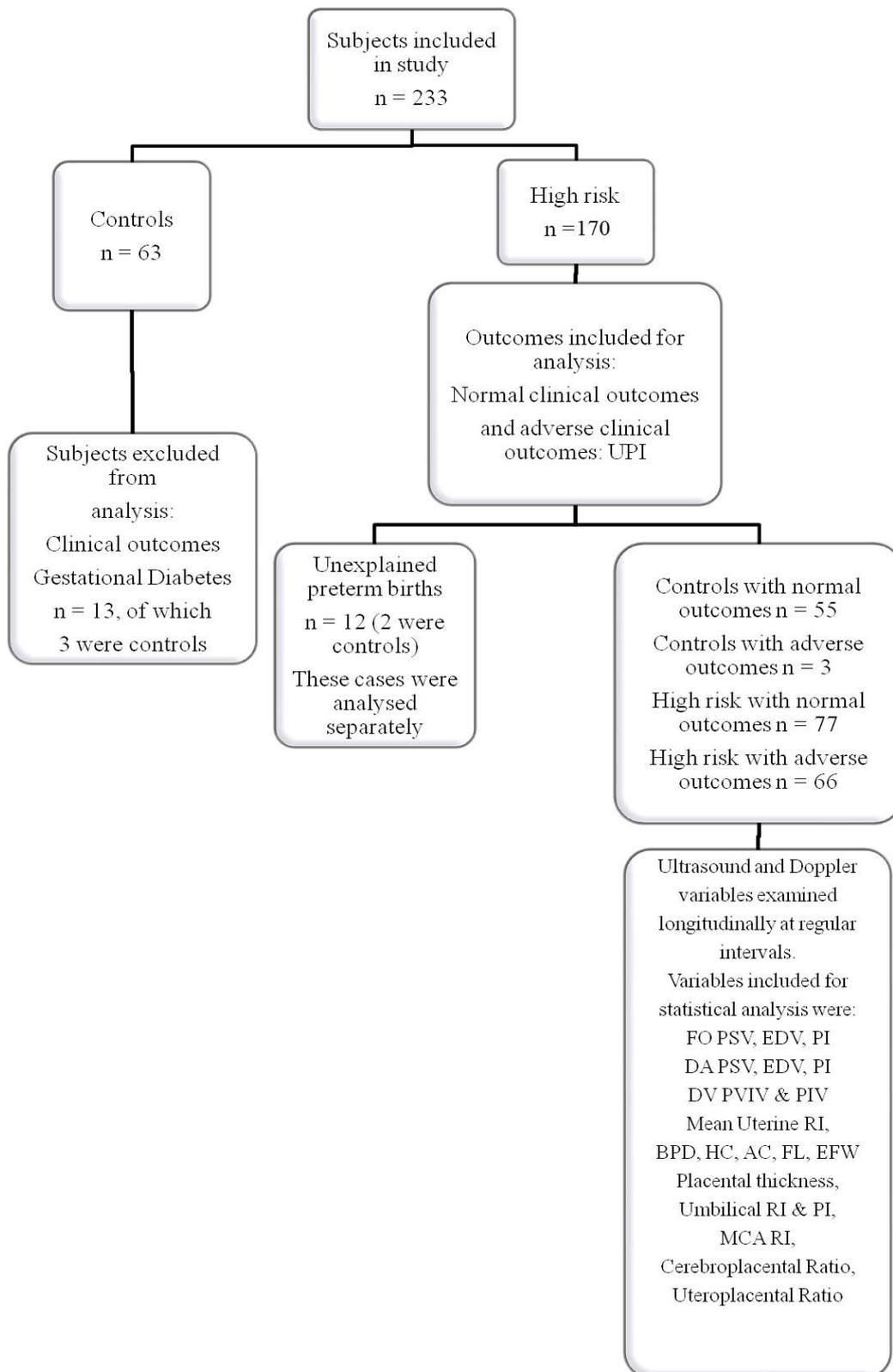


Figure 66 Flow diagram describing the study design for Study 3

The Doppler Indices Pulsatility Index (PI) of fetal foramen ovale (FO), ductus arteriosus (DA), ductus venosus (DV) and Resistance Index (RI) of Middle cerebral artery (MCA), Umbilical artery (UA) and mean RI of maternal uterine arteries(Ut A) were measured. Table 38 and Table 39 describe the number of observations included for statistical analysis, with specific number of observations at each time point respectively. Ultrasound scanning protocols have been described in Chapter 3.

Table 38 Number of Ultrasound and Doppler observations in study 3

Variable	n = total number of observations
Umbilical artery RI	1954
Umbilical artery PI	1919
MCA RI	1930
Cerebroplacental ratio	1905
Uteroplacental ratio	1844
DV Preload index (s-a)/s	1916
DV Peak Velocity index(s-a)/d	1545
DV - PIV (s-a)/tamx	1390
DV s/a ratio	1916
DV systolic / diastolic ratio	1551
DA PI	1839
FO PI	1816
uterine artery notch	1879
Mean Ut RI	1866
placental thickness	1737
Bi Parietal Diameter	1941
Head Circumference	1942
Abdominal Circumference	1933
Femur Length	1937
Amniotic Fluid index	1201
HC/AC ratio	1933
Estimated fetal weight	1928

Table 39 Number of observations at every time point for study 3

		Weeks of gestation									
		16	20	24	26	28	30	32	34	36	38
DA PI	n	132	161	200	204	198	202	194	198	168	77
	%	7.2	8.8	10.9	11.1	10.8	11.0	10.6	10.8	9.1	4.2
FO PI	n	136	155	195	203	192	198	195	196	161	82
	%	7.5	8.5	10.7	11.2	10.6	10.9	10.7	10.8	8.9	4.5
DV PIV	n	89	107	146	157	150	151	157	151	139	65
	%	6.4	7.7	10.5	11.3	10.8	10.9	11.3	10.9	10.0	4.7
Umb RI	n	161	174	203	206	207	208	202	202	179	94
	%	8.2	8.9	10.4	10.5	10.6	10.7	10.3	10.3	9.2	4.8
Umb PI	n	151	168	201	209	204	206	200	199	178	93
	%	7.9	8.8	10.5	10.9	10.6	10.7	10.4	10.4	9.3	4.8
Mean RI	n	162	175	206	204	208	205	201	200	165	34
	%	8.6	9.3	11.0	10.9	11.1	10.9	10.7	10.6	8.8	1.8
Pla thi	n	151	163	188	182	180	183	175	182	151	78
	%	8.7	9.4	10.8	10.5	10.4	10.5	10.1	10.5	8.7	4.5
MCA RI	n	159	173	205	214	204	207	200	202	172	77
	%	8.2	9.0	10.6	11.1	10.6	10.7	10.4	10.5	8.9	4.0
CPR	n	159	173	203	206	204	207	200	202	172	77
	%	8.2	9.0	10.4	10.5	10.6	10.7	10.4	10.5	8.9	4.0
BPD	n	162	175	206	203	204	208	200	204	173	88
	%	8.4	9.0	10.6	10.5	10.5	10.7	10.3	10.5	8.9	4.5
HC	n	162	175	206	203	204	208	200	204	174	88
	%	8.4	9.0	10.6	10.5	10.5	10.7	10.3	10.5	9.0	4.5
AC	n	160	174	206	202	204	207	199	204	171	88
	%	8.3	9.0	10.7	10.5	10.6	10.7	10.3	10.6	8.9	4.6
FL	n	160	175	206	203	204	208	200	203	172	88
	%	8.3	9.0	10.6	10.5	10.5	10.7	10.3	10.5	8.9	4.5
EFW	n	160	174	206	202	204	207	199	204	171	88
	%	8.3	9.0	10.7	10.5	10.6	10.7	10.3	10.6	8.9	4.6

* missing data has been addressed in detail in chapter 3.

Endpoint

The primary outcome was UPI.

Outcome measures for statistical analysis

Clinical outcomes: UPI and unexplained preterm birth analysed separately.

To evaluate growth in the fetuses, three different classification methods were used to classify fetal outcomes, these being ‘small for gestational age’ (SGA), based on Australian national birthweight centile charts by Roberts and Lancaster charts [283], IUGR based on customized centile charts [284] as well as IUGR based on

ultrasound growth trajectory. All definitions for classification of clinical history as well as outcomes have been described in chapter 3.

Statistical analysis

For statistical analysis, patients with unexplained preterm births and gestational diabetes were excluded from the UPI analysis. Unexplained preterm births were analysed separately. Individual analyses were also performed for preeclampsia and IUGR.

For the final statistical analysis for study 3, a total of 201 patients were included, of which 132 patients had normal outcomes and were compared to 69 patients with adverse outcomes as defined above.

Statistical analysis methods for longitudinal data

Mixed linear models were used for longitudinal analysis, details of which are described in Chapter 3. Longitudinal analysis of all variables was performed with F test-UNIANOVA, to compare the clinical and Doppler data among the groups of subjects, p values <0.05 were considered significant. The F test was based on the linearly independent pair wise comparisons of EMMEANS for adverse outcomes as well as normal outcomes. The main effects were compared with least significant difference (LSD) confidence level adjustment; mean difference was significant at the 0.05 level.

Statistical methods for analysis of categorical data

Pearson bivariate correlation analysis was used to evaluate relationships between the Doppler Indices and clinical outcomes. All tests were 2-tailed, and probability values were considered significant at the .05 level.

A possible confounding factor was treatment with thromboprophylaxis in high-risk patients, where some patients were treated with aspirin alone and some were treated with enoxaparin in addition to aspirin. To address this potential confounder, separate longitudinal analyses were performed variables, for ultrasound and Doppler using enoxaparin and aspirin as covariates.

RESULTS

(A) CLINICAL OUTCOMES

Pregnancy outcomes in high risk and control pregnancies

Of 63 controls, 9.5% developed UPI, 4.8% IUGR, 3.2% gestational hypertension, unexplained preterm birth in 3.2%, and 1.6% developed gestational diabetes. Not one of the controls developed preeclampsia.

Of 170 high-risk pregnancies, 40% developed UPI, 12.4% developed preeclampsia, 7.1% gestational hypertension, 21.8% IUGR, 7% developed gestational diabetes.

Severity of adverse clinical outcomes

All clinical outcomes were further classified according to severity of outcomes as per definitions described earlier in the methods section. Table 40, Table 41 and Table 42 describe the clinical outcomes in relation to the severity of adverse outcomes.

Table 40 Severity of outcomes in UPI and preeclampsia

Total number of patients = 233
UPI
UPI all (n = 72) 32.6%
UPI delivered full term (n = 38) 16.3%
Preeclampsia
Preeclampsia -all (n = 23) 9.9%
Preeclampsia late onset (n =10) 4.3%
Preeclampsia - severe-delivered \leq 37 weeks (n =13) 5.6%
Preeclampsia - severe-delivered \leq 30 weeks (n=8) 3.4%
Gestational hypertension (n =14) 6%
Placental abruption (n = 2) 0.9%
Liver infarct (n =1) 0.4%

Fetal outcomes in IUGR are described in Table 41.

Table 41 Severity of outcomes in IUGR

IUGR Severity by Ultrasound classification	SGA by Australian centiles	IUGR by Customised centiles classification
CSA n = 7 (3%)		
Mild IUGR n = 26 (11.2 %)	3rd to 10 th centile n= 13 (5.6%)	3rd to 10th centile n= 15 (6.4%)
Moderate IUGR n = 7 (3%)		
Severe IUGR (n= 8) 3.4%	< 3rd centile n = 10 (4.3%)	< 3rd centile n= 18 (7.7%)

Key-CSA –constitutionally small fetuses, SGA-Small for gestational age, <10th centile using population centile charts based on birth weight, IUGR- intrauterine growth restriction

After excluding iatrogenic causes of preterm birth such as IUGR, preeclampsia, diabetes or other known causes leading to intervention of labour, it was found that there were 19 unexplained preterm births in the current study (8.2 %).

Comparison of outcomes according to the clinical history

The incidence of adverse outcomes was higher in high-risk pregnancies. Women with high-risk pregnancies, especially with a past history of preeclampsia had a higher incidence of UPI, IUGR, preeclampsia and gestational hypertension.

Preeclampsia also occurred in a high proportion of women with oocyte donation or women with a history of recurrent spontaneous miscarriages (RMC) as shown in Table 42. Women with RMC, once beyond the first trimester, were also prone to developing IUGR, and/or preterm labour. Table 42 describes the outcomes in relation to the clinical history.

Table 42 Clinical Histories and outcomes

History	UPI (n=74)		Preeclampsia (n=21)		IUGR (n=40)		gestational hypertension (n=14)		true preterm (n=19)	
		%		%		%		%		%
control	6	8.1%	0	0%	3	7.5%	2	14.3%	2	10.5%
High risk P/H/O	68	91.9%	21	100%	37	92.5%	12	85.7%	17	89.5%
Preeclampsia P/H/O	15	20.3%	6	28.6%	10	25%	5	35.7%	1	5.3%
IUGR P/H/O	8	10.8%	2	9.5%	6	15%	0	0%	2	10.5%
Preterm birth P/H/O	13	17.6%	1	0.5%	5	12.5%	2	14.3%	3	15.8%
Recurrent miscarriage P/H/O	13	17.6%	3	14.3%	7	17.5%	2	14.3%	4	21%
fetal loss >12 weeks Current H/O	8	10.8%	1	0.5%	4	10%	2	14.3%	6	31.5%
oocyte donation other causes for high risk	7	9.5%	4	19%	3	7.5%	0	0%	4	21%
	4	5.4%	4	19%	13	32.5%	3	21.4%	3	15.8%

Key- P/H/O- past history of.

Comparison of outcomes with South Australian state data

The clinical outcomes were compared with the South Australian pregnancy outcome (Table 43), [318], showing that the incidence of adverse outcomes was higher in the present study.

The incidence of preterm in our study was 8.2 % versus 7.3 % in SA data. SA data showed that the incidence of pregnancy hypertension, which was as defined by the SA Pregnancy Outcome Unit a combination of gestational hypertension and preeclampsia, was 7.4 % statewide, whereas it was 16 % in the present study. The presence of SGA, using Robertson Lancaster charts identified 9.9 % SGA babies in our study, versus 8.9 % SGA babies state-wide. From our own data, customised centile charts identified a greater proportion of babies with IUGR babies at birth, in comparison to Robertson Lancaster charts, corroborating with previously published observations [284, 319]. Antenatally, ultrasound growth trajectory monitoring proved superior to any other method of identification of IUGR, by identifying the greatest proportion of growth disorders in the fetus.

Table 43 Comparison of IUGR outcomes with South Australian data

study cohort	SGA using Australian centiles	IUGR using customised centiles	IUGR with Ultrasound
SA	8.9 %	--	--
Present study	9.9 %	14.2 %	13 %

Key: SGA-Small for gestational age, <10th centile using population centile charts based on birth weight.

IUGR- intrauterine growth restriction

Birth weight and gestational age in adverse clinical outcomes

UPI, IUGR and preeclampsia were associated with lower birth weights and gestational ages with an increased severity of disease. Table 44 and Table 45 describe the birth weights and gestational ages in adverse outcomes respectively.

Table 44 Birth Weight in adverse clinical outcomes

Birth weight	Mean	5 to 95 %	CI
UPI	2582.7	250	4700
Normal outcome	3534.8	2670	4500
Preeclampsia in present pregnancy	2205	540	3360
Normal	3524.3	2660	4500
Gestational hypertension	3532.9	2580	4700
Placental abruption	1720	1620	1820
Liver infarct n=1	250	--	--
Other	2798.9	730	4720
SGA-small normal	2658.6	2090	3090
Mild IUGR	2651.2	730	3190
Moderate IUGR	2357.1	2090	2730
Severe IUGR	598.8	250	860
IUGR by customised centiles	2003	250	3240
No IUGR	3364	1410	4720
Less than 3rd centile customised centiles	1369.4	250	2670
More than 3rd but less than 10th centile	2672.9	1870	3240
Normal - above 10th centile	3364.5	1410	4720
IUGR by Robertson Lancaster charts	2077.6	250	3090
Normal	3333.4	730	4720
Less than 3rd centile Robertson Lancaster charts	1127.5	250	2650
More than 3rd but less than 10th centile	2065	570	3030
Normal - above 10th centile	3302.1	730	4720

Table 45 Gestational age in adverse clinical outcomes

Gestational age in weeks at birth	Mean	5 to 95 %	CI
UPI	36.1	22	42
Normal outcome	39.2	37	41.6
Diabetes	37.6	35.4	39.5
Pregnancy hypertension			
Preeclampsia in present pregnancy	34.6	26.9	39.3
Normal	39.3	34	41.6
Gestational hypertension	38.8	36	42
Abruption	30.2	29	31.4
Liver infarct	22		
Other	36.3	26	40.8
IUGR by ultrasound			
Normal	38.5	28	42
SGA-small normal	38.4	34.6	40.9
Mild IUGR	37.2	26	40.8
Moderate IUGR	36.7	34.6	39.1
Severe IUGR	26.8	22	30
IUGR by customised centiles			
Less than 3rd centile by customised centiles	31.3	22	40.7
More than 3rd but less than 10th centile	38.2	33	40.9
Normal-above 10th centile	38.4	28	42
SGA by Australian centiles			
Less than 3rd centile Australian classification	30.3	22	40.7
More than 3rd but less than 10th centile	35.2	27	40.8
Normal-above 10th centile	38.2	26	42

(B) LONGITUDINAL ANALYSIS OF ALL VARIABLES IN ADVERSE PREGNANCY OUTCOMES

UPI

A longitudinal evaluation of all ultrasound and Doppler variables in UPI in comparison to normal outcomes showed that there were significant differences in the Doppler flow dynamics of uteroplacental and fetoplacental circulation in addition to changes in biometry.

Uteroplacental haemodynamic evaluation included uterine artery Doppler mean RI and uteroplacental ratio; both showed higher values in comparison to those in normal outcomes; the trends throughout gestation were statistically significant (Figure 67). Umbilical artery Doppler PI was used to evaluate fetoplacental circulation, and it was observed that umbilical PI was significantly higher in UPI (Figure 67).

Placental thickness was higher and biometric measurements BPD, HC, AC and FL were significantly lower in later gestation in UPI (Figure 67).

MCA Doppler RI and cerebroplacental ratios (CPR) were used to evaluate cerebral circulation and “brain sparing” effect and it was seen that MCA RI and CPR was significantly lower in UPI (Figure 68).

Evaluation of PI of central shunts foramen ovale (FO PI) and ductus arteriosus (DA PI) also showed significantly higher values in earlier gestation in UPI (Figure 68).

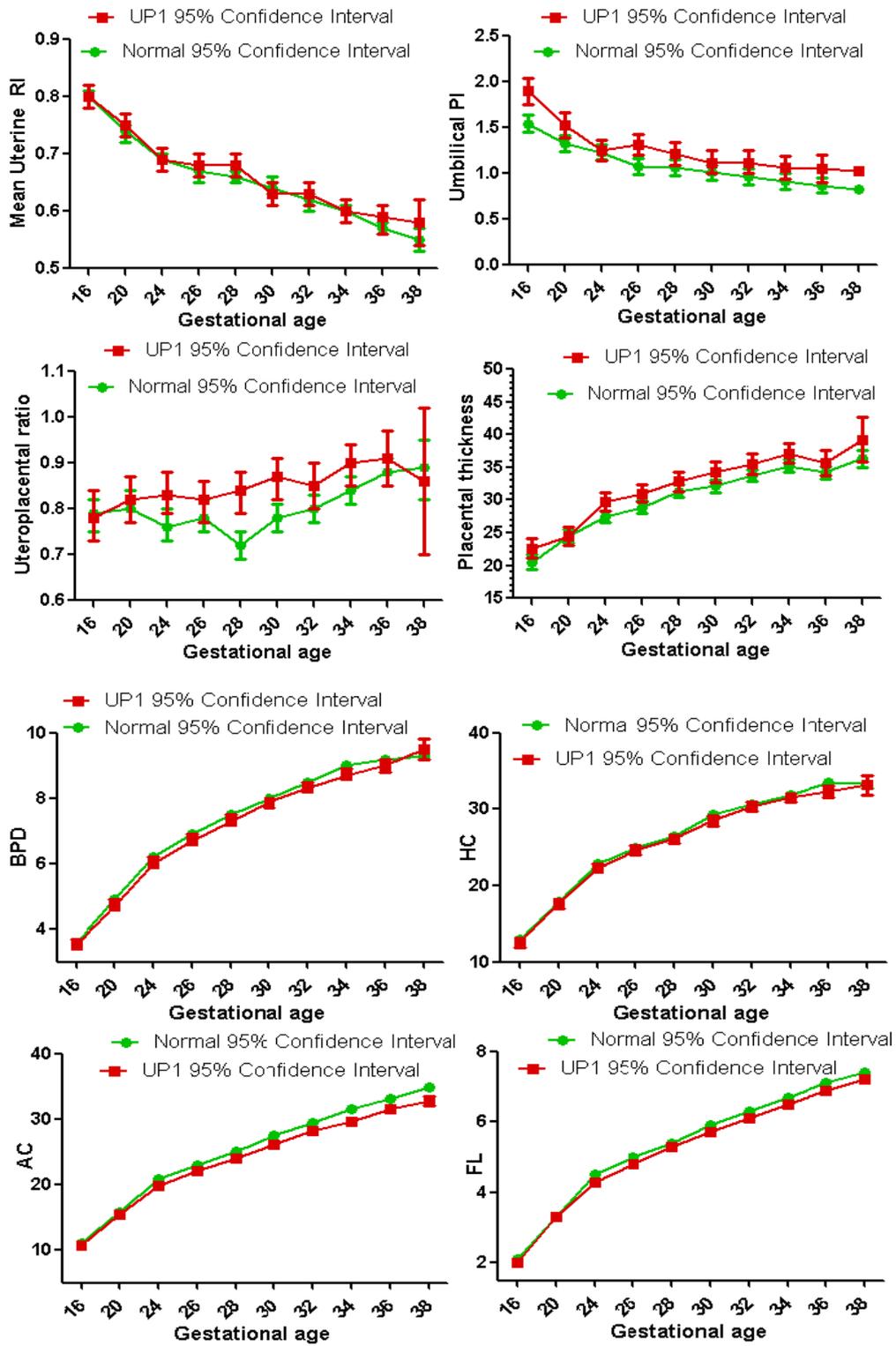


Figure 67 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in uteroplacental insufficiency and normal pregnancy outcome

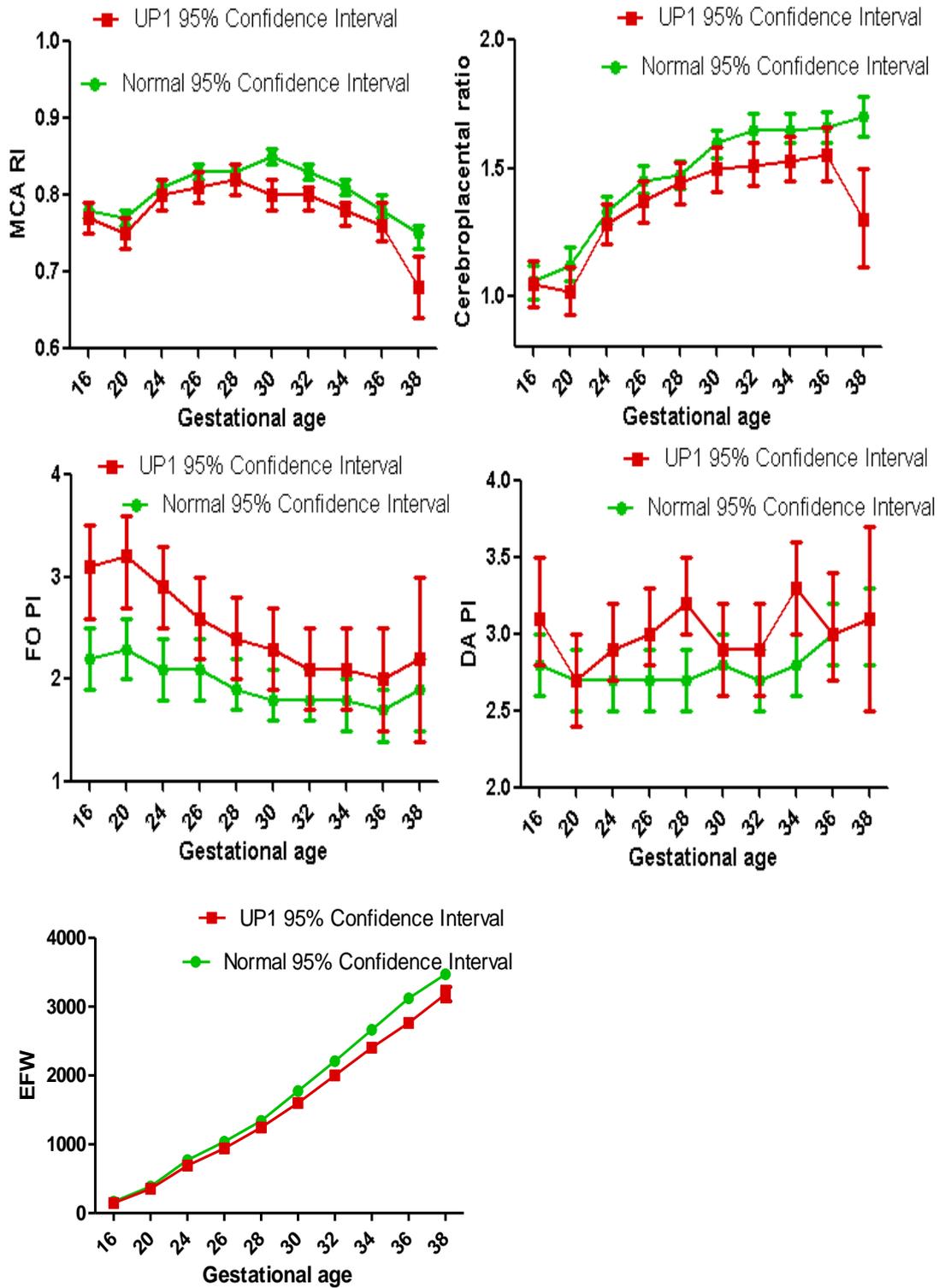


Figure 68 Longitudinal evaluation of fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in uteroplacental insufficiency and normal pregnancy outcome

Sequence of observed significant haemodynamic changes in UPI

Significant differences were present in UPI as compared to normal pregnancies, in the Doppler flow dynamics of uteroplacental and fetoplacental circulation in addition to changes in biometry.

FO PI demonstrated significant changes in those fetuses with adverse outcome, as early as 16 weeks. This was associated with an increase in placental thickness as well as a high umbilical artery PI. At 20 weeks, uterine artery Doppler flow waveforms also showed significant changes, followed by a reduction in fetal biometry at 24 weeks. MCA began to demonstrate a reduction in RI along with an increase in DA PI.

Table 46 describes the p values obtained after longitudinal pairwise comparison of estimated marginal means at the different ages of gestation. Figure 69 describes the schematic sequence of changes in different ultrasound and Doppler variables in pregnancies complicated by UPI.

The actual values for estimated marginal means for all the variables in UPI and normal outcomes are described in the appendix. (S File 1).

Table 46 Comparison of all Doppler and ultrasound variables in UPI versus normal outcomes

p values based on F Test UNIANOVA for different gestational ages in weeks										
Dependant variable	16	20	24	26	28	30	32	34	36	38
FO PI	0.003	0.001	0.001	0.026	0.035	0.053	0.214	0.147	0.287	0.453
Umbilical PI	0.000	0.018	0.779	0.001	0.044	0.129	0.039	0.054	0.039	0.151
Placental thickness	0.026	0.980	0.007	0.016	0.114	0.022	0.059	0.044	0.189	0.114
Mean Uterine RI	0.599	0.033	0.003	0.018	0.000	0.001	0.016	0.016	0.019	0.048
EFW	0.579	0.743	0.008	0.002	0.002	0.000	0.000	0.000	0.000	0.000
BPD	0.521	0.158	0.029	0.086	0.119	0.150	0.078	0.014	0.046	0.326
AC	0.245	0.124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FL	0.066	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
uteroplacental ratio	0.927	0.524	0.012	0.236	0.000	0.002	0.063	0.061	0.307	0.776
MCA RI	0.604	0.463	0.354	0.047	0.469	0.000	0.001	0.002	0.095	0.002
DA PI	0.126	0.960	0.181	0.039	0.003	0.681	0.372	0.001	0.954	0.918
cerebroplacental ratio	0.952	0.062	0.275	0.086	0.563	0.057	0.009	0.022	0.077	0.000

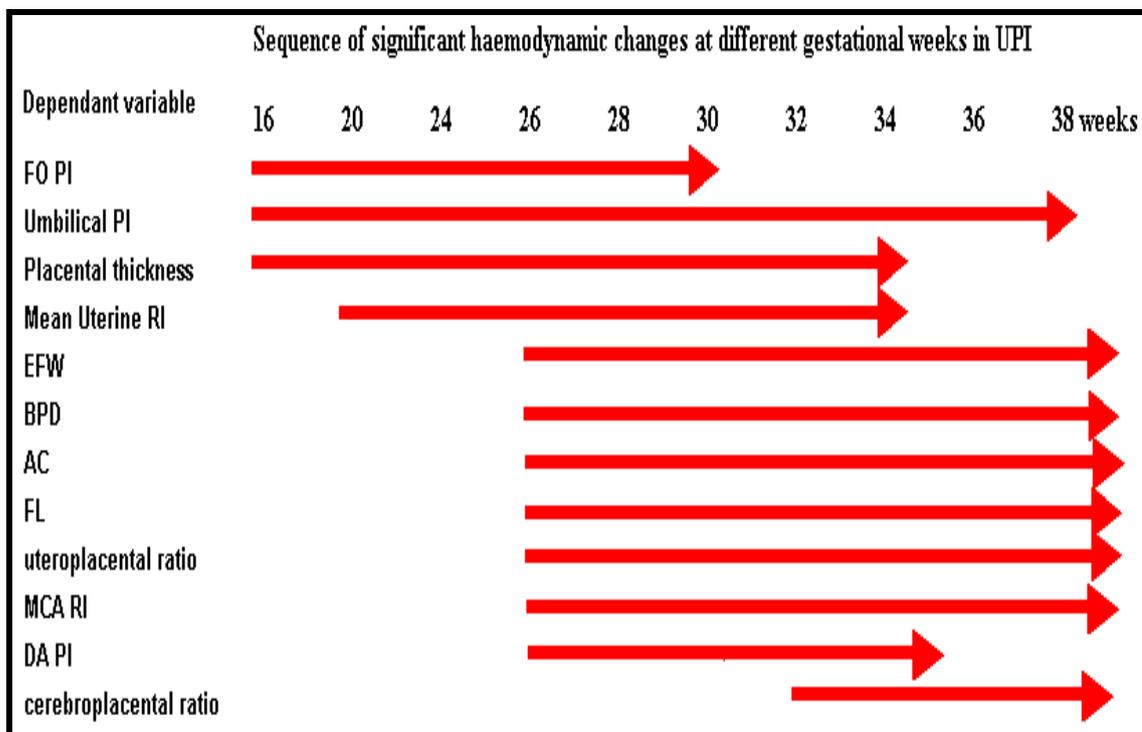


Figure 69 Schematic representation of the sequence of changes in UPI

The results of longitudinal analysis of ultrasound and Doppler variables in UPI are summarized in the Table 47. As seen from Table 47, the pair-wise comparisons using the F test showed significant differences although the degree of correlation was weak overall.

Table 47 Ultrasound and Doppler parameters in Uteroplacental insufficiency (UPI)

Direction of change in the variable and p values in UPI based on longitudinal evaluation by F test							
Dependant variable	Direction of change in the variable in UPI in comparison to normal outcomes	df , F for UPI	pair wise comparison mean difference *(95 % CI for difference) between UPI to normal mean lower upper bound bound			significance by F test	Pearson's correlation coefficient for UPI
Uteroplacental and fetoplacental haemodynamics							
Mean Uterine RI	↑	1, 1457; 33.563	0.06	0.04	0.08	0.000	0.15**
Umbilical RI	↑	1, 1537; 6.009	0.02	0	0.03	0.014	0.07**
Umbilical PI	↑	1, 1505; 14.317	0.11	0.05	0.17	0.000	0.10**
Uteroplacental ratio	↑	1, 1436; 10.324	0.06	0.02	0.09	0.001	0.09**
Cerebral flows and 'brain sparing'							
MCA RI	↓	1, 1516; 11.977	0.02	0.04	0.01	0.001	-0.18**
Cerebroplacental ratio	↓	1, 1494; 13.693	0.12	0.18	0.06	0.000	-0.12**
Haemodynamics of the central shunts							
DA PI	↑	1, 1434; 15.361	0.26	0.13	0.4	0.000	0.08**
FO PI	↑	1, 1416; 30.459	0.53	0.34	0.72	0.000	0.09**
Biometry and placental thickness							
Placental thickness	↑	1, 1348; 33.361	2.06	1.36	2.76	0.000	0.06*
EFW	↓	1, 1516; 146.175	235.62	73.8	197.4	0.000	-0.13**
BPD	↓	1, 1529; 66.910	0.46	0.57	0.35	0.000	-0.09**
HC	↓	1, 1529; 48.735	1.64	2.1	1.18	0.000	-0.08**
AC	↓	1, 1520; 315.459	2.3	2.55	2.04	0.000	-0.13**
FL	↓	1, 1525; 347.213	0.46	0.51	0.41	0.000	-0.10**
HC/AC	↑	1, 1520; 13.897	0.05	0.02	0.07	0.000	0.14**

- The F test in mixed linear models tests the effect of uteroplacental insufficiency longitudinally. ** denotes highly significant degree of correlation.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.
- * 95 % confidence interval adjustment for difference in the variable between UPI and normal after multiple pair wise comparisons of estimated marginal means was performed with Least Significant Difference test (equivalent to no adjustments).
- Pearson's correlation coefficient evaluates the degree of correlation of the ultrasound variable for UPI.

IUGR

A longitudinal evaluation of all ultrasound and Doppler variables in IUGR in comparison to normal outcomes showed that there were significant differences in the Doppler flow dynamics of uteroplacental and fetoplacental circulation in addition to changes in biometry.

Longitudinal analysis was also performed for the ultrasound and Doppler variables after classifying IUGR into grades based on severity of IUGR

Uterine artery Doppler mean RI and uteroplacental ratio were significantly higher values in IUGR in comparison to those in normal outcomes (Figure 70). Umbilical artery Doppler PI and RI was used to evaluate fetoplacental circulation, and it was observed that Umbilical PI was significantly higher in IUGR (Figure 70). MCA Doppler RI and Cerebroplacental ratios (CPR) were used to evaluate cerebral circulation and “brain sparing” effect; MCA RI and CPR were significantly lower in IUGR (Figure 70).

Evaluation of central shunts foramen ovale (FO) and Ductus Arteriosus (DA) also showed significantly higher PI values in IUGR (Figure 71).

DV indices, BPD, HC, FL/AC and placental thickness did not show significant differences in IUGR in comparison to normal pregnancies.

EFW and all biometric data i.e. BPD, HC, AC and FL, were significantly lower in Mild, Moderate and Severe IUGR when compared to normal outcomes. FL measurements showed a pronounced effect of severe IUGR.

DA PI was significantly higher in mild and moderate IUGR but showed no difference in severe IUGR when compared to normal outcomes (Table 51).

FO PI was significantly higher in mild IUGR but was not statistically significant on comparison with moderate and severe IUGR (Table 51).

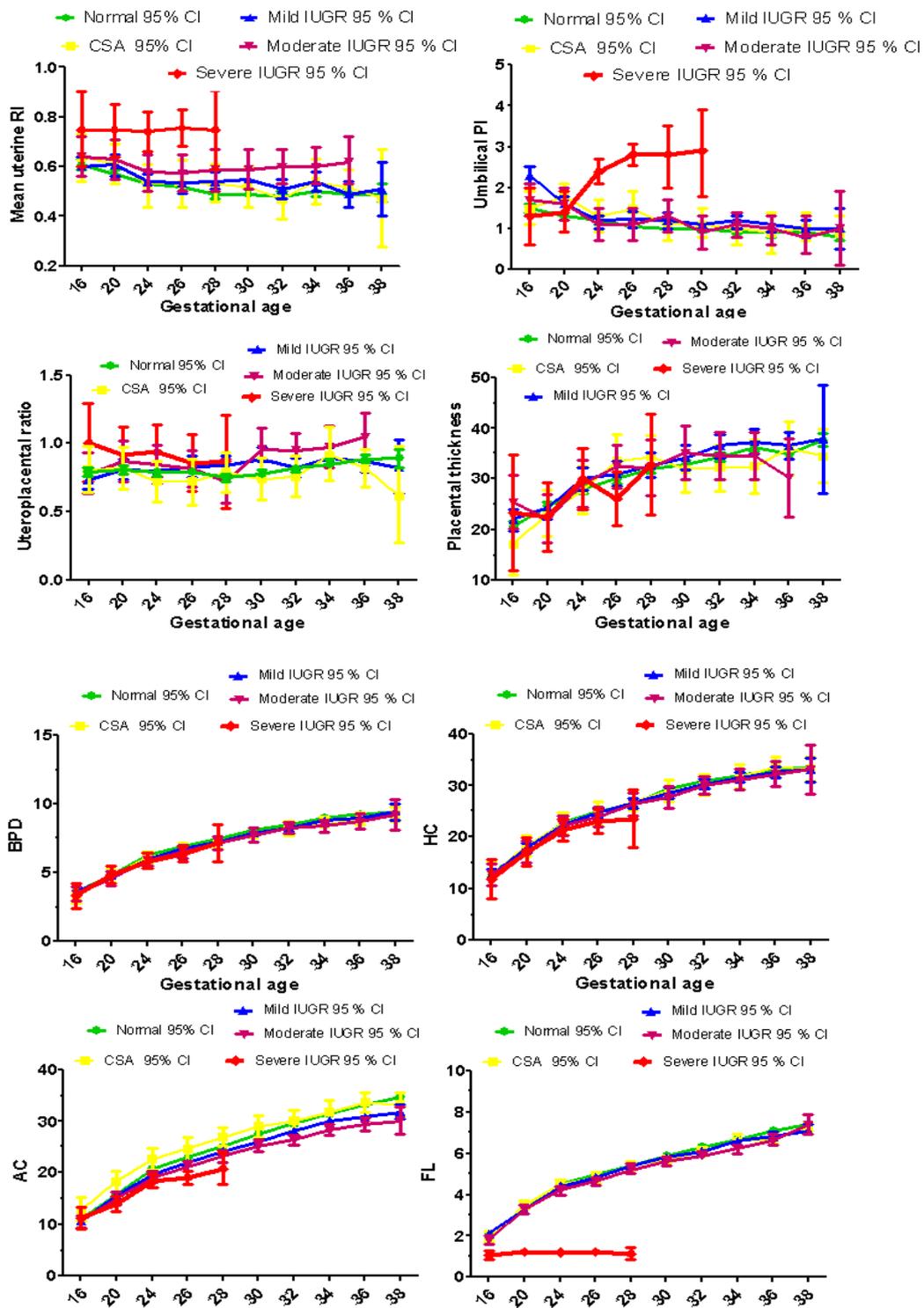


Figure 70 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in IUGR and normal pregnancy outcome

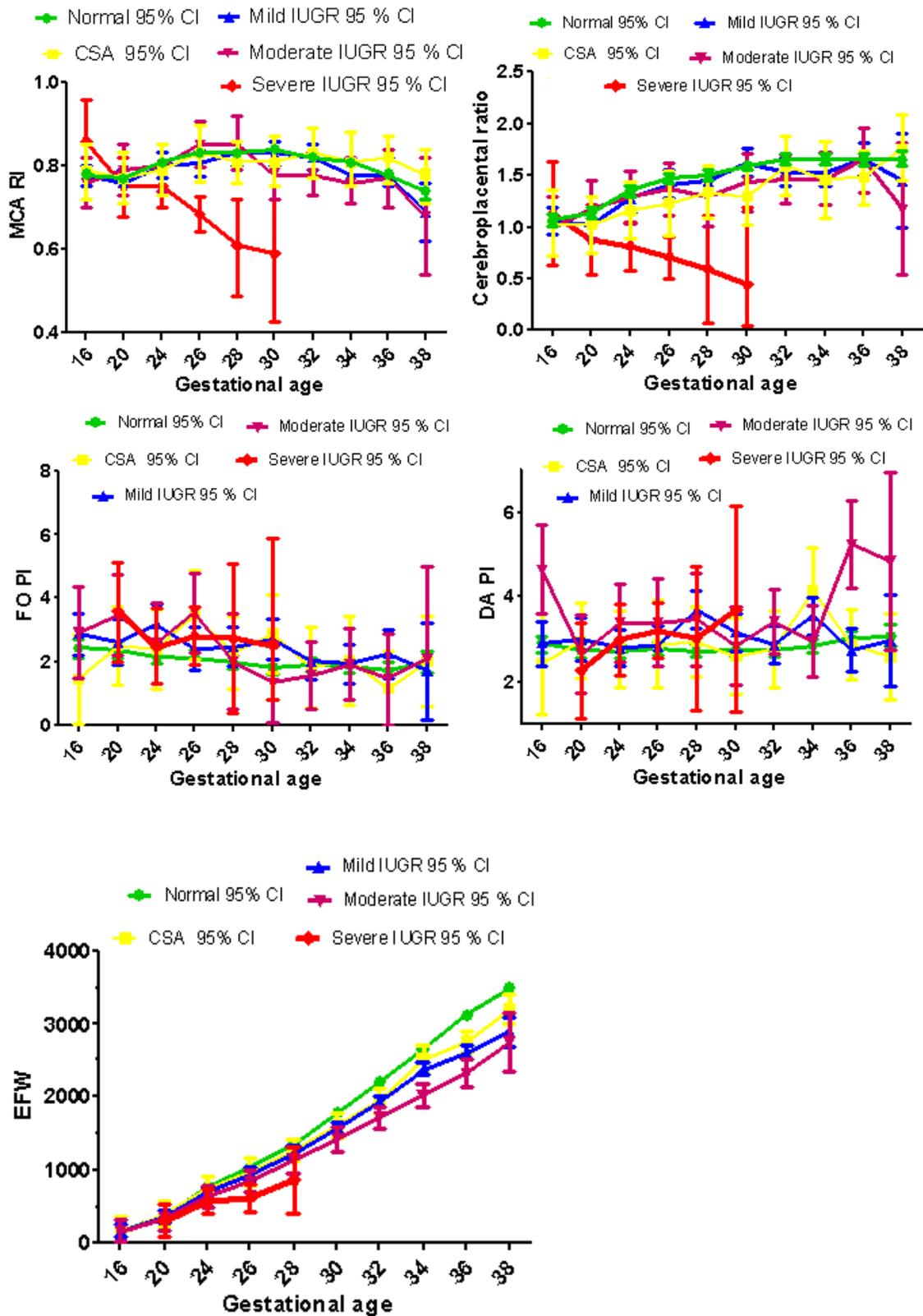


Figure 71 Longitudinal evaluation of estimated fetal weight, fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in IUGR and normal pregnancy outcome

Sequence of observed significant haemodynamic changes in IUGR

Significant differences were present in the Doppler flow dynamics of uteroplacental and fetoplacental circulation in addition to changes in biometry in IUGR, as early as 16 weeks, in comparison to normal pregnancies. In general, the Doppler flow waveforms changes followed the same sequence as described in UPI. FO PI and DA PI demonstrated dynamic changes throughout pregnancy. Sequence of changes in the central shunts DA PI and FO PI was different, depending on the severity of IUGR.

These changes were evident as early as 16 weeks of gestation FO PI demonstrated significant changes in those fetuses with adverse outcome, as early as 16 weeks. This was associated with an increase in placental thickness as well as a high umbilical artery PI. At 20 weeks, uterine artery Doppler flow waveforms also showed significant changes, followed by a reduction in fetal biometry at 24 weeks. MCA began to demonstrate a reduction in RI along with an increase in DA PI. Figure 72 describes the sequence of changes in different ultrasound and Doppler variables in pregnancies complicated by IUGR.

Table 48 describes the p values obtained after longitudinal pair-wise comparison of estimated marginal means at the different ages of gestation for IUGR. Figure 72 describes the schematic sequence of changes in different ultrasound and Doppler variables in pregnancies complicated by IUGR.

The actual values for estimated marginal means for all the variables in IUGR and normal outcomes are described in the appendix (S File 1).

Table 48 Comparison of all Doppler and ultrasound variables in IUGR versus normal outcomes

p values based on F Test UNIANOVA for different gestational ages in weeks										
Dependant variable	16	20	24	26	28	30	32	34	36	38
FO PI	0.003	0.001	0.001	0.026	0.035	0.053	0.214	0.147	0.287	0.453
Umbilical PI	0	0.018	0.779	0.001	0.044	0.129	0.039	0.054	0.039	0.151
Placental thickness	0.026	0.98	0.007	0.016	0.114	0.022	0.059	0.044	0.189	0.114
Mean Uterine RI	0.599	0.033	0.003	0.018	0	0.001	0.016	0.016	0.019	0.048
Uteroplacental ratio	0.927	0.524	0.012	0.236	0	0.002	0.063	0.061	0.307	0.776
EFW	0.579	0.743	0.008	0.002	0.002	0	0	0	0	0
BPD	0.521	0.158	0.029	0.086	0.119	0.15	0.078	0.014	0.046	0.326
AC	0.245	0.124	0	0	0	0	0	0	0	0
FL	0.066	0.2	0	0	0	0	0	0	0	0.011
FL/AC	0.031	0.672	0.845	0.775	0.555	0.752	0.917	0.668	0.831	0.513
MCA RI	0.604	0.463	0.354	0.047	0.469	0	0.001	0.002	0.095	0.002
DA PI	0.126	0.96	0.181	0.039	0.003	0.681	0.372	0.001	0.954	0.918
HC	0.409	0.638	0.183	0.277	0.529	0.03	0.391	0.371	0.006	0.793
Cerebroplacental ratio	0.952	0.062	0.275	0.086	0.563	0.057	0.009	0.022	0.077	0

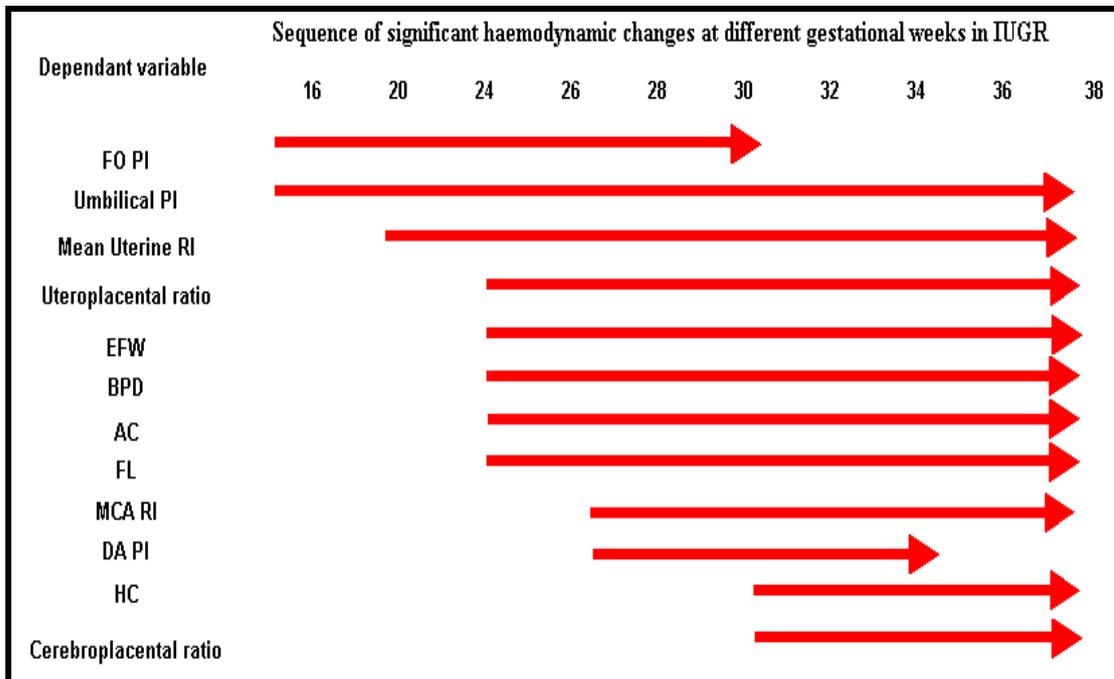


Figure 72 Schematic representation of the sequence of changes in IUGR

The results of longitudinal analysis of ultrasound and Doppler variables in IUGR are summarized in the Table 49. As seen from Table 49, the pair-wise comparisons using the F test showed significant differences although the degree of correlation was weak overall.

Table 49 Ultrasound and Doppler variables in all IUGR versus normal outcomes

Dependant variable	Direction of change in the variable in IUGR in comparison to normal outcomes	df , F for IUGR	pairwise comparison mean difference *(95 % CI for difference) between IUGR to normal			p value by F test	Pearson's correlation coefficient for IUGR
			mean	lower bound	upper bound		
Uteroplacental and fetoplacental haemodynamics							
Mean Uterine RI	↑	1, 1610; 79.150	0.14	0.11	0.17	0.000	0.25**
Umbilical RI	↑	1, 1696; 29.555	0.07	0.05	0.1	0.000	0.19**
Umbilical PI	↑	1, 1664; 28.318	0.37	0.23	0.5	0.000	0.25**
Uteroplacental ratio	↑	1, 1588; 11.543	0.1	0.04	0.16	0.001	0.09**
Cerebral flows and 'brain sparing'							
MCA RI	↓	1, 1673; 23.071	0.06	0.08	0.03	0.000	-0.11**
Cerebroplacental ratio	↓	1, 1648; 33.990	0.32	0.43	0.21	0.000	-0.21**
Haemodynamics of the central shunts							
DA PI	↑	1, 1583; 8.057	0.48	0.15	0.81	0.005	0.07**
FO PI	↑	1, 1561; 0.963	0.23	0.23	0.69	0.326	0.05*
Biometric measurements							
EFW	↓	1, 1669; 38.589	230.8	-303.68	157.93	0.000	-0.16**
AC	↓	1, 1674; 26.645	1.27	1.75	0.79	0.000	-0.17**
FL	↓	1, 1678; 3.274	0.09	0.18	0.01	0.071	-0.14**
HC/AC	↑	1, 1674; 6.619	0.06	0.01	0.11	0.010	0.15**

- The F test in mixed lineal models tests the effect of IUGR longitudinally.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.
- * 95 % confidence interval adjustment for difference in the variable between IUGR and normal after multiple pair wise comparisons of estimated marginal means was performed with Least Significant Difference test (equivalent to no adjustments).
- Pearson's correlation coefficient evaluates the degree of correlation of the ultrasound variable for IUGR.

Longitudinal analysis was also performed for the ultrasound and Doppler variables after classifying IUGR into grades based on severity of IUGR. Mean uterine RI, umbilical RI, umbilical PI, uteroplacental ratio and placental thickness were significantly higher in mild, moderate and severe IUGR when compared to normal outcomes (Table 50).

CPR, MCA RI (Table 51), EFW and all biometric data i.e. BPD, HC, AC and FL, were significantly lower in Mild, Moderate and Severe IUGR when compared to normal outcomes.

DA PI was significantly higher in mild and moderate IUGR when compared to normal outcomes (Table 51). In severe IUGR, there was a slight insignificant increase in DA PI and FO PI up to 24 to 26 weeks, after which the values began decreasing after 26 weeks, reaching 'normal' values just before delivery, around 28 weeks (Figure 71).

FO PI was significantly higher in mild IUGR and remained significantly high throughout pregnancy but was not statistically significant on comparison with moderate and severe IUGR (Table 51). Moderate IUGR was also associated with a significant increase in FO PI initially, followed by a decrease to 'normal values' mid-gestation and decreased further below "normal" levels, around 28 weeks of gestation (Figure 71).

Table 50 Uteroplacental and fetoplacental haemodynamics and placental thickness in IUGR with differing severity

Direction of change in the variable and p values in 'severity of IUGR' categories based on longitudinal evaluation by F test							
Dependant Variable	df , F for IUGR severity	Direction of change in the variable in IUGR categories compared with normal outcomes	IUGR classification based on ultrasound	pair wise comparison mean difference * (95 % CI for difference) between IUGR categories to normal			p value by F test
				mean	lower bound	upper bound	
Mean Uterine RI	4, 1583; 35.482	ns	CSA	0.02	0.06	0.01	0.186
		↑	Mild IUGR	0.04	0.06	0.02	0.000
		↑	Moderate IUGR	0.08	0.11	0.05	0.000
		↑	Severe IUGR	0.22	0.26	0.18	0.000
Umbilical RI	4, 1668; 23.716	↑	CSA	0.03	0.06	0.01	0.01
		↑	Mild IUGR	0.02	0.04	0.01	0.009
		↑	Moderate IUGR	0.04	0.07	0.02	0.001
		↑	Severe IUGR	0.17	0.2	0.13	0.000
Umbilical PI	4, 1635; 18.357	ns	CSA	0.1	0.24	0.04	0.175
		↑	Mild IUGR	0.15	0.23	0.07	0.000
		↑	Moderate IUGR	0.13	0.3	0.04	0.000
		↑	Severe IUGR	0.8	1	0.6	0.000
Uteroplacental Ratio	4, 1560; 3.597	ns	CSA	0.03	0.03	0.09	0.372
		ns	Mild IUGR	0.03	0.07	0.01	0.114
		↑	Moderate IUGR	0.06	0.11	0	0.038
		↑	Severe IUGR	0.11	0.19	0.03	0.007
Placental thickness	4, 1454; 3.609	ns	CSA	0.51	1.23	2.25	0.560
		↑	Mild IUGR	1.33	2.61	0.05	0.040
		ns	Moderate IUGR	0.22	2	1.56	0.810
		↑	Severe IUGR	4.51	1.63	7.39	0.000

- Ns-not significant
- The F test in mixed lineal models tests the effect of IUGR categories longitudinally.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.
- * 95 % confidence interval adjustment for difference in the variable between IUGR categories and normal after multiple pair wise comparisons of estimated marginal means was performed with Least Significant Difference test (equivalent to no adjustments).
- Pearson's correlation coefficient evaluates the degree of correlation of the ultrasound variable for IUGR.

Table 51 Cerebral circulation, 'Brain sparing' and central shunts in IUGR with differing severity

Direction of change in the variable and p values in 'severity of IUGR' categories based on longitudinal evaluation by F test							
Dependant Variable	df , F for IUGR severity	Direction of change in variable in IUGR categories compared with normal outcomes	IUGR classification based on ultrasound	pair wise comparison mean difference * (95 % CI for difference) between IUGR categories to normal			p value by F test
				mean	lower bound	upper bound	
Cerebral flows and 'brain sparing'							
MCA RI	4, 1644; 8.129	ns	CSA	0.01	0.01	0.03	0.341
		↓	Mild IUGR	0.02	0.00	0.03	0.035
		ns	Moderate IUGR	0.02	0.00	0.04	0.086
		↓	Severe IUGR	0.08	0.05	0.11	0.000
Cerebroplacental Ratio	4, 1619; 20.949	↓	CSA	0.13	0.03	0.24	0.012
		↓	Mild IUGR	0.1	0.03	0.18	0.008
		↓	Moderate IUGR	0.15	0.04	0.25	0.007
		↓	Severe IUGR	0.63	0.48	0.78	0.000
Haemodynamics of the central intrauterine shunts							
DA PI	4, 1554; 5.073	ns	CSA	0.02	0.32	0.35	0.93
		↑	Mild IUGR	0.18	0.37	0.000	0.05
		↑	Moderate IUGR	0.87	1.3	0.45	0.000
		ns	Severe IUGR	0.23	0.32	0.77	0.41
FO PI	4, 1532; 2.654	ns	CSA	0.09	0.55	0.37	0.70
		↑	Mild IUGR	0.37	0.63	0.11	0.01
		ns	Moderate IUGR	0.39	0.96	0.19	0.19
		ns	Severe IUGR	0.54	1.3	0.23	0.17

- Ns- not significant
- The F test in mixed lineal models tests the effect of IUGR categories longitudinally.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.
- * 95 % confidence interval adjustment for difference in the variable between IUGR categories and normal after multiple pair wise comparisons of estimated marginal means was performed with Least Significant Difference test (equivalent to no adjustments).
- Pearson's correlation coefficient evaluates the degree of correlation of the ultrasound variable for IUGR categories.

PREECLAMPSIA

Longitudinal analysis in preeclampsia, including severe preeclampsia needing delivery ≤ 37 weeks showed significant increase in mean uterine RI, Umbilical RI, Umbilical PI, DA PI, FO PI and HC/AC ratio (Figure 73 and Figure 74). Significant decrease was also seen in MCA RI, cerebroplacental ratio, EFW, BPD, HC, AC and FL. (Table 55, Figure 74).

All the DV indices, placental thickness and FL/AC were similar in normal outcomes on comparison with severe preeclampsia needing delivery below 37 weeks.

A separate longitudinal analysis in severe preeclampsia needing delivery below 30 weeks showed significant positive correlation with Mean Uterine RI and umbilical PI but significant negative correlation with MCA RI, cerebroplacental ratio, EFW, BPD, HC, AC and FL.

Our study showed that DA PI and FO PI were significantly higher in severe preeclampsia requiring delivery below 37 weeks but above 30 weeks, as well as in late onset preeclampsia, however, DA PI and FO PI were not statistically different in normal outcomes on comparison with severe preeclampsia needing delivery below 30 weeks (Table 56).

However, after exclusion of patients with iatrogenic preterm delivery, when pregnancies with late onset preeclampsia alone was considered for analysis, mean uterine RI, umbilical RI, cerebroplacental ratio, uteroplacental ratio, BPD, HC, AC, FL, HC/AC and FL/AC ratio showed no statistically significant differences. The only significant parameters in late onset preeclampsia delivering full term were DA PI, FO PI, Placental thickness and MCA RI (Table 53).

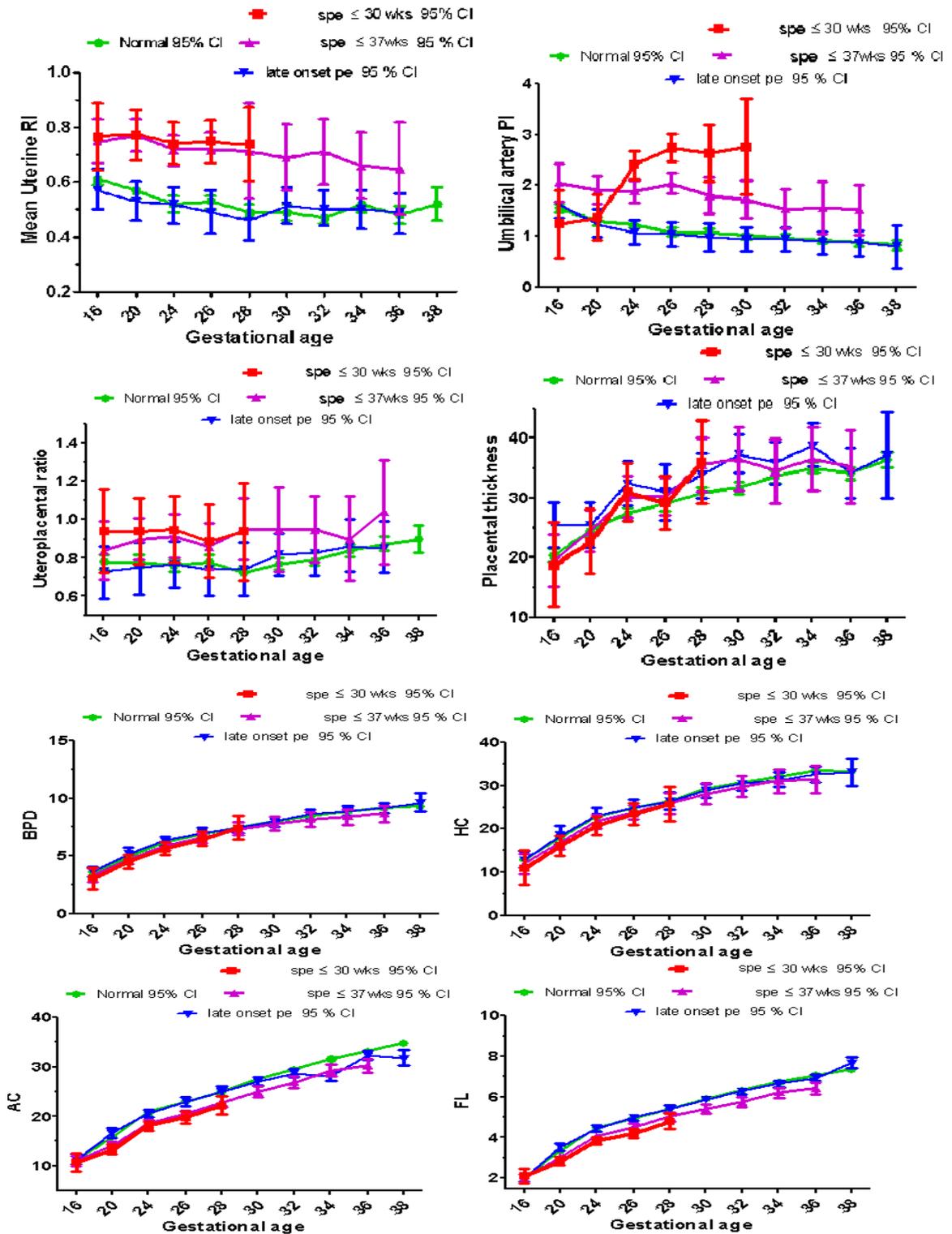


Figure 73 Longitudinal evaluation of uteroplacental, fetoplacental haemodynamics and biometric measurements: profile plots of estimated marginal means in preeclampsia and normal pregnancy outcome

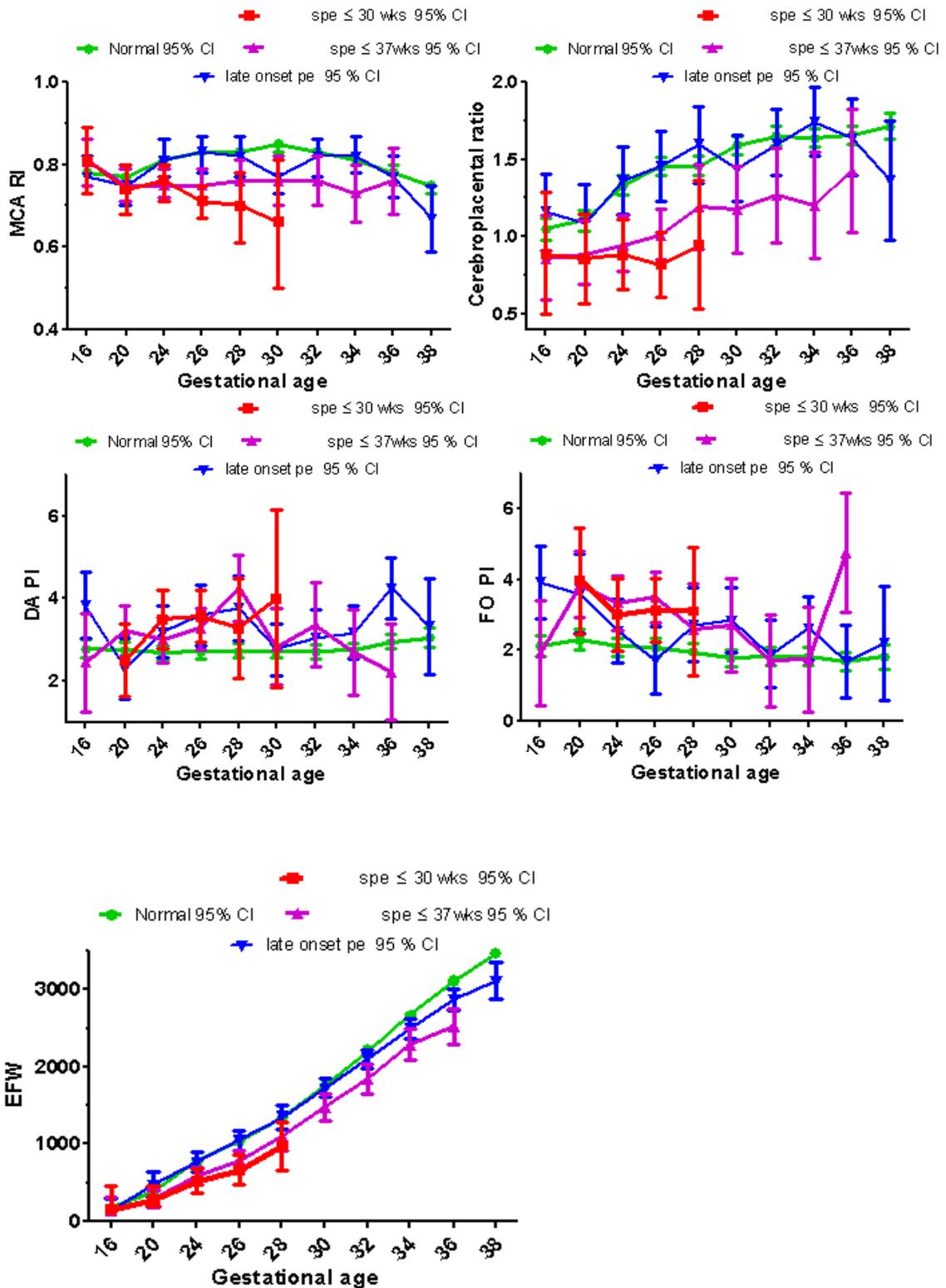


Figure 74 Longitudinal evaluation of estimated fetal weight, fetal cerebral flow and central shunt haemodynamics: profile plots of estimated marginal means in preeclampsia and normal pregnancy outcome

Key-spe-severe preeclampsia

Sequence of observed significant haemodynamic changes in Preeclampsia

Mean Uterine RI was higher at 16 weeks in severe preeclampsia and remained persistently high throughout pregnancy (Figure 73). DA PI and FO PI were significantly higher in preeclampsia, as early as 16 weeks, followed by an increase in Umbilical RI, Umbilical PI, Uteroplacental ratio and cerebroplacental ratio followed by a decrease in fetal biometry and MCA RI (Figure 74)

Table 52 describes the p values obtained after longitudinal pairwise comparison of estimated marginal means at the different ages of gestation for preeclampsia. Figure 75 describes the schematic sequence of changes in different ultrasound and Doppler variables in pregnancies complicated by preeclampsia.

The actual values for estimated marginal means for all the variables in preeclampsia, including all the different categories based on gestational age at onset, and normal outcomes are described in the appendix (S File 1).

Table 52 Comparison of all Doppler and ultrasound variables in Preeclampsia versus normal outcomes

p values based on F Test UNIANOVA for different gestational ages in weeks										
Dependant variable	16	20	24	26	28	30	32	34	36	38
Umbilical PI	0.002	0.01	0.006	0	0.014	0.004	0.008	0.013	0.006	0.142
DA PI	0.059	0.604	0.387	0.003	0.483	0.365	0.743	0.131	0.41	0.394
FO PI	0.061	0.01	0.188	0.244	0.482	0.062	0.53	0.53	0.16	0.787
Mean Uterine RI	0.096	0.023	0	0.005	0.001	0	0	0.003	0.019	
Umbilical RI	0.25	0.34	0.07	0.01	0.99	0.25	0.09	0.2	0.03	0.63
FL	0.03	0.57	0	0	0	0	0	0	0	0.47
MCA RI	0.683	0.21	0.046	0.005	0.023	0	0.03	0.244	0.305	0.015
Uteroplacental ratio	0.843	0.291	0.046	0.671	0.015	0.012	0.025	0.118	0.217	
Cerebroplacental ratio	0.62	0.372	0.087	0	0.317	0.001	0.006	0.11	0.23	0.035
Placental thickness	0.085	0.58	0.004	0.338	0.062	0.003	0.365	0.113	0.991	0.99
EFW	0.362	0.909	0.044	0.015	0.131	0.004	0	0	0	0
AC	0.217	0.429	0	0	0.011	0	0	0	0	0

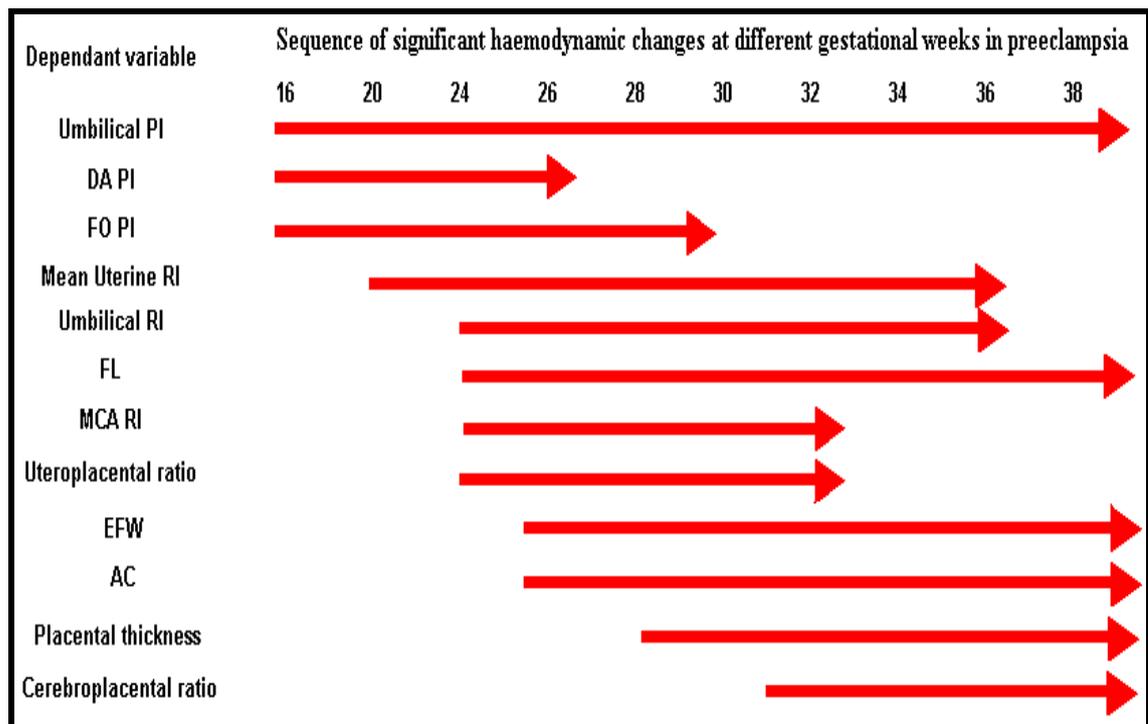


Figure 75 Schematic representation of the sequence of changes in preeclampsia

Of interest, in late-onset preeclampsia, mean uterine artery RI was no different from normal outcomes, however, DA PI and FO PI were significantly higher at 16 weeks, followed by a decrease in MCA RI, much later in pregnancy, around 30 weeks (Table 53). Table 53 describes the p values obtained after longitudinal pair-wise comparison of estimated marginal means at the different ages of gestation for late onset preeclampsia. Figure 76 describes the schematic sequence of changes in different ultrasound and Doppler variables in pregnancies complicated by late onset preeclampsia.

Table 53 Comparison of all Doppler and ultrasound variables in late-onset Preeclampsia versus normal outcomes

p values based on F Test UNIANOVA for different gestational ages in weeks										
Dependant variable	16	20	24	26	28	30	32	34	36	38
DA PI	0.014	0.264	0.132	0.018	0.012	0.882	0.36	0.219	0.001	0.637
FO PI	0.001	0.029	0.316	0.492	0.156	0.022	0.881	0.092	0.969	0.629
Placental thickness	0.013	0.643	0.012	0.381	0.142	0.002	0.245	0.06	0.924	0.838
MCA RI	0.726	0.266	0.921	0.882	0.697	0.001	0.45	0.649	0.669	0.048

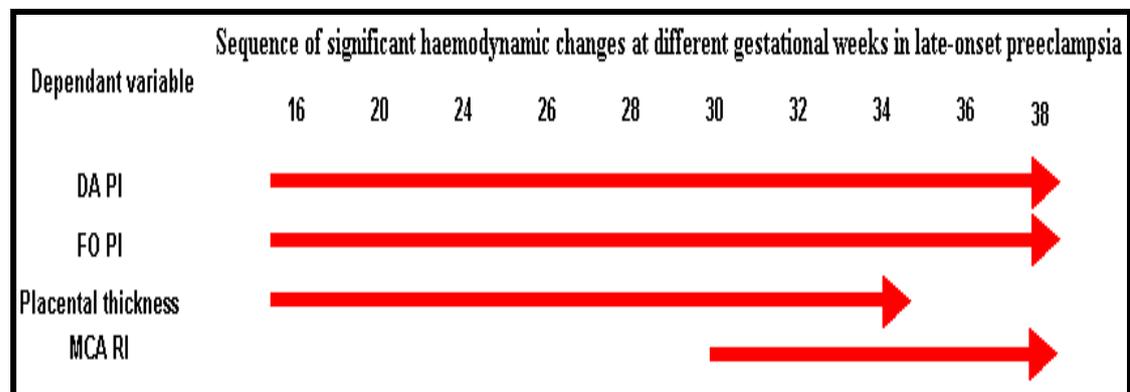


Figure 76 Schematic representation of the sequence of changes in late onset preeclampsia

The results of longitudinal analysis of ultrasound and Doppler variables in preeclampsia are summarized in the Table 54. As seen from Table 54, the pair-wise comparisons using the F test showed significant differences although the degree of correlation was weak overall.

Table 54 Comparison of all Doppler and ultrasound variables in all Preeclampsia versus normal outcomes

Dependant variable	Direction of change in the variable in preeclampsia in comparison to normal outcomes	df , F for Preeclampsia	Pair wise comparison mean difference			p value by F test	Pearson's correlation coefficient for preeclampsia
			mean	lower bound	upper bound		
Uteroplacental and fetoplacental haemodynamics							
Mean Uterine RI	↑	1, 1089; 70.077	0.09	0.07	0.11	0.000	0.21**
Umbilical RI	↑	1, 1145; 26.727	0.04	0.03	0.06	0.000	0.12**
Umbilical PI	↑	1, 1144; 19.307	0.21	0.11	0.3	0.000	0.21**
Uteroplacental ratio	↑	1, 1070; 8.669	0.06	0.02	0.1	0.004	0.09**
Cerebral flows and 'brain sparing'							
MCA RI	↓	1, 1136; 24.194	0.04	0.05	-0.02	0.000	-0.12**
Cerebroplacental ratio	↓	1, 1125; 30.984	0.2	0.28	-0.13	0.000	-0.16**
Haemodynamics of the intrauterine shunts							
DV PIV	↓	1, 836; 3.894	0.12	0.23	0	0.044	0.03
DA PI	↑	1, 1088; 12.738	0.46	0.21	0.71	0.000	0.12**
FO PI	↑	1, 1085; 35.740	1	0.67	1.32	0.000	0.12**
Biometric measurements and placental thickness							
Placental thickness	↑	1, 1016; 8.510	1.96	0.64	3.28	0.001	0.08**
EFW	↓	1, 1129; 52.060	162.4	206.6	118.3	0.000	-0.11**
HC	↓	1, 1137; 2.925	0.52	1.12	0.08	0.005	-0.07**
AC	↓	1, 1132; 105.334	1.57	1.87	1.27	0.000	-0.12**
FL	↓	1, 1134; 40.318	0.18	0.23	0.12	0.000	-0.09**
HC/AC	↑	1, 1132; 16.118	0.07	0.03	0.1	0.000	0.14**

- Ns- not significant
- The F test in mixed lineal models tests the effect of preeclampsia categories longitudinally.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.
- * 95 % confidence interval adjustment for difference in the variable between preeclampsia categories and normal after multiple pair wise comparisons of estimated marginal means was performed with Least Significant Difference test (equivalent to no adjustments).
- Pearson's correlation coefficient evaluates the degree of correlation of the ultrasound variable for preeclampsia categories.

Table 55 Uteroplacental and fetoplacental haemodynamics and placental thickness in Preeclampsia with differing severity

Dependant Variable	Direction of change in the variable in ' severity of preeclampsia' categories in comparison to normal outcomes	df , F for severity of preeclampsia	Categories for preeclampsia	pair wise comparison mean difference * (95 % CI for difference) between preeclampsia categories to normal			p value by F test
				mean	lower bound	upper bound	
Mean Uterine RI	↑	1, 997; 135.432	Spe ≤ 30 weeks	0.23	0.19	0.27	0.000
	↑		Spe ≤ 37 weeks	0.16	0.12	0.19	0.000
	ns		Late pe				ns
Umbilical RI	↑	1, 1077; 50.948	Spe ≤ 30 weeks	0.14	0.11	0.18	0.000
	↑		Spe ≤ 37 weeks	0.08	0.06	0.1	0.000
	ns		Late pe				ns
Umbilical PI	↑	1, 1076; 39.917	Spe ≤ 30 weeks	0.73	0.55	0.92	0.000
	↑		Spe ≤ 37 weeks	0.41	0.28	0.53	0.000
	ns		Late pe				ns
Uteroplacental Ratio	↑	1, 1009; 16.792	Spe ≤ 30 weeks	0.14	0.07	0.21	0.000
	↑		Spe ≤ 37 weeks	0.12	0.07	0.18	0.000
	ns		Late pe				ns
Placental thickness	↓	1, 965; 17.683	Spe ≤ 30 weeks	2.88	5.26	0.5	0.018
	ns		Spe ≤ 37 weeks				ns
	↑		Late pe	3.78	2.02	5.55	0.000

Key: Spe- severe preeclampsia
Late pe-late onset preeclampsia

Table 56 Cerebral circulation, 'Brain sparing' and central shunts in preeclampsia with differing severity

Dependant Variable	Direction of change in the variable in 'severity of preeclampsia' categories in comparison to normal outcomes	df, F for severity of preeclampsia	Categories for preeclampsia	pair wise comparison mean difference * (95 % CI for difference) between preeclampsia categories to normal			p value by F test
				mean	lower bound	upper bound	
Cerebral flows and 'brain sparing'							
MCA RI	↓	1, 1069; 12.888	Spe ≤ 30 weeks	-0.06	-0.09	-0.03	0.000
	↓		Spe ≤ 37 weeks	-0.04	-0.06	-0.02	0.000
	↓		Late pe	-0.02	-0.04	0	0.025
Cerebroplacental Ratio	↓	1, 1059; 36.317	Spe ≤ 30 weeks	-0.53	-0.67	-0.38	0.000
	↓		Spe ≤ 37 weeks	-0.3	-0.4	-0.2	0.000
	↓		Late pe				ns
Haemodynamics of the central intrauterine shunts							
DA PI	↑	1, 1028; 7.740	Spe ≤ 30 weeks	0.18	0.3	0.66	0.463
	↑		Spe ≤ 37 weeks	0.31	0.02	0.63	0.063
	↑		Late pe	0.43	0.13	0.73	0.005
FO PI	↑	1, 1023; 11.790	Spe ≤ 30 weeks	1.04	0.35	1.74	0.003
	↑		Spe ≤ 37 weeks	1.16	0.71	1.61	0.000
	↑		Late pe	0.72	0.31	1.14	0.001

Key: Spe-severe preeclampsia
Late pe-late onset preeclampsia

Key findings

Study 3: Fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in UPI was a longitudinal study performed to explore the correlation between Doppler of maternal and fetal circulation as well as placental thickness in adverse placental outcomes in pregnancies complicated by UPI.

The results of study 3 illustrated that, on longitudinal examination of changes in Ultrasound and Doppler variables, FO PI and DA PI were the earliest parameters to demonstrate significant changes in UPI, IUGR and preeclampsia, preceding the deviations seen in biometry and other measures for UPI.

UPI: The sequence of changes documented that FO PI increased as early as 16 weeks, associated with an increase in umbilical artery PI and mean uterine artery RI around 16 to 20 weeks. This was followed by a reduction in biometric measurements around 24 weeks, and then followed by brain-sparing, observed as a reduction in MCA RI and CPR, at around 26 weeks.

IUGR: The earliest parameter to show significant differences was an increase in FO PI. Interestingly, Mild and moderate IUGR showed significant differences in FO PI and DA PI. Severe IUGR did not show statistically significant differences.

Preeclampsia: DA PI, FO PI and mean Uterine RI were all significantly increased in Preeclampsia. This was particularly demonstrated in early onset preeclampsia requiring intervention before 37weeks. This happened as early as 16 weeks, before a reduction in biometric measurements, MCA RI or CPR.

In late onset preeclampsia, mean Uterine RI did not show any significant differences. However, FO PI and DA PI were higher as early as 16 weeks. Placental thickness was also higher around 20 weeks.

CHAPTER 7

**Study 4: Fetal and Maternal Doppler flow
haemodynamics: correlation with adverse clinical
and placental outcomes**

Introduction

The objective of this study was to evaluate abnormalities in maternal and fetal circulation and correlate these findings with placental bed and placental morphological findings.

Histopathology studies have demonstrated that in uncomplicated pregnancies, the 100 or so spiral arteries in the placental bed undergo a series of changes that convert them from small diameter, high resistance vessels into large caliber low-resistance vessels. This process is called endovascular cytotrophoblast invasion; it starts with conception and stops by the end of the second trimester, and contributes to an increase in uteroplacental flow, from 45 ml per minute in the non-pregnant state to almost 750 ml per minute at term [83]. There is a concomitant increase in maternal cardiac output by 30 to 35 percent, where the trophoblast invasion in normal pregnancies leads to an increase in the uteroplacental circulation, contributing about 25 % of the total increase in maternal cardiac output [84].

Conversely, in pregnancies with UPI, i.e. preeclampsia and IUGR, studies have shown that there is defective cytotrophoblastic invasion [85], with superficial and/or absent transformation of the about 100 spiral arteries [320]. Placental lesions such as uteroplacental and /or foeto-placental ischaemic or thrombotic vascular lesions may be present, especially in severe forms of the disease affecting the mother as well as fetus, sometimes leading to leading to stillbirths [321]. Vasoconstriction, uteroplacental thrombosis and subsequent placental ischaemia have been proposed as leading pathophysiological pathways involved in UPI [322, 323].

Adverse pregnancy outcomes such as IUGR and/ or preeclampsia may be associated with abnormality in placental size. A sonographically abnormally thin or thick placenta appear both associated with adverse pregnancy outcomes and increased perinatal risk, in particular, IUGR [304, 324, 325].

In addition, placental ischaemic lesions have been shown to correlate with abnormalities in umbilical artery Doppler findings [326-328]. Reduced and absent

or reversed end diastolic flow in umbilical arteries is associated with increased placental resistance [74, 329, 330]. Giles et al proposed that this increased placental resistance could be due to obliteration or a reduced number of tertiary stem villous arteries [79] and Trudinger proposed that vascular sclerosis with obliteration of the tertiary stem villi could be the Doppler defined lesion in UPI [331]. Montenegro et al found that abnormalities in umbilical artery Doppler flow waveforms were associated with placental infarcts and massive perivillous fibrin deposition. [328]

Abnormal uterine artery Doppler flow velocity waveforms have also been observed with placental lesions suggesting that abnormality in the Doppler flow waveforms of uterine artery depict abnormal spiral artery transformation [332] and a reduction in uteroplacental flow [333, 334] in UPI.

Thus, placental lesions have been shown to be a key feature of the various UPI clinical phenotypes and an abnormality in umbilical and uterine Doppler flow velocity waveforms could probably reflect fetoplacental and uteroplacental circulatory responses to abnormal placentation. However, there is a paucity of data in the literature correlating abnormal foeto-placental and uteroplacental Doppler flow waveforms and the type and severity of placental lesions.

This study was based on the premise that placental lesions, in addition to changes in uteroplacental and foeto-placental circulation, may also be associated with fetal adaptive responses such as brain sparing as reflected in changes in the fetal cerebral circulation. In addition, these changes might also be reflected in alterations in Doppler flow waveforms in fetal central shunts, as the shunts probably play a pivotal role in redistribution of flows in UPI.

Hypothesis

The haemodynamic changes in fetal shunts and uteroplacental and foeto-placental haemodynamics are associated with abnormal placentation.

Aims

The specific aim is to assess the association between Doppler haemodynamics of fetal central shunts, fetal arterial circulation and uteroplacental haemodynamics with placental bed and placental histopathology.

Study design

A prospective longitudinal observational study was undertaken in a cohort of high risk and low risk pregnancies.

Study 4 was a study of comparison of Doppler variables in clinical and placental outcomes. Hence all patients from study 3 (n = 233) were eligible for participation in study 4.

For participation in study 4, to enter the “low risk “ cohort, patients had satisfy the “low risk” criteria mentioned in study 1 (Chapter 3)

To be eligible as a participant in the “high risk” cohort, they were required to fulfil the criteria considered at risk for developing UPI (Chapter 3)

Informed consent was obtained from all the participants in the study. Serial scans were then performed at 10 time points throughout pregnancy, at 16, 20, 24, 26, 28, 30, 32, 34, 36 and 38 weeks.

Scan variables for study 4

The variables evaluated for the study 4 are as follows.

Shunts: Doppler PI of FO and DA, Doppler PIV, PVIV, S/A ratio and S/D ratio of ductus venosus ***Uteroplacental and fetoplacental haemodynamics:*** Doppler PI and RI for umbilical artery (umb RI), Doppler RI for both uterine arteries, from which mean uterine RI (mean RI) was calculated. Uteroplacental ratio was calculated as a ratio of mean RI to umbilical RI.

Cerebral flow evaluation: Doppler RI of middle cerebral artery (MCA RI) and cerebroplacental ratio (CPR). CPR was defined as a ratio of MCA PI to umbilical RI.

Biometry: The serial measurements for the trial also included evaluation of fetal growth trajectory and well being with biometric measurements BPD, HC, AC, FL and amniotic fluid Index. In addition, placental thickness was also measured.

Scan protocols have been described in detail in chapter 3.

Placental samples: At birth, placentas were collected by research midwives; further histopathological analysis was performed by placental pathologists using a standard protocol in accordance with recommendations in the literature[335]. A total of 206 placentas were obtained. Placental bed biopsies were performed by the operating surgeon during the time of Lower segment Caesarian section (LSCS).

58 placental bed biopsy samples were obtained, of which 22 samples were true placental bed samples, and therefore included for statistical analysis. Non-placental bed samples as well as indeterminate samples were excluded from the statistical analysis of placental bed biopsies.

Endpoint

The primary outcome was clinical and histological UPI and unexplained preterm birth analysed separately.

Clinical outcomes for statistical analysis: clinical UPI

- UPI, including preeclampsia, gestational hypertension, placental abruption and
- Ultrasound diagnosis of IUGR due to placental insufficiency.

Placental adverse outcomes for statistical analysis: Histological UPI was further classified as uteroplacental pathology, intraplacental (also referred to as placental) pathology and fetoplacental pathology. ‘Any’ pathology included any of the placental outcomes mentioned above, including any placental pathology of uncertain significance. All definitions for clinical and placental outcomes have been described in detail in chapter 3. For statistical analysis of study 4, all subjects in whom placenta and/ or placental bed biopsy was available, were considered.

Clinical outcomes such as gestational diabetes and unexplained preterm births were excluded from the analysis for UPI, and unexplained preterm births were analysed separately.

After excluding gestational diabetes and preterm births, 184 placentas were available for histological examination, and therefore these patients were included in

the final statistical analysis for study 4. Figure 77 describes the flow chart regarding study design for study 4.

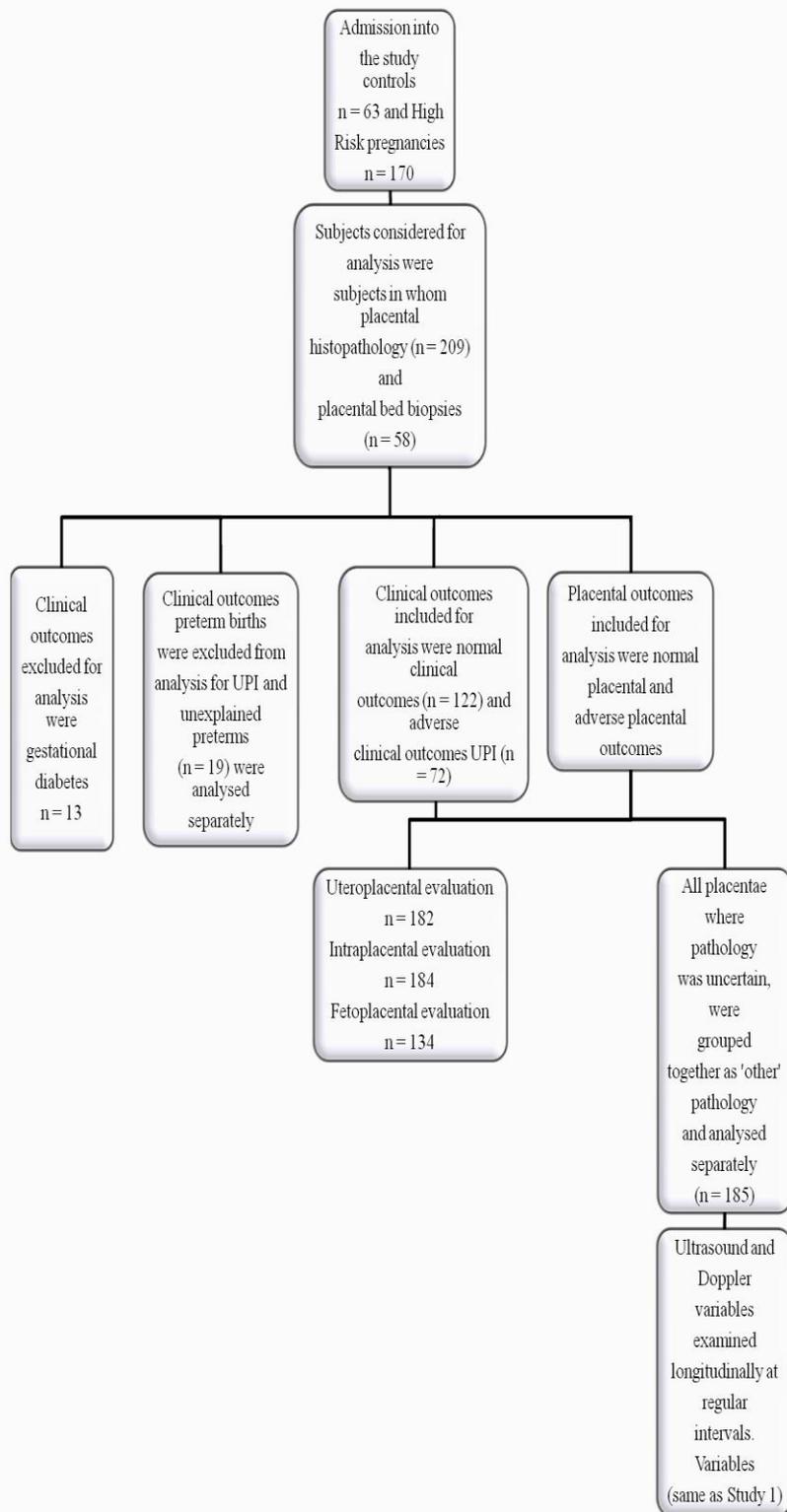


Figure 77 Flow chart describing study design for study 4

Statistical analysis

SPSS version 14 software package for Windows (SPSS Inc, Chicago, IL, 2005) and GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA), were used for statistical analysis.

Fisher's exact test was used to detect statistical significance in categorical data, i.e. to examine the significance of association between clinical and placental outcome.

The linear mixed effects regression model was used for longitudinal analysis. For each of the ultrasound and Doppler variables, longitudinal analysis by univariate analysis of variance using the linear mixed effects regression model. Linear pair wise comparisons of estimated marginal means (EMMEANS), also known as modified population marginal means or predicted means, of the variables were done for placental outcomes at different gestational ages. Longitudinal analysis of all variables was performed with F test-UNIANOVA, to compare the placental and Doppler data among the groups of subjects. The test statistic for UNIANOVA is the F statistic, which measures the ratio of between-group variability to within-group variability, p values <0.05 were considered significant. The F test was based on the linearly independent pair wise comparisons of EMMEANS for adverse placental outcomes as well as normal outcomes.

Details of statistical methods and analysis are explained in chapter 3.

Table 57 describes the number of total ultrasound and Doppler observations in study 4.

Table 58 describes the number of observations for placental histology.

Table 57 Number of observations for Ultrasound and Doppler variables in Study 4

Ultrasound and Doppler variable	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
Umbilical artery - resistance index	1638	82.0	359	18.0	1997	100
Umbilical artery – Pulsatility index	1604	80.3	393	19.7	1997	100
MCA RI	1615	80.9	382	19.1	1997	100
Cerebroplacental ratio	1597	80.0	400	20.0	1997	100
Ductus venosus preload index (s-a)/s	1603	80.3	394	19.7	1997	100
Ductus venosus peak velocity index(s-a)/d	1289	64.5	708	35.5	1997	100
Ductus venosus - PIV (s-a)/tamx	1134	56.8	863	43.2	1997	100
Ductus venosus s/a ratio	1603	80.3	394	19.7	1997	100
Ductus venosus - systolic / diastolic ratio	1295	64.8	702	35.2	1997	100
Ductus arteriosus - peak systolic velocity	1551	77.7	446	22.3	1997	100
Ductus arteriosus - end diastolic velocity	1549	77.6	448	22.4	1997	100
DA PI	1531	76.7	466	23.3	1997	100
Foramen ovale - peak systolic velocity	1557	78.0	440	22.0	1997	100
Foramen ovale - end diastolic velocity	1549	77.6	448	22.4	1997	100
FO PI	1512	75.7	485	24.3	1997	100
Mean Ut RI	1559	78.1	438	21.9	1997	100
Placental thickness	1455	72.9	542	27.1	1997	100
Bi Parietal Diameter	1628	81.5	369	18.5	1997	100
Head Circumference	1628	81.5	369	18.5	1997	100
Abdominal Circumference	1619	81.1	378	18.9	1997	100
Femur Length	1624	81.3	373	18.7	1997	100
Estimated fetal weight	1615	80.9	382	19.1	1997	100

* Explanation regarding missing data in chapter 3.

Table 58 Placental outcomes evaluated for histopathology

placental outcomes	n/N where n= pathology and N= Number of samples included for analysis
Uteroplacental morphology	26/ 182, (12.8%)
Placental morphology	28 / 184, (13.7%)
Fetoplacental morphology	5 / 175, (2.6%)
Other placental morphology	124 /185 (60.2%)
Placental bed biopsies	7 / 22 (12.1%)

Results

(A) Placental histopathology in adverse clinical outcomes including UPI, PE and IUGR.

UPI and placental outcomes

As shown in Table 59, when placental histopathology of pregnancies with UPI were compared to those with normal clinical outcomes, only the incidence of fetoplacental pathology was significantly higher in UPI, i.e. 6.9 % in the group with UPI, compared to 0.9 % in the patients with normal outcomes.

Table 59 Uteroplacental Insufficiency and placental outcomes

Placental outcome	Clinical outcome		
	UPI (n=72)	normal outcomes (n = 122)	Fisher test p value
Uteroplacental pathology	10 of 72	16 of 122	0.509
Placental pathology	12 of 72	16 of 122	0.283
Fetoplacental pathology	4 of 72	1 of 122	0.042
Other pathology	43 of 72	71 of 122	0.15
Placental bed biopsies	2 of 22	5 of 22	1

Statistically significant observations are colour-coded in yellow.

Preeclampsia and placental outcomes

The incidence of fetoplacental pathology was significantly higher in preeclampsia (15.8 %), when compared to normal clinical outcomes (0.9 %), Table 60 describes the placental outcomes in preeclampsia. Uteroplacental pathology was seen in preeclampsia, although it was not statistically significant.

Placental evaluation for pathology did not show any significant difference in the group of patients with normal outcomes in comparison with preeclampsia occurring after 37 week's gestation.

Table 60 Preeclampsia and placental outcomes

Placental outcome	Clinical outcome					
	PE n=23	Fisher test p value	SPE ≤ 37 wks n =13	Fisher test p value	late onset PE n =10	Fisher test p value
Uteroplacental pathology	5 of 23	0.068	2 of 13	0.245	3 of 10	0.132
Placental pathology	4 of 23	0.306	1 of 13	0.709	3 of 10	0.151
Fetoplacental pathology	3 of 23	0.009	3 of 13	0	0	--
Other pathology	14 of 23	0.216	5 of 13	0.603	9 of 10	0.05
Placental bed biopsies	1 of 23	1	1 of 13	0.333	0	0.839

PE includes all types of preeclampsia, irrespective of gestational age of onset. SPE ≤ 37 wks - severe preeclampsia where intervention was necessary, leading to termination of pregnancy before 37 weeks. Late onset PE-Pregnancies with preeclampsia with late onset, delivered at term. Statistically significant observations are colour-coded in yellow.

IUGR and placental outcomes

When placental evaluation was performed in pregnancies complicated by IUGR and compared to pregnancies with normal clinical outcomes, statistically significant differences in placental outcomes were observed in the severe IUGR subgroup alone. A greater proportion of placentas in severe IUGR revealed uteroplacental and fetoplacental pathology in comparison with normal clinical outcomes. Table 61 describes the placental outcomes in IUGR.

Table 61 IUGR and placental outcomes

Placental outcome	Clinical outcome					
	mild IUGR n = 26	Fisher test p value	moderate IUGR n = 7	Fisher test p value	severe IUGR n = 8	Fisher test p value
Uteroplacental pathology	3 of 26	1	--	--	2 of 8	0.045
Placental pathology	4 of 26	0.531	1 of 7	0.568	1 of 8	0.43
Fetoplacental pathology	1 of 26	0.233	--	--	3 of 8	0.0001
Other pathology	16 of 26	0.51	2 of 7	0.403	4 of 8	0.147
Placental bed biopsies	1 of 26	0.5	--	--	1 of 8	--

IUGR=intrauterine growth restriction. Mild, moderate and severe IUGR classification is based on fetal growth trajectory as observed on ultrasound. Statistically significant observations are colour-coded in yellow.

Preterm birth and placental outcomes

There was no significant difference in placental outcomes in unexplained preterm births, in comparison to normal placental outcomes.

(B) Fetal and maternal Doppler flow haemodynamics in the presence of abnormal placental histopathology

This section describes the various Doppler flow patterns and ultrasound biometric measurements throughout gestation in pregnancies associated with defined placental pathology in comparison to pregnancies with normal placental histological findings. P values for all the variables for all placental outcomes have been described in the appendix (S File 2).

Uteroplacental flow haemodynamics in adverse placental outcomes

Uterine artery Doppler was used for evaluation for uteroplacental haemodynamics. When compared to mean RI in normal placental outcomes, it was observed that the mean uterine artery flow waveforms demonstrated a significantly high resistance flow pattern throughout pregnancy, in the presence of fetoplacental pathology. Trends for increase in mean RI were evident up to 20 weeks of gestation, in the presence of other forms of placental pathology as well, but this was not significant. Table 62 and Figure 78 describe the estimated marginal means of Mean Uterine RI in all adverse placental pathology.

Table 62 Uterine artery RI and placental outcomes

Placental outcomes	Estimated marginal means of Uterine RI at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	0.67	0.63	0.56	0.56	0.54	0.53	0.52	0.53	0.54	0.58
placental pathology	0.60	0.59	0.53	0.53	0.52	0.53	0.49	0.51	0.50	0.46
fetoplacental pathology	0.74	0.59	0.58	0.69	0.66	0.64	0.57	0.62	0.69	
any pathology	0.63	0.60	0.56	0.56	0.53	0.51	0.51	0.53	0.51	0.54
Normal	0.62	0.60	0.54	0.53	0.52	0.52	0.49	0.53	0.47	0.51

The table describes the estimated marginal means of uterine artery Resistance Index (RI) at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant observations are colour-coded in yellow.

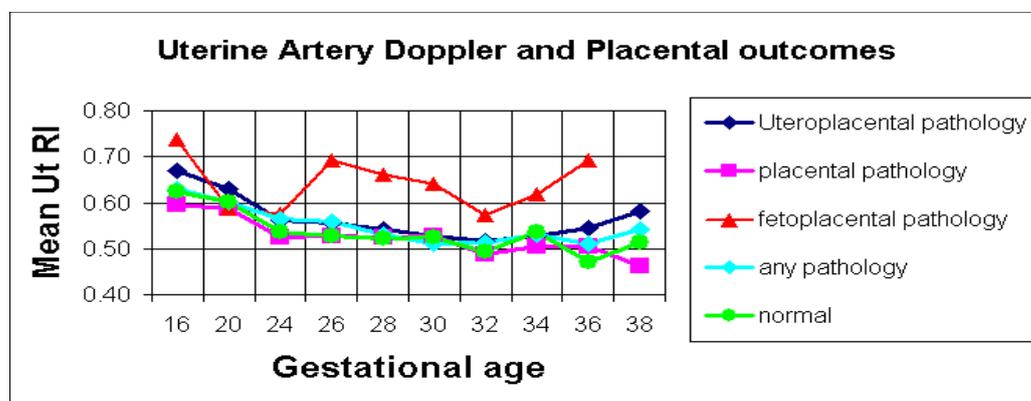


Figure 78 Longitudinal evaluation of uterine artery Doppler RI: profile plots of estimated marginal means in adverse placental and normal placental outcome

Fetoplacental flow haemodynamics in adverse placental outcomes

In pregnancies with normal placental outcomes, umbilical artery Doppler flow patterns showed a high resistance pattern in earlier gestation, which gradually transformed to a low resistance pattern in early second trimester and mean umbilical artery PI decreased with advancing gestation. When compared to mean PI in normal placental outcomes, it was observed that the mean umbilical artery flow waveforms demonstrated a significantly high pulsatility throughout pregnancy, in the presence of fetoplacental pathology (Table 63, Figure 79). Trends for increase in mean RI were evident up to 20 weeks of gestation, in the presence of other forms of placental pathology as well, relative to pregnancies with normal placental outcomes, but these differences were not significant.

Table 63 Umbilical artery PI and placental outcomes

Placental outcomes	Estimated marginal means of Umbilical artery PI at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	1.85	1.32	1.38	1.44	1.25	1.15	1.14	1.11	1.04	1.02
placental pathology	1.43	1.42	1.53	1.01	1.00	0.96	0.96	0.82	0.89	0.85
fetoplacental pathology	1.93	1.32	2.43	2.05	1.76	1.69	1.43	1.28	1.24	
any pathology	1.72	1.37	1.32	1.29	1.17	1.07	1.06	1.03	1.01	0.96
Normal	1.70	1.40	1.11	1.07	1.03	0.97	0.97	0.97	0.85	0.83

The table describes the estimated marginal means of umbilical artery Pulsatility Index (PI) at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant observations are colour-coded in yellow.

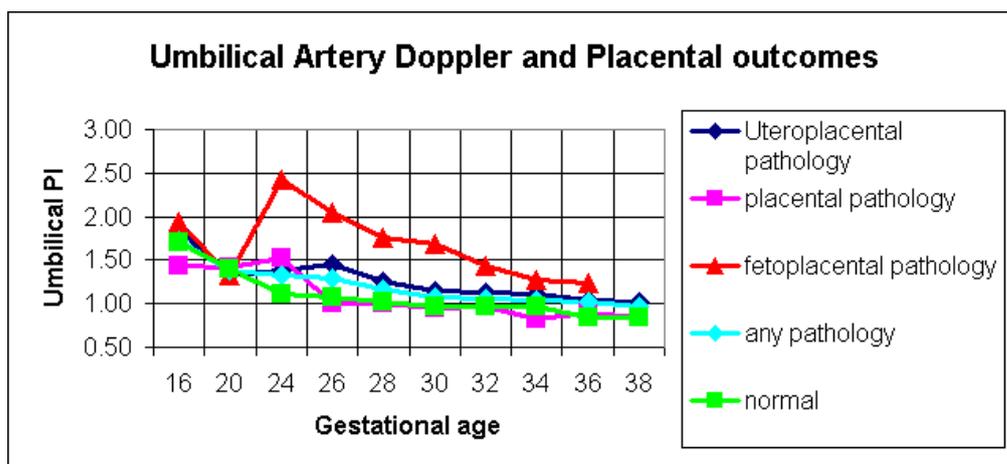


Figure 79 Longitudinal evaluation of umbilical artery Doppler PI: profile plots of estimated marginal means in adverse placental and normal placental outcome.

Placental thickness

On sonographic evaluation of placental thickness, it was observed that the placental thickness increased with gestational age in pregnancies with normal placental outcomes. When compared to mean placental thickness in normal placental outcomes, mean placental thickness reduced significantly with advancing gestation in fetoplacental pathology; contrarily, uteroplacental pathology was associated with a significant decrease in later gestation, as shown in Table 64 and Figure 80.

Table 64 Placental thickness and placental outcomes

Placental outcomes	Estimated marginal means of placental thickness at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	20.6	24.6	29.7	28.8	30.8	31.1	35.0	32.2	33.4	35.1
placental pathology	21.3	24.9	28.4	29.6	35.1	33.6	35.0	36.1	34.8	37.0
fetoplacental pathology		22.7	32.3	22.9	25.3	20.9	26	30.1	31.5	
any pathology	20.7	24.1	28.7	30.2	32.9	33.1	34.4	36.5	34.8	37.9
normal	20.8	24.8	27.4	30.2	31.5	32.7	35.0	36.2	35.5	37.3

The table describes the estimated marginal means of sonographic placental thickness at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant observations are colour-coded in yellow.

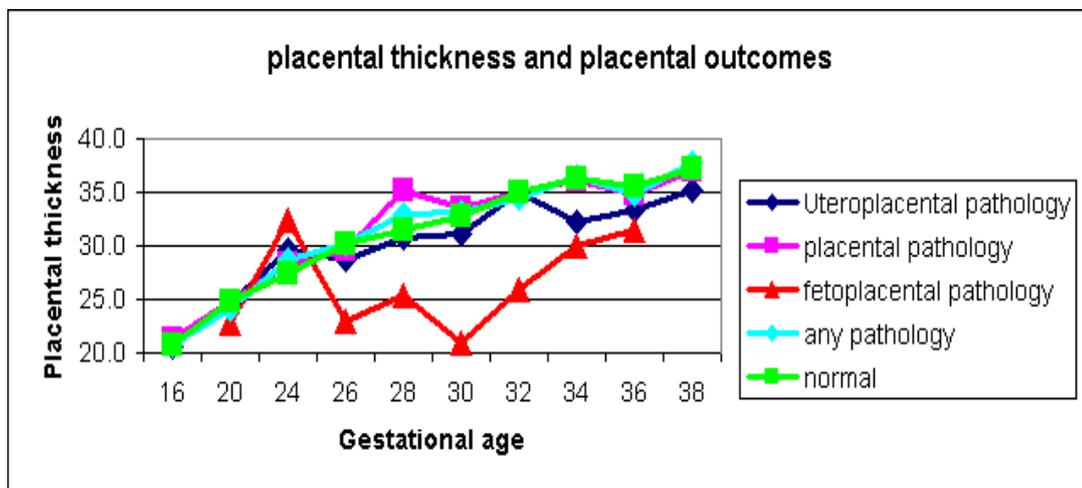


Figure 80 Longitudinal evaluation of sonographic placental thickness: profile plots of estimated marginal means in adverse placental and normal placental outcome

Fetal cerebral circulation in adverse placental outcomes

Middle cerebral artery (MCA) Doppler and cerebroplacental ratio was used to evaluate fetal cerebral circulation and brain sparing in the presence of adverse placental pathology. In pregnancies with normal placental outcomes, MCA RI followed an inverse parabolic curve, with resistance initially increasing till the late second trimester and then gradually reducing in the third trimester.

When compared to mean MCA RI in normal placental outcomes, mean MCA RI showed lower resistance in all forms of placental pathology. The reduction in resistance as evidenced by lower MCA RI was statistically significant in all forms of pathology. However, this reduction was more pronounced clinically in fetoplacental pathology (Table 65, Figure 81).

Table 65 Middle cerebral artery RI and Placental outcomes

The table describes the estimated marginal means of middle cerebral artery Resistance Index (RI) at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant

Placental outcomes	Estimated marginal means of Middle cerebral artery RI at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	0.80	0.80	0.81	0.84	0.79	0.84	0.84	0.80	0.80	0.70
placental pathology	0.74	0.76	0.79	0.82	0.79	0.82	0.80	0.78	0.79	0.66
fetoplacental pathology	0.73	0.82	0.83	0.74	0.56	0.74	0.80	0.79	0.75	
any pathology	0.78	0.77	0.81	0.83	0.83	0.83	0.82	0.80	0.77	0.74
normal	0.78	0.76	0.81	0.81	0.83	0.84	0.81	0.81	0.79	0.73

observations are colour-coded in yellow.

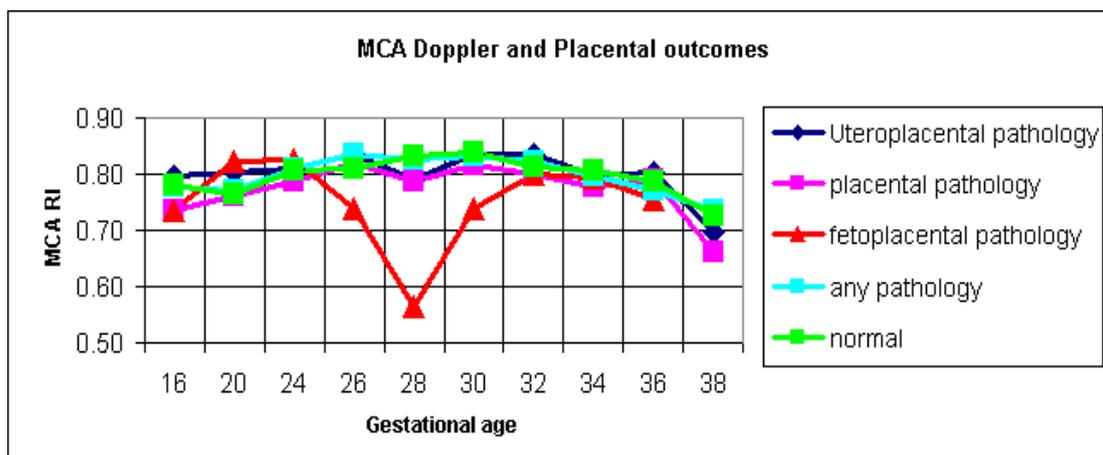


Figure 81 Longitudinal evaluation of middle cerebral artery RI: profile plots of estimated marginal means in adverse placental and normal placental outcome

Cerebroplacental ratio (CPR)

CPR was calculated as a ratio of MCA pulsatility index to umbilical artery pulsatility index (MCA PI/ UA PI). In normal placental outcomes, the mean cerebroplacental ratio increased slightly linearly with gestational age. In comparison to CPR in normal placental outcomes, CPR demonstrated a statistically significant reduction of CPR in fetoplacental pathology, which became clinically evident by around 24 weeks of gestation (Table 66 and Figure 82). There was no statistically significant difference in other forms of placental outcomes.

Table 66 Cerebroplacental ratio and placental outcomes

Placental outcomes	Estimated marginal means of cerebroplacental ratio at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	0.95	1.25	1.30	1.44	1.33	1.61	1.58	1.60	1.63	1.53
placental pathology	1.03	1.19	1.31	1.48	1.46	1.58	1.57	1.61	1.65	1.48
fetoplacental pathology	0.70	1.09	1.10	1.17	0.79	1.18	1.16	1.31	1.48	
any pathology	1.06	1.13	1.30	1.46	1.48	1.56	1.63	1.60	1.60	1.54
normal	1.04	1.07	1.32	1.40	1.46	1.60	1.63	1.63	1.73	1.78

The table describes the estimated marginal means of cerebroplacental ratio at different gestational ages in normal as well as abnormal placental outcomes.

Statistically significant observations are colour-coded in yellow.

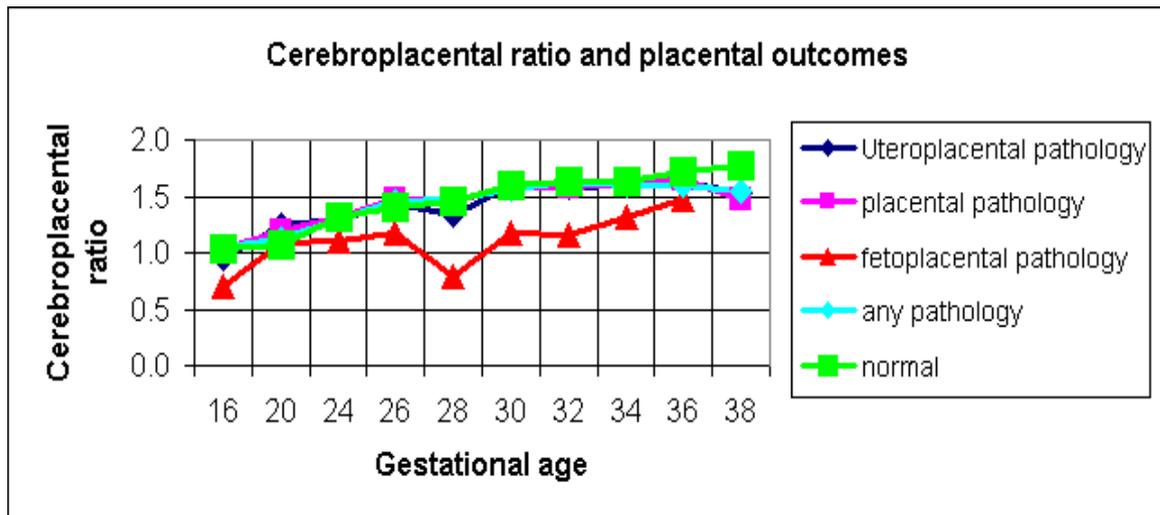


Figure 82 Longitudinal evaluation of cerebroplacental ratio: profile plots of estimated marginal means in adverse placental and normal placental outcome

Intrauterine fetal shunts in adverse placental outcomes

Fetal ductus venosus, DA and FO were examined serially throughout pregnancy and a longitudinal analysis was performed to assess them for any changes in the Doppler flow waveforms in the presence of adverse placental outcomes.

Ductus venosus did not show any significant differences in the flow patterns when ductal venosus flows in adverse outcomes were compared to normal outcomes.

Fetal Ductus arteriosus and placental outcomes

In pregnancies with normal placental outcomes, Ductus arteriosus (DA) PI was relatively constant throughout pregnancy. In comparison to DA PI in normal outcomes, DA PI showed fluctuating changes throughout pregnancy with a statistically significant increase in intraplacental pathology alone (Table 67 and Figure 83). DAPI also appeared to show an increasing trend in earlier gestation followed by a dynamic change with advancing gestation in fetoplacental pathology, however these dynamic changes were not statistically significant.

Table 67 Ductus arteriosus PI and placental outcomes

Placental outcomes	Estimated marginal means of Ductus arteriosus PI at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	2.94	2.87	2.61	2.93	2.75	2.74	2.75	2.60	2.72	2.62
placental pathology	3.06	2.51	3.24	2.67	2.77	3.02	3.06	3.45	3.90	3.52
fetoplacental pathology	2.71	3.67	2.65	3.00	2.25	2.77	2.72	1.71	2.40	
any pathology	2.98	2.73	2.86	2.78	2.91	2.81	2.80	2.96	3.05	3.32
normal	2.89	2.78	2.60	2.84	2.80	2.85	2.80	3.08	3.13	2.89

The table describes the estimated marginal means of ductus arteriosus Pulsatility Index (PI) at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant observations are colour-coded in yellow.

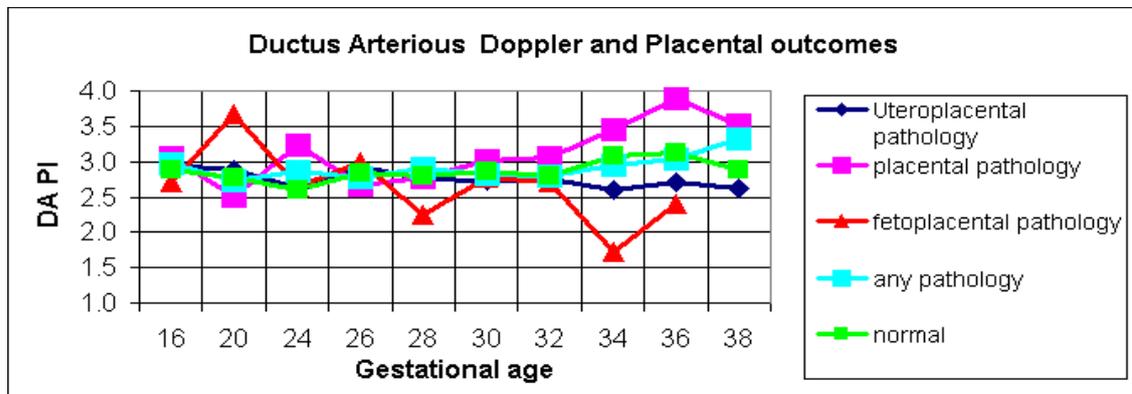


Figure 83 Longitudinal evaluation of ductus arteriosus PI: profile plots of estimated marginal means in adverse placental and normal placental outcome

Fetal Foramen ovale and placental outcomes

Serial fetal foramen ovale (FO) evaluation in normal placental outcomes revealed a gradual decrease with advancing gestation. In comparison to FO PI in normal placental outcomes, FO PI was significantly higher in intraplacental pathology in early stages of gestation, i.e., around 16 to 20 weeks after which it reduced with advancing gestation, whereas, in uteroplacental pathology, it was significantly lower at 16 to 20 weeks and remained so throughout pregnancy (Table 68, Figure 84). In fetoplacental pathology, no statistically significant changes were found.

Table 68 Foramen ovale PI and Placental outcomes

Placental outcomes	Estimated marginal means of Foramen Ovale PI at different gestational ages in weeks									
	16	20	24	26	28	30	32	34	36	38
Uteroplacental pathology	1.99	1.72	1.73	1.47	1.58	1.74	1.30	1.49	1.29	1.40
placental pathology	3.43	2.85	3.08	2.38	2.52	2.44	2.65	2.25	1.82	3.19
fetoplacental pathology	1.35	2.19	1.14	1.62	1.59	1.76	1.45	1.66	1.81	
any pathology	2.91	2.45	2.50	2.44	2.16	2.26	2.08	2.33	1.70	1.74
normal	2.64	2.94	3.12	3.22	2.93	2.74	2.21	2.27	2.44	2.59

The table describes the estimated marginal means of foramen ovale Pulsatility Index (PI) at different gestational ages in normal as well as abnormal placental outcomes. Statistically significant observations are colour-coded in yellow.

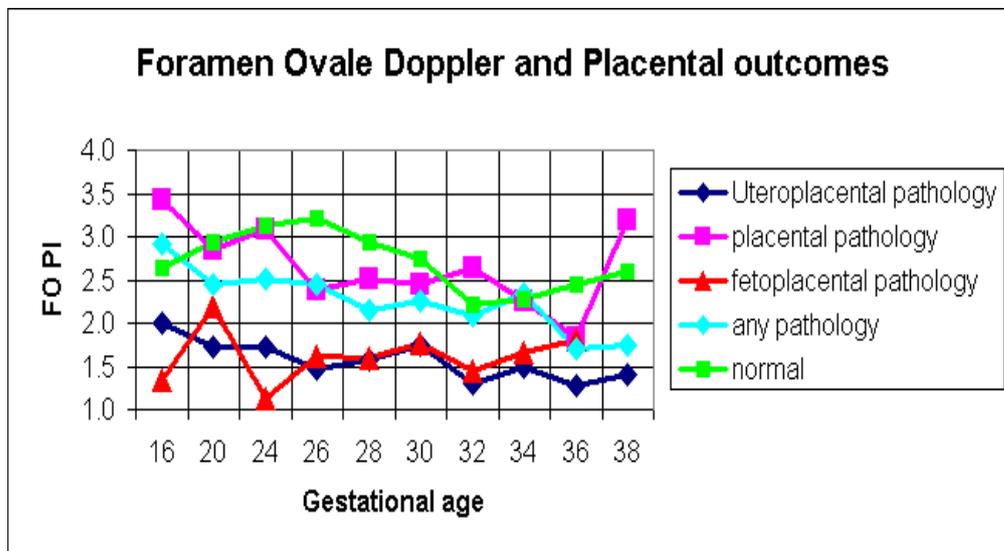


Figure 84 Longitudinal evaluation of foramen ovale PI: profile plots of estimated marginal means in adverse placental and normal placental outcome.

Doppler haemodynamics and Placental bed biopsies

After exclusion of clinical outcomes such as diabetes and preterm births as well as ‘non placental bed’ and ‘indeterminate’ samples, 22 PBB samples were available for analysis. It was observed that in PBBs demonstrating “closed” arteries, significant reductions in MCA RI and CPR were present, along with significant dynamic variations in DA PI, where the PI was initially higher around 16 to 20 weeks followed by a reduction in PI with advancing gestation. BPD, FL and HC/AC ratio were also significantly lower in placentas with “closed” PBBs.

The following table, Table 69 summarises the observations in the different ultrasound and Doppler variables after statistical analysis with the F test.

Table 69 Longitudinal analysis of all ultrasound and Doppler variables in adverse placental outcomes

Direction of change in the variable and p values based on longitudinal evaluation by F test				
Dependant variable	placental bed biopsy	fetoplacental pathology	placental pathology	uteroplacental pathology
Mean Uterine RI	↑ 0.398	↑ 0.00	ns 0.075	ns 0.11
Umbilical RI	ns 0.317	↑ 0.032	↑ 0.112	↑ 0.38
Umbilical PI	↑ 0.881	↑ 0.00	↑ 0.105	↑ 0.423
Uteroplacental ratio	ns 0.799	ns 0.151	ns 0.188	ns 0.531
Placental thickness	ns 0.543	↓ 0.01	ns 0.093	↓ 0.006
MCA RI	↓ 0.002	↓ 0.005	↓ 0	↓ 0.023
Cerebroplacental ratio	↓ 0.003	↓ 0.002	↓ 0.217	↓ 0.923
DV Preload Index	ns 0.676	ns 0.379	ns 0.959	ns 0.644
DV PVIV	ns 0.903	ns 0.32	ns 0.464	ns 0.522
DV PIV	ns 0.263	ns 0.968	ns 0.067	ns 0.603
DV S/a Ratio	ns 0.135	ns 0.615	ns 0.307	ns 0.802
DV S/D	ns 0.517	ns 0.962	ns 0.64	ns 0.669
DA PI	↑ 0.027	ns 0.691	↑ 0.007	ns 0.198
FO PI	ns 0.104	ns 0.133	↑ 0.00	↓ 0.00
EFW	ns 0.253	↓ 0.006	↓ 0.00	↓ 0.003
BPD	↓ 0.00	ns 0.673	↓ 0.00	↓ 0.00
HC	ns 0.555	ns 0.946	↓ 0.00	↓ 0.00
AC	ns 0.685	↓ 0.043	↓ 0.00	↓ 0.00
FL	↓ 0.017	↓ 0.00	↓ 0.00	↓ 0.00
HC/AC	↓ 0.021	ns 0.275	ns 0.121	ns 0.38
FL/AC	ns 0.214	ns 0.589	ns 0.30	ns 0.858

- The F test in mixed lineal models tests the effect of adverse placental outcomes longitudinally.
- This test is based on the linearly independent pair-wise comparisons at different gestational ages among the estimated marginal means. The mean difference is significant at the 0.05 level.

Thromboprophylaxis and placental outcomes

Of the total 233 women included in the study, 90 (38.6 %) women with “high risk” pregnancies had been treated with thromboprophylaxis. Of the 90 women on thromboprophylaxis, 38 women (16.3 %) were on aspirin alone, while 52 women (22.3%) were on a combination of aspirin and enoxaparin. This was a potential confounding factor, so separate longitudinal analyses of the different ultrasound and Doppler variables were performed using thromboprophylaxis, aspirin and enoxaparin as covariates.

In addition, Fisher’s exact test analysis was performed to evaluate for any association of thromboprophylaxis on placental outcomes. Fisher’s exact test was also performed separately to test the association of adverse outcomes with enoxaparin in comparison with aspirin.

Of the 170 high risk pregnancies, 149 samples were available (86.5 %) and 21 samples were missing (12.4 %) for evaluation of effect of thromboprophylaxis, either by aspirin, or with a combination of aspirin and enoxaparin, in high risk pregnancies. Of these samples, 22 cases with uteroplacental pathology were evaluated and it was found 17 (77.3 %) of 22 had been treated with some form of prophylaxis. 78.5 %, i.e. 62 pregnancies treated with thromboprophylaxis had normal outcomes ($p = 0.02$).

The significant difference on treatment with thromboprophylaxis was observed in uteroplacental outcomes alone, irrespective of mode of treatment, i.e., either with aspirin alone or a combination of aspirin and enoxaparin. 79 samples were available for testing the effect of enoxaparin on placental outcomes.

There were no differences in placental outcomes between the aspirin subgroup and the aspirin plus enoxaparin subgroup

Key findings

Study 4, “Fetal and Maternal Doppler flow haemodynamics – correlation with adverse clinical and placental outcomes”, was a longitudinal study performed to test the hypothesis that the haemodynamic changes in fetal shunts and maternal fetal uteroplacental haemodynamics are associated with abnormal placentation.

Key findings: (A) Placental histopathology in adverse clinical outcomes including UPI, preeclampsia and IUGR

- Uteroplacental pathology was associated with severe preeclampsia.
- Fetoplacental pathology was associated with IUGR and preeclampsia.
- A combination of uteroplacental and fetoplacental pathology was associated with severe adverse outcomes such as severe preeclampsia or severe IUGR.
- Late onset preeclampsia was not associated with any observable significant placental pathology.

Key findings: (B) Fetal and maternal Doppler flow haemodynamics in the presence of abnormal placental histopathology

Uteroplacental pathology, which was defined as pathology within maternal vasculature, and manifest as unopened spiral arteries, acute atherosclerosis, placental infarction or intraluminal endovascular trophoblast in the third trimester, interestingly showed no difference in mean uterine RI, umbilical RI or umbilical PI, however, a significant reduction of placental thickness, reduced MCA RI, **reduced FO PI** and a reduction in all biometric parameters-BPD, HC, AC, FL, EFW.

Fetoplacental pathology, defined as pathology within the fetal vasculature, and manifest as fetal thrombotic vasculopathy (avascular villi, fetal artery thrombosis), showed a significant association with an increased mean Uterine RI, increase in Umbilical RI and PI, reduced placental thickness, reduction in MCA RI and CPR, as well as reduced biometric parameters EFW, AC, FL. There were **no differences in FO PI or DA PI**.

Intraplacental pathology, defined as pathology affecting only the placental villi, showed no difference in Uterine RI or umbilical RI, but was associated with a significant reduction was observed in MCA RI, **increase in DA PI, increase in FO PI**, reduction in all biometric parameters EFW, BPD, HC, AC, FL.

Summary and Discussion

Introduction

Fetal and maternal wellbeing can be compromised by uteroplacental insufficiency (UPI). Consequently, when UPI is suspected, clinical care has been focussed towards appropriate maternal and fetal surveillance to optimise timing of delivery in order to reduce perinatal mortality as well as morbidity. However, despite this care, preeclampsia and IUGR are still major contributors to fetal mortality and morbidity worldwide even in developed countries with the most advanced tertiary care facilities.

Clearly, preeclampsia and IUGR are multifactorial in origin and must be considered from several perspectives. For this thesis, I chose to examine pregnancies complicated by preeclampsia and IUGR from the 'UPI' perspective. Most of the earlier studies have focussed on trying to determine the best time for delivery. However, no testing regime has been shown to identify the fetus at risk, in very early stages of the disease, when the fetus is still able to adapt to the progressing placental insufficiency.

Earlier studies have demonstrated that the earliest documented ultrasound change in fetal compromise due to placental disease appears to be a reduction in fetal biometry. This is associated with redistribution of blood flow seen as 'brain sparing' in the fetus. The cerebroplacental ratio (CPR) has been established as an effective parameter to reflect 'brain sparing' in the fetus. A decrease in CPR has also been recognized as the earliest parameter to predict adverse pregnancy outcomes. However, it had not been established if the fetus demonstrates any adaptive changes before either a reduction in biometric measurements or a decrease in CPR. It is also not known if there are any adaptive haemodynamic changes in the fetal heart, in early stages of redistribution of flow, before a reduction in fetal biometric measurements or CPR.

Viewed from a clinical fetal surveillance perspective, several research questions were addressed in this thesis:

1. What are the haemodynamic patterns throughout the gestation in normal pregnancies?
2. What are the early haemodynamic changes demonstrated by the fetus in uteroplacental insufficiency?

3. Can these changes be detected clinically before the fetus is obviously growth restricted?
4. Do these clinical changes correlate with placental pathology?

In order to answer these questions, 4 prospective studies were undertaken in a prospective cohort of control pregnancies with normal outcomes and high- risk pregnancies with adverse clinical outcomes. This thesis has studied the early changes occurring in the fetal and maternal vascular haemodynamics in pregnancies complicated by uteroplacental insufficiency, leading to IUGR and preeclampsia. Specifically, fetal central shunts, the foramen ovale (FO) and ductus arteriosus (DA) have been examined in relation to fetal biometry and fetal cerebral circulation as well as fetoplacental and uteroplacental circulation (Figure 85). This thesis also documents normograms of their Doppler flow patterns as well as their short-term and long-term responses to stress, in fetal anaemia-intrauterine transfusion model and uteroplacental insufficiency model respectively. The findings in this thesis from these studies have provided novel data on the pathophysiology of fetal central shunts FO and DA in pregnancy.

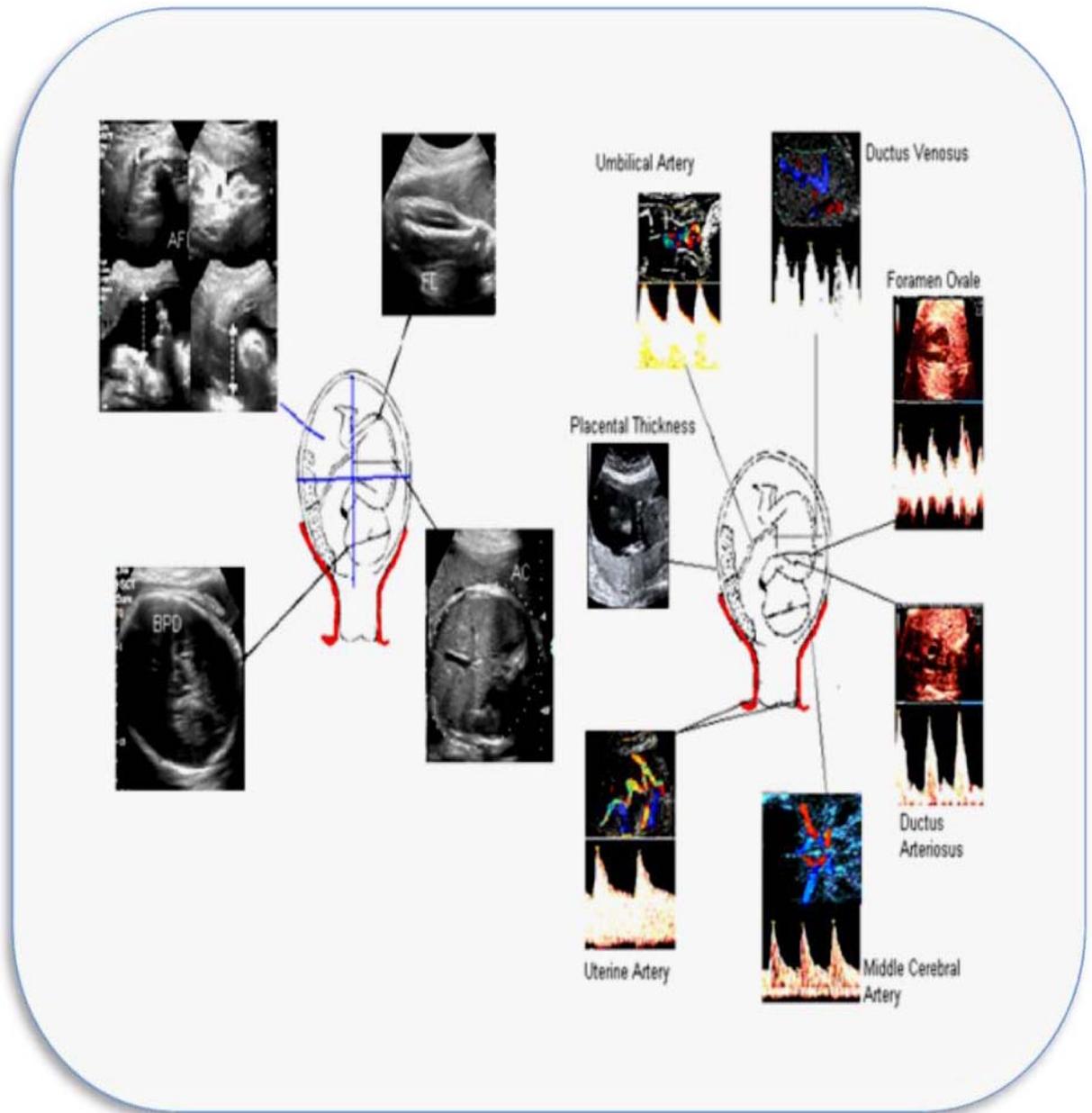


Figure 85 Summary of all ultrasound and Doppler measurements in the present thesis

The first chapter provides an overview of the different intrapartum as well as antenatal tests for fetal wellbeing and discusses the evidence obtained from clinical trials.

Evidence from a review of literature, (Chapter 2) suggests that Doppler ultrasound is useful in decreasing the adverse perinatal outcome in high risk pregnancies [13]. Serial ultrasound and Doppler monitoring have the potential to identify severe fetal compromise, and several longitudinal studies have documented the sequential

changes in circulatory haemodynamics in the compromised fetus by evaluation of the fetus for signs of brain sparing and severity of redistribution of circulation [137, 154, 155, 157, 158, 336, 337]. However, there still remains no marker for identifying the “high-risk” AGA fetuses. There is also no marker available to identify the fetus at risk before IUGR becomes evident as a reduction in AC.

The main difference between fetal and neonatal circulation is the presence of intrauterine shunts, which work closely with the placenta to ensure appropriate nutrition and oxygenation of the fetus. Since the fetal heart is vital to enable redistribution of flow, and the presence of intrauterine shunts DA, FO and DV has already been documented as a means to efficiently deliver oxygen and nutrition to fetal tissues from placenta after bypassing the lungs, the hypothesis was put forward that the fetal heart and the fetal shunts may be involved in redistribution even before any brain sparing becomes evident by currently established parameters. To explore this concept further, a review of fetal cardiovascular physiology has been provided, in relation to intrauterine fetal shunts.

Chapter 3 has described the research methodology and rationale as well as the specific aims and hypotheses used in the thesis.

It also describes the methodology used in this thesis,

1. To explore flows through all shunts-DA, DV, FO to identify flow changes early in disease and document sequential changes in flows through all fetal vessels and shunts irrespective of fetal size.
2. To evaluate the interrelation of flows within the shunts themselves and their relation with fetal growth, fetal cerebral, fetoplacental and uteroplacental circulation, and explore the usefulness of flows through central shunts, specifically, DA and FO, as possible predictors of adverse outcome.

The next section summarises the key hypotheses and aims for all the four studies, along with the observed key findings.

Results of hypothesis testing

The studies addressed four hypotheses.

The first hypothesis was that “Fetal circulation is a shunt – dependant circulation. There exists a relationship between flow haemodynamics of fetal central shunts and fetal cerebral resistance. “

To address this hypothesis, the study first established reference ranges for the Doppler flows

Aim of Study 1 (Normograms study)

To establish reference ranges for the Doppler flows of central shunts i.e. DA and FO as well as evaluate fetal Biometry and Doppler waveforms of maternal uterine arteries, umbilical artery, ductus venosus, middle cerebral artery with serial measurements , in the same set of control pregnancies, in a longitudinal study

Key findings: New normograms for central shunts and placental thickness were established and previously published normograms for other variables were validated with our longitudinal observations (Chapter 4).

The second hypothesis was that “the fetal central shunts are involved in adaptation mechanisms in acute and chronic fetal cardiovascular stress.”

Acute cardiovascular stress was assessed using the ‘fetal anaemia’ model, where fetuses underwent fetal transfusion for correction of their anaemia. This model was chosen as the anaemic fetus is placed under considerable acute haemodynamic stress due to volume overload when it undergoes intrauterine transfusion.

Thus, we assessed acute cardiovascular stress by investigating the acute haemodynamic alteration in intracardiac and other fetal shunts as a response to the acute cardiovascular stress of fetal transfusion with Doppler ultrasound (**Study 2- Fetal anaemia-intrauterine transfusion study**).

Study 2 was thus undertaken to test the **hypothesis:**

There is an adaptive redistribution through all the fetal intrauterine shunts in addition to blood flow changes in the brain as an acute response to fetal intrauterine transfusion.

Key findings:

- The peak systolic velocity of middle cerebral artery reduced significantly as expected. The PI, RI and S/D ratio of umbilical artery and PIV of ductus venosus also reduced immediately after the procedure.
- However, **there was a rising trend in the PI of fetal foramen ovale and fetal ductus arteriosus although it did not achieve statistical significance. No statistically significant difference in PSV, EDV or PI of the central shunts DA and FO after transfusion was observed (Chapter 5).**

The **third hypothesis** was that **“The changes in fetal central shunt flows precede the reduction in fetal biometric measurements and the ‘brain-sparing’ effect in fetal hypoxia or maternal disease in UPI. This would suggest that there is earlier intracardiac redistribution of the blood flow in pregnancies complicated by uteroplacental insufficiency and /or fetal growth restriction.” (Study 3-Clinical outcomes study)**

The objective of this study was to evaluate chronic fetal cardiovascular stress using the UPI model.

The specific aims were

1. To evaluate maternal uterovascular insufficiency with serial evaluation of uterine artery Doppler flows **(Study 3).**
2. To identify fetal compromise and reduction of fetal growth with serial evaluation of fetal biometry as well as umbilical artery and ductus venosus Doppler flows **(Study 3).**
3. To identify brainsparing in adverse pregnancy outcomes with middle cerebral artery Doppler and cerebroplacental ratio and then compare foramen ovale and ductus arteriosus doppler flows in these fetuses with flows in fetuses with normal pregnancy outcomes **(Study 3).**

Study 3: Fetal shunts and chronic adaptive mechanisms: fetal shunts and other vessels in UPI was a longitudinal study performed to explore the correlation between Doppler of maternal and fetal circulation as well as placental thickness in adverse placental outcomes in pregnancies complicated by UPI.

Key findings: The results of study 3 illustrated that, on longitudinal examination of changes in Ultrasound and Doppler variables , FO PI and DA PI were the earliest parameters to demonstrate significant changes in UPI, IUGR and preeclampsia, preceding the deviations seen in biometry and other measures for UPI (Chapter 6).

Study 4 was designed to address the **fourth hypothesis** which stated, “**The haemodynamic changes in fetal shunts and maternal fetal uteroplacental haemodynamics are associated with abnormal placentation.**” (Study 4- **Placental outcome study**).

The objective of this study was to examine the relationship between adverse clinical, adverse placental outcomes and Doppler ultrasound.

Study 4, “Fetal and Maternal Doppler flow haemodynamics – correlation with adverse clinical and placental outcomes”, was a longitudinal study performed to assess the association between Doppler haemodynamics of fetal central shunts, fetal arterial circulation and uteroplacental haemodynamics with placental histopathology and placental bed biopsies in adverse placental outcomes in pregnancies complicated by UPI (**Chapter 7**).

Key findings: (A) Correlation of clinical and placental outcomes

- Uteroplacental pathology was associated with severe preeclampsia.
- Fetoplacental pathology was associated with IUGR and preeclampsia.
- A combination of uteroplacental and fetoplacental pathology was associated with severe adverse outcomes such as severe preeclampsia or severe IUGR.
- Late onset preeclampsia was not associated with any significant observable placental pathology

Key findings: (B) Doppler flow haemodynamics correlation with adverse placental outcomes.

Uteroplacental pathology(UPI) was defined as a pathology within maternal vasculature, and manifest as unopened spiral arteries, acute atherosclerosis, placental infarction or intraluminal endovascular trophoblast in the third trimester. UPI showed no difference in mean uterine RI, umbilical RI or umbilical PI. However, a comparison of UPI to controls showed a significant reduction of placental thickness, reduced MCA RI , **reduced FO PI** and a reduction in all biometric parameter-BPD, HC, AC, FL, EFW.

Fetoplacental pathology, defined as a pathology within the fetal vasculature, and manifest as fetal thrombotic vasculopathy (avascular villi, fetal artery thrombosis), showed a significant association with an increased mean Uterine RI, increase in Umbilical RI and PI, reduced placental thickness, reduction in MCA RI and CPR, as well as reduced biometric parameters EFW, AC, FL when compared to controls. There were **no differences in FO PI or DA PI**.

Intraplacental pathology, defined as a pathology affecting only the placental villi, showed no difference in Uterine RI or umbilical RI, but was associated with a significant reduction in MCA RI, **increase in DA PI, increase in FO PI**, reduction in all biometric parameters EFW, BPD, HC, AC, FL.

Discussion

The final section of chapter 8 of this thesis explores the key findings of this thesis in the context of anatomical and physiological principles in existing literature. The contribution of the four studies to the existing body of knowledge and its clinical significance with implications in fetal surveillance is discussed. Limitations as well as further avenues for research are also explored.

Clinical adverse outcomes and placental histopathology in UPI

This research has shown that spiral artery disease, in many but not all cases, leads to adverse clinical outcomes such as preeclampsia and IUGR, leading to fetal compromise, which is in accordance to previously published data. Fetoplacental pathology was significantly higher in pregnancies complicated by severe preeclampsia and IUGR. It should be noted that a proportion of pregnancies with abnormal placentation had good clinical outcomes. It is possible that this could be due to the relatively limited sample size [338]. Another explanation of this observation could be that the ‘placental reserve’ capacity, which is much more considerable than generally thought, guards against fetal compromise in the presence of placental pathological changes [81, 338].

Furthermore, our data did not demonstrate a significant incidence of uteroplacental disease in histopathology. The results from our placental bed biopsy data also did not show any statistically significant differences in normal and adverse clinical outcomes. However, these results should be interpreted with caution, as the sample size was inadequate, given that only 22 placental bed biopsy samples could be included for analysis. Another explanation for this observation is that placental histopathology may not be a sensitive marker for uteroplacental disease.

Doppler flow velocity waveforms of maternal-fetal circulation and UPI

As a new finding, this work has shown that fetal intrauterine shunts FO and DA demonstrate circulatory responses to UPI as early as 16 weeks which can be identified with the help of Doppler ultrasound, suggesting a key pathway for redistribution of fetal flows in complicated pregnancies. These **precede** changes demonstrated in fetal biometry, fetal cerebral circulation, fetoplacental circulation and materno-placental circulation. This is a novel finding, of considerable potential clinical relevance, as it provides a potential new strategy for fetal surveillance,

which could be validated with further clinical investigation. Figure 86 describes the proposed sequence of changes in fetal compromise.

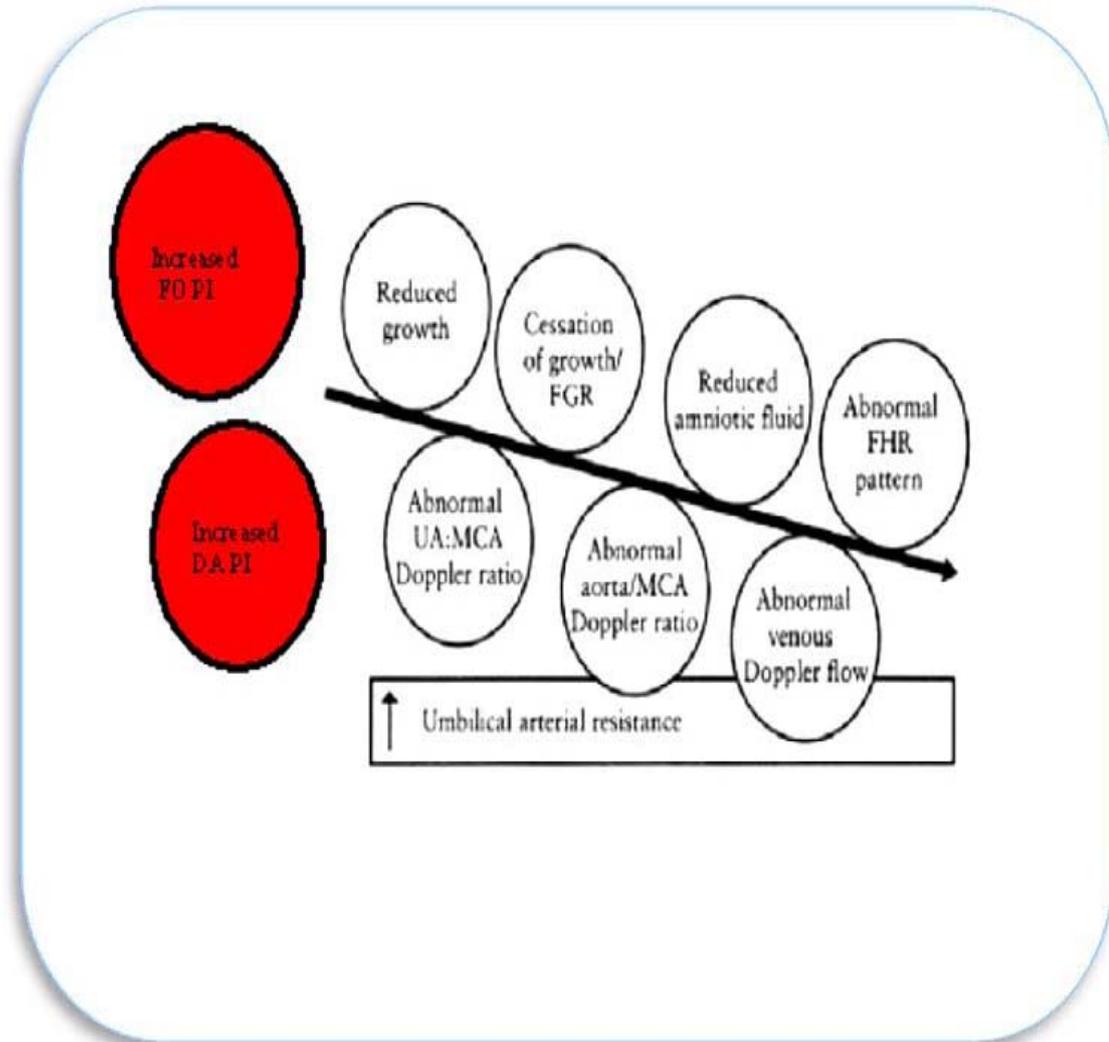


Figure 86 Sequence of changes in ultrasound parameters in UPI

Modified from Harrington et al 2000 [339]

In the next section of the chapter, the pathophysiology of abnormal flows through central shunts DA and FO will be discussed with reference to a review of previously established anatomical and physiological concepts.

PATHOPHYSIOLOGY OF FORAMEN OVALE

FO PI flows demonstrated a decrease in PI with advancing gestational age, in normal fetuses (Study 1: Normogram study).

An increase in FO PI was found as early as 16 weeks of gestation, in fetuses complicated with UPI, Preeclampsia and IUGR, followed by a ‘pseudonormalisation in progressive severe compromise. (Study 3: longitudinal study in UPI). This increase in FO PI in early gestation was associated with Placental pathology (Study 4).

FO PI also showed a consistent and immediate increase in anaemic fetuses after intrauterine transfusion (Study 2), although this increase was not statistically significant.

In normal pregnancies, we have found that FO PI reduces with advancing gestational age, perhaps due to a decrease in proportion of shunting of flows. It has been established that foramen ovale flows decrease from 34% combined cardiac output at 20 weeks to 18% at 30 weeks, and then remain unchanged from 30 to 38 weeks [271].

We observed an increase in FO PI in early gestation, in pregnancies complicated by UPI, which was associated with placental pathology. It is possible that this increase in FO PI is an adaptive mechanism, reflecting intracardiac redistribution. This could have several possible explanations. In a dynamic organ such as the beating fetal heart, PI can be affected by numerous causes. We hypothesise that the changes in FO PI that we have observed can be explained on the basis of:

- Anatomy of the FO valve.
- Physiology of flows through FO: relation with IVC.
- Physiology of flows through FO: relation to pressures in atria and ventricles.
- Physiology of FO in relation to aorta
- Increased placental resistance causing backward reflection of pressure wave.

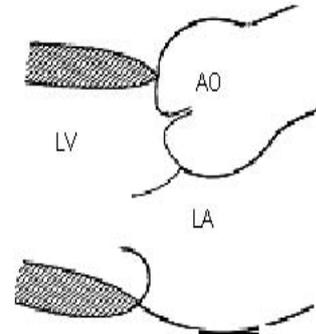
The following section will provide a detailed discussion of these proposed pathophysiological mechanisms.

Figure 87 summarises all the proposed hypotheses for the pathophysiology of abnormal flows through FO.

(a) FO in relation to IVC

NOTE:
This figure is included on page 255 of the print copy of the thesis held in the University of Adelaide Library.

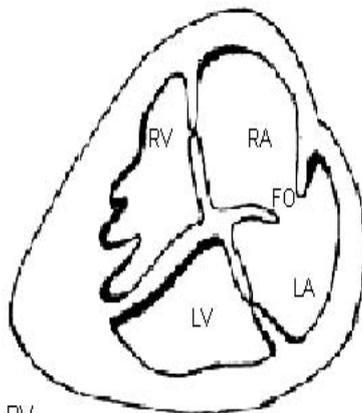
(d) Long axis view: Aortic root



Aorta

- ↑Aortic impedance
- ↑Elasticity
- ↑distensibility of aortic root
- "Spinnaker effect"
- immature windkessel function
- ↑backward reflection of pressure wave from placenta

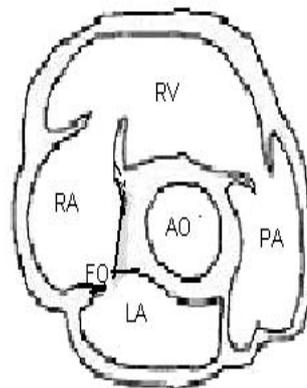
(b) 4 chamber view:
FO in relation to RA, RV, LA, LV



RV
Pressure loading
Volume loading

LV
Pressure loading
Volume loading

(c) 5 chamber view:
FO in relation to Aorta



RA
Pressure loading
Volume loading

LA
Pressure loading
Volume loading

(e) Windkessel function of Aorta

NOTE:
This figure is included on page 255 of the print copy of the thesis held in the University of Adelaide Library.

Figure 87 Hypothetical pathophysiological mechanisms for increased Doppler pulsatility index in foramen ovale in fetal compromise associated with uteroplacental insufficiency.

This figure illustrates the anatomical aspects and physiological basis for alteration in flows through foramen ovale, based on its relation to both atria, ventricles, the IVC and the aorta.

This schematic diagram has been adapted from the following resources:

a: Amoroso et al 1942. [185]

b and c: AIUM normal views Am Society of Echocardiography 2004[340]

d: Dean JCS Heart 2002[341]

e: <http://www.akdeniz.edu.tr/tip/fizyoloji/d2/windkessel.htm> accessed on 5th Sept 2007

Anatomical basis for pathophysiology of abnormal flows through FO

FO is a tubular and pliable valve [69, 193], its diameter is less than half of the IVC diameter [342], and the atrial septum is situated further to the right and is tilted over the IVC, thereby designed to receive flows directly from right atrium [66]. Amoroso et al in 1941 [185], introduced the concept of the FO being the left terminal portion of a functional 'posterior caval channel' which has subsequently been confirmed by angiographic studies and Doppler exams [180, 343]. Kieserud and co-workers extended this hypothesis further by including ductus venosus in this functional channel [180].

Recent studies have demonstrated that position of FO allows a preferential streaming of flows across FO [240]. Blood flow across FO is preferentially distributed via a mechanism similar to a windsock, the left arm of the windsock being the area between FO valve and the atrial septum, to enter the left atrium, whereas the right arm is directed towards the tricuspid valve to join the flow from the SVC and coronary sinus [240]. The restricting area in FO for flow into the left atrium is not the oval shaped ostium of the septum, but, rather, the horizontal area between the FO and the atrial septum above the FO [317] (

Figure 87). Increased pulsatility through FO may be caused due to increased shunting of flows through FO with increased pressure across the windsock, coupled with an increased pulsatility of flow through the functionally 'restrictive area' between the FO valve and atrial septum. Increase in pulsatility of FO PI in early stages of UPI could be considered as a necessary adaptive response, to ensure constant forward cerebral perfusion, to enable maintenance of critical cerebral pressure, to protect the brain from hypoxic ischaemic injury.

Pathophysiology of flows through FO in relation to preload-afterload interactions

Flows through FO are influenced by pressure loading conditions in the atria. It has been observed that FO flows allow flow between atria according to the kinetic energy of the caval flow and their respective pressure in right and left atria [344]. Right atrial pressures are slightly higher than left atrial pressures, thereby ensuring flow through FO is from the right to the left. Right and left ventricles have common arterial outflow pressures. Ventricles pump in parallel, so the stroke volumes may differ [345].

Ventricular pressures increase with gestation. Atrial pressures depend on the preload-afterload interactions between atria and ventricles. As a parallel circuit, FO flows will reflect haemodynamic events in both of the atria. Fetal atrial function is vital for ventricular filling, and failure of atrial function is almost the last stage in myocardial decompensation in hypoxia.

There is a greater contribution of 'atrial systole to ventricular end diastolic volume in the fetus than in the adult [346]. A coordinated atrial contraction is necessary to fill its less compliant ventricles [267, 347]. In acute pressure loading conditions, lamb experiments showed that atrial myocardial flow response was much greater than ventricular myocardial flow. A significant increase in right atrial 'a' wave pressure, i.e. active atrial contraction was seen in acute ventricular pressure loading, suggesting an increase in atrial wall stress and right atrial myocardial oxygen demand [348]. Atrial flow exceeded ventricular flow; a two-fold increase in ventricular flows was associated with a five-fold increase in fetal atrial myocardial flows reflected as a reversal in 'a' wave in the ductus venosus [348]. This suggests that atrial blood flow is regulated independently in the fetus and there is no evidence of any compromise of atrial flow with increasing work load [349]. It is probable that FO exhibits an adaptive response with an increase in pressure or volume loading conditions in atria, which is reflected as an increase in FO PI. This mechanistic hypothesis is supported by our own observations in Study 2, where FO PI increased immediately after intrauterine transfusions, although this increase did not reach statistical significance. Study 2 also supported the hypothesis that cerebral responses to fetal transfusion is not only due to cerebral autoregulation alone but also has an associated central component with cardiac involvement.

Another important feature of the fetal heart myocardium is that it functions at the upper limit of the Frank-Starling law. In the fetus, the myocardium is less compliant than the adult, and this is even more pronounced in the younger fetus [266-268], therefore even slight alterations in intracardiac pressures might be reflected as alterations in pulsatility of intracardiac blood flow. Preferential streaming of the oxygen-rich blood from IVC to the left atrium is enabled by the presence of the Eustachian valve in the IVC, which is anatomically situated opposite the left atrium [240]. The crista dividens is the free upper edge of atrial septum primum. Together

with the crista dividens, the Eustachian valve directs venous flows from the IVC into two streams. This preferential streaming has been confirmed with lamb experiments [237] as well as by Doppler studies on human fetuses [240]. Even though the pressure in the IVC is low, the flow is more than two thirds of the total cardiac venous return. Flows through FO have also been shown to be dependant on the kinetic energy of IVC [344]. An increase in flows and pressure through IVC might therefore lead to increase in flows and pressure through the FO, leading to an increase in FO PI in early stages of gestation.

Pathophysiology of abnormal flows through FO based on aortic biomechanics

It is likely that FO PI will also be influenced by altered biomechanics of the aorta. The aorta and some of the proximal large arterial vessels store about 50% of the left ventricular volume during systole and propel this 50% of the volume forwards in diastole. This ventricular systolic- aortic diastolic interplay represents the windkessel function and is responsible for forward propulsion of flows [350]. The windkessel function is a property of the aortic wall, which functions as a reservoir in diastole and propels blood forward in systole, thus converting pulsatile flow into steady, continuous flow. It also results in a reduction of left ventricular afterload and improvement in coronary flow and left ventricular relaxation [350].

Ventricular ejection generates a pressure pulse which is propagated throughout the arterial system [351]. This pressure pulse depends on the elastic and geometric properties of vessels as well as the viscosity of blood [352]. Since blood is incompressible and flowing through elastic conduits, the arteries, the pressure propagation is along the arterial walls. Pulse wave velocity and the arterial elastic modulus are highly dependant on arterial wall tension and therefore blood pressure. It has been observed that in IUGR due to UPI, there is an increase in diastolic pressure increase of afterload. This increase in afterload results in a decrease of aortic distensibility during the neonatal period, suggesting an alteration of aortic wall structure [353]. In the uncompromised fetus, it has been demonstrated that the pulsatility is lower in the aorta, while IUGR is associated with an increase in fetal arterial diastolic as well as aortic pulse pressure [354, 355].

With increasing peripheral resistance in UPI, there is increased back-pressure, as well as a compensatory increase in the left ventricular outflow tract (LVOT) pressure gradient. This causes increased pulsatile load and a possible increase in distensibility of aortic root [356]. Increased pulsatility with increased back pressure leads to an increased amplitude of aortic distension waveform and an increase of amplitude to end-diastolic diameter ratio [357, 358], which is associated with increased cardiac contractility [357]. This increase in cardiac contractility may enable increased pulsatile flows through aortic root with increased distension through aortic root and LVOT.

Windkessel function necessitates the vessel wall to have enough elastin to be able to withstand the pressure of a large volume of blood and act as a capacitor. This requires that the arterial wall have enough elastin. However, it has been observed that in early stages, the artery predominantly consists of collagen, and the elastin deposition in the media occurs at a much slower stage, and exponentially increases in the 2nd half of pregnancy, and depends on the increasing haemodynamic forces and pressures in fetal circulation [359]. Experimental data suggests that there is an impaired elastin synthesis during a critical period of vascular formation in the fetus [360]. This can lead to abnormal compliance of the aorta affecting the cardiac output.

The impedance of aorta also depends on aortic diameters and cross-sectional area, in addition to the aortic wall elasticity. Pulsatility of flow is determined by viscoelastic properties of aorta, i.e., the Young's modulus. Young's modulus is an estimate of arterial stiffness that is independent of wall thickness. Young's modulus in aortic root and ascending aorta is lower as compared to descending aorta, which may facilitate increased distension of the aortic root in early hypoxaemia. The radius and Young's modulus exponentially tapers from ascending to descending aorta. Distension of aortic root with increased pulsatility is transmitted to the right atrium. This transmitted pulsatility may also cause the right atrium to be compressed slightly. When the right atrium undergoes slight compression, the FO valve being pliable, probably acts like a spinnaker, directing more flows through the FO from RA into LA. This effect has been demonstrated in adults [356]. If the same hypothesis is extrapolated in the fetal circulation, the spinnaker effect could also contribute to increase in FO PI.

Intracardiac pressures and flows are affected by downstream resistance. Mathematical and computer models of fetal circulation have described fetal circulation as a transmission line [361]. The frequency domain model of fetal circulation [361, 362] implies that after ventricular ejection and a shock wave generated by the origin of thoracic aorta, a forward pressure wave travels along the arterial tree at a given pulse velocity. In a flow system with low impedance and steady flows, there is hardly any backward reflection. In UPI, increased peripheral resistance and increased placental resistance leads to increased impedance mismatch [352]. An increase in the impedance mismatch leads to an increase in back pressure causing increased wave reflection of pressure waves in pulsatile flow, augmenting forward systolic flow and reducing diastolic flows [363]. An increased placental resistance leads to backpressure causing backward reflection of aortic pressure waves [364, 365]. This increased reflection of pressure waves may cause an augmentation of systolic velocities, and thereby an increase in PI.

Pseudonormalisation of FO PI

We observed that the FO PI was higher in earlier gestation in UPI, in comparison to the FO PI in fetuses with normal outcomes. However, when the flows in IUGR fetuses were compared to normal fetuses in later stages of compromise, when the CPR and biometry were abnormal, there was no difference in FO PI values in both the groups. We describe this effect as pseudonormalisation, because even though the Doppler indices appear normal, we speculate that this normalisation occurs due to an increasing severity of fetal compromise with abnormal pathophysiology. There could be several explanations for pseudonormalisation of Doppler flows.

A previous study on distribution of cardiac output in normal fetuses showed that the in fetuses with uncomplicated pregnancies with normal outcomes, the flows through FO decreased from 34 % combined cardiac output at 20 weeks to 18% at 30 weeks, after which it remained unchanged from 30 to 38 weeks [271]. Lamb experiments showed that near-term, about 44% of the umbilical venous blood was distributed to DV. With a 50% reduction of umbilical flow, there was a reduction of umbilical flow to the liver, with an increase of umbilical flow from 44 % to 72 % to ductus venosus, and the proportion of O₂ delivery to upper body organs derived from the ductus

venous increased through the FO (33.2 vs. 49.4%) [273]. It was also observed that the ostium of FO is decreased, despite a normal sized heart, in severely growth restricted fetuses [242]. The diameter of RA was also observed to increase in IUGR fetuses, in the same study. This was speculated to be either due to increasing congestion of RA or an increasing volume flow to the heart.

To summarise, previously published observations from literature have established that increased backward reflection of the pressure wave with UPI leads to increased right ventricular afterload which then leads to deteriorating right ventricular contraction and myocardial dysfunction [366]. Impaired right ventricular relaxation is further compounded by increasing right atrial congestion [367], increased right ventricular preload [143], augmented right atrial contraction [368] and a possible increase in right atrial pressure. We speculate that these haemodynamic responses in deteriorating cardiac function in UPI, combined with, a possible reduction in the ostium of FO as discussed above, as well as reduction in proportion of oxygenated flow through the FO, would therefore be reflected as a pseudonormalisation of the Doppler indices, with a reduction in FO PI. This pseudonormalisation would therefore actually reflect a more serious form of shunt derangement with circulatory compromise.

PATHOPHYSIOLOGY OF DUCTUS ARTERIOSUS

DA PI flows demonstrated a constant PI with advancing gestational age, in normal fetuses. (Study 1: Normogram study).

An increase in DA PI was found as early as 16 weeks of gestation, in fetuses complicated with UPI, Preeclampsia and IUGR, followed by a 'pseudonormalisation' in progressive severe compromise. (Study 3: longitudinal study in UPI). Severe placental pathology was associated with this increase in DA PI in early gestation (Study 4)

DA PI also showed an immediate increase in anaemic fetuses after intrauterine transfusion (Study 2), although this increase did not reach statistical significance.

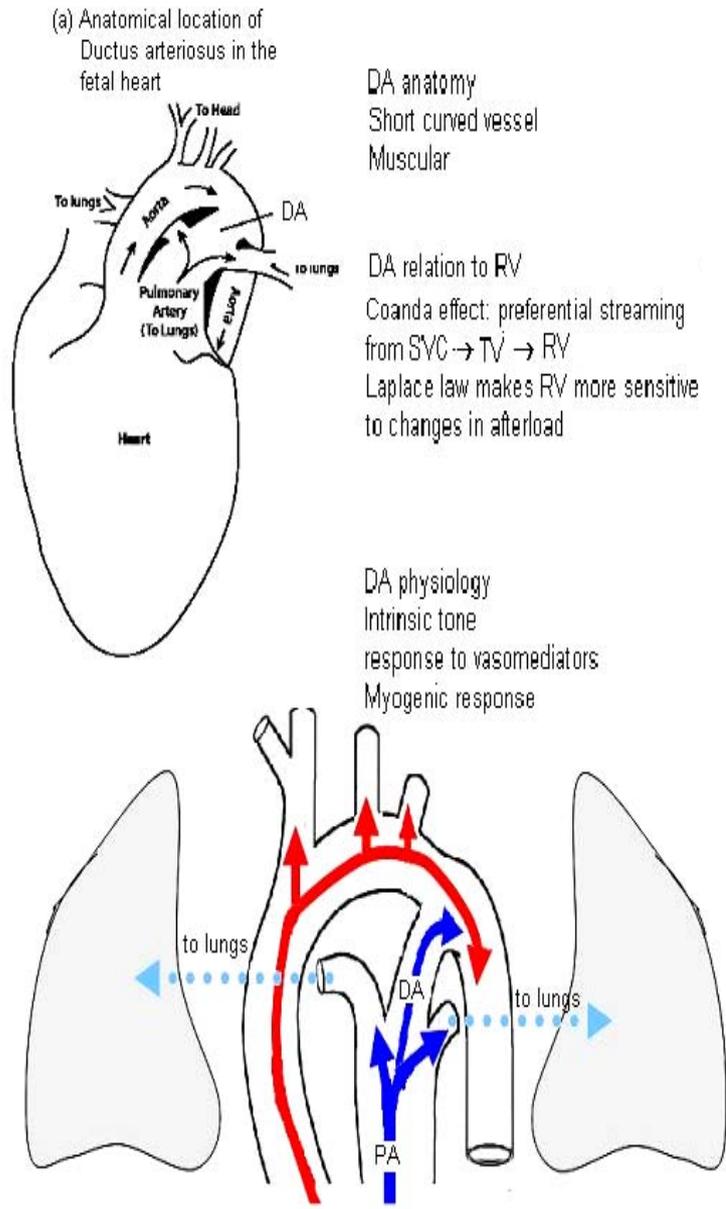
We have shown that DA has a significantly increased PI in fetuses of pregnancies complicated by UPI, as early as 16 to 20 weeks. With progressing severity of fetal compromise, this increase in PI reversed and appeared normal, which we have termed as 'pseudonormalisation', in view of the severity of compromise.

First of all, the possible mechanisms for an increase in DA PI in early gestation will be discussed, after which, an attempt will be made, to discuss pseudonormalisation with advancing age and gestation.

This increase in DA PI may be explained on the basis of:

- Anatomy of DA
- Developmental histology and morphology of DA.
- Intrinsic tone of DA
- Physiology of flows through DA
- Preferential streaming of flows through FO
- Myogenic response to increasing afterload
- Response to vasomediators

The proposed pathophysiological mechanisms are discussed in detail. Figure 88 summarises all the proposed hypotheses for the pathophysiology of abnormal flows through DA.



(b) Ductus arteriosus in relation to right ventricle

NOTE:
This figure is included on page 263 of the print copy of the thesis held in the University of Adelaide Library.

(c) DA in relation to PA, AO, Lungs, aortic isthmus
 PA -AO pressure gradient
 AO → Ao isthmus → cerebral flows
 Ao isthmus opens into DA at an acute angle, with narrowing
 DA diameter smaller than PA and AO → Venturi effect
 Pulmonary hypoxic constriction → ↑ PA pressure with increasing hypoxia

Figure 88 Pathophysiology of abnormal flows through Ductus arteriosus
 This figure demonstrates the anatomical relations of the ductus arteriosus and describes the proposed pathophysiological mechanisms leading to an increase in the Pulsatility Index in its Doppler flow waveforms.

This schematic diagram has been adapted from the following resources:
 b: Merck manuals online. <http://www.merck.com/mmhe/sec23/ch265/ch265b.html> accessed on 25th May 2008., c: Kiserud 2001 [317]

Pathophysiology of flows through DA based on morphological characteristics

The DA is a unique arterial vessel connecting the pulmonary artery to the descending aorta. The aortic isthmus, which is the junction between the cerebral and placental resistance systems, is a short portion of the aorta between the origin of the subclavian artery and ductus arteriosus. It has a narrow lumen, 25 percent smaller than ascending and descending aorta[369] and opens into the ductus arteriosus at an angle, the ductus arteriosus –isthmus angle measuring less than 90 degrees [370].

The DA is a curved, narrow vessel, measuring 3.95 mm in length at 15 weeks to 12.2 mm in length at 34 weeks [371]. It is narrower than pulmonary artery and aorta. The length and curvature of DA increases with gestational age. Recent observations suggest that even though the absolute diameter of DA increases in size, with gestation, the relative diameter, in comparison to aortic isthmus, reduces with advancing gestation [371].

Histologically, it is a muscular vessel, unlike pulmonary artery and aorta, which are elastic [205, 206]. The smooth muscle cells within the DA are highly sensitive to vasomediators [218] and the intimal cushions present within the DA developmentally prepare it for effective closure at birth. [208]. This unique anatomy and histology of DA makes it sensitive to any changes in flow haemodynamics caused by UPI.

Pathophysiology of flows through DA in relation to preload-afterload interactions

The amount of flow entering DA is determined by the preferential streaming of flows from FO. The crista dividens, which forms the upper edge of FO, along with the Eustachian valve, helps direct highly oxygenated flows from IVC and ductus venosus into LA [254]. The Lower's tubercle, present in the lateral aspect of right atrium at the SVC-right atrial junction, preferentially streams flows from SVC into tricuspid valve [372]. This dual preferential streaming has been attributed to the 'Coanda effect', described as the tendency of a moving fluid, either liquid or gas, to follow a nearby curved surface if the angle is not too sharp, rather than follow a straight line. It is a unique mechanism, which keeps the two streams separate, and has been observed in the heart on Colour Doppler studies [373] as well as Magnetic Resonance Imaging [374].

The pulmonary artery lumen is bigger than the lumen of DA, the aortico-isthmic junction is also narrow, therefore, blood flow from DA into aorta must speed up while entering the descending aorta, reducing its pressure, producing a Venturi effect derived from the Bernoulli's equation. Venturi effect is a well-known effect in fluid dynamics in which the increased kinetic energy of blood flow through the constriction corresponds to a pressure drop and consequent constriction of the vessel walls. In UPI, we reason that there is redistribution of flows in earlier gestation, with preferential streaming of oxygenated flows in favour of the FO. This preferential streaming would possibly lead to an increasing return of flows through the SVC, subsequently leading to more flows through tricuspid valve and therefore through RV into pulmonary artery. This increase in flows through the muscular DA would lead to dilatation of DA, thereby increasing the intrinsic pressure within the DA lumen, being reflected as an increase in PI. Thus, it could be postulated that the increased PI in DA is a reflection of flow-mediated dilatation in DA caused by UPI.

Lamb experiments have shown that the direction of flow in DA is from right to left [375]. This is because of a pressure gradient which favours the pulmonary artery. In the fetus, the pulmonary arteries have a high arterial pressure as the lungs are collapsed. The pulmonary system is a high pressure high resistance system whereas the systemic circulation system is low resistance, low pressure flow system. This leads to a pressure gradient so that flow can bypass lungs via the DA and enter the descending aorta. Increase in shunting through FO would be expected to lead to an increase in this pressure gradient, and as a consequence, increased flows through aortic isthmus, to maintain critical cerebral perfusion in UPI. This is probably achieved by increasing the LVOT pressure gradient, in early stages of fetal compromise. This increase in pressure gradient could be reflected as increased PI in DA.

Pseudonormalisation of DA PI

We observed that the DA PI was higher in earlier gestation in UPI, especially in IUGR, in comparison to the normal fetuses. However, when the flows in IUGR fetuses were compared to normal fetuses in later stages of compromise, in the presence of abnormal CPR and biometry, the DA PI flow velocity waveforms were similar in both the groups. We describe this effect as pseudo-normalisation, because even though the Doppler indices appear normal, we speculate that this apparent normalisation is actually decompensation. We propose that pseudo-normalisation occurs due to increasing severity of fetal compromise with abnormal pathophysiology, and put forth several possible mechanisms to support our theory.

First of all, the volume of flows in an arterial vessel will be influenced by its size and length. Szpinda's study [371] indicated that the volume of the ductus arteriosus revealed an increase approximately according to the cubic function $y=0.0007x^{3.3782}$. In the examined age range, the volume of the ductus arteriosus increased about 23 times (from $5.08\pm 2.03 \text{ mm}^3$ to $117.30\pm 8.50 \text{ mm}^3$), the length increased 3.1-fold and the diameter 2.6-fold, respectively. Therefore, increase in length and volume of DA with gestation, may affect the impedance of flows within DA.

Secondly, forward flow into the DA is due to the mass acceleration effect created by flow from the RV into the pulmonary artery leading to inertial effects and forward propulsion [376]. In the fetus, the RV has a greater wall stress, as per Laplace law, which predicts that the RV will have a higher resting wall stress due to its larger radius of curvature-to free wall thickness ratio [347]. The trans-luminal pressure varies inversely with vessel radius and is directly proportional to tension that develops in the vessel walls. In the normal fetus, RV stroke volume is larger than LV stroke volume and RV function curve is elevated [349]. RV chamber is larger than the LV chamber. With similar ejection fractions, the larger RV ejects 50% more blood with each beat. Transfer of flow through DA depends on asynchrony, which exists between ejections of two ventricles. Because of the asynchrony, RV ejects its column of blood before the LV, thereby propelling flow into DA before aortic pressure reaches its peak [375].

There is low ventricular compliance in the fetus due to a stiffer myocardium. In UPI with increasing severity of compromise, there is increased afterload, impaired stroke volume, probably leading to a right sided congestion and decreased cardiac contractility, which possibly leads to a reduction in systolic flow velocities and consequently reduction in pulsatility, in DA, reflected as a 'pseudonormalisation' of PI.

Shunting across the DA with advancing gestation is also probably dependant on autoregulation within DA. Autoregulation is the phenomenon where vascular resistance within the organ changes in response to the changes in organ-perfusion pressure, enabling the organ to maintain constant levels of flow. Myogenic response is a type of autoregulation, where the vascular pressure is regulated by intraluminal pressure. An increase in intraluminal pressure stimulates vasoconstriction of vessels and a decrease in pressure leads to relaxation. Myogenic response varies from organ to organ and has also been demonstrated in the ductus arteriosus in vivo [377]. At what gestation this response becomes functional, is not known. It is possible that in early gestation, there is functional immaturity; therefore myogenic response may not be so well developed. With advancing gestation, the fetus probably enters a higher level of maturation and redistribution mechanisms along with regional autoregulation become well-developed, therefore, influencing the flow velocities and DA pressures. This may contribute to a 'pseudonormalisation' of DA PI.

DA flows are also directly dependant on pulmonary artery pressures. PA pressure is increased in the presence of hypoxia, as a consequence to hypoxic pulmonary vasoconstriction.

Rasanen described an increase in the proportion of flows through the lungs, with advancing gestation [271]. This increase in pulmonary artery pressure, combined with an increasing afterload, probably leads to an increased proportion of flows through the DA directed towards the placenta. This may be associated with an increased pressure within DA and consequently lesser velocities and reduction of PI, clinically recognisable as 'pseudonormalisation'.

Another important contributor to flow alterations in DA is its sensitivity to

vasomediators such as oxygen tension, prostaglandins and nitric oxide [231, 378-381]. Experimental models have demonstrated that the patency and closure of DA can be influenced by altered expression of prostaglandin receptors PGE2, endothelin receptors ET and cyclooxygenase COX 1 and COX 2 receptors COX2 [382-384].

A previously published study by Mari and colleagues [234] suggested that there were no alterations in flow in DA in IUGR. Mari's study Doppler methodology was different from ours. One of the main differences in Mari's study was the use of Continuous Wave (CW Doppler), with the scan site of insonation being the sagittal plane in their study. Their definition of growth restriction in the fetus was also different from our definition, where they relied on menstrual age for gestational age estimation. Our study utilised pulsed Doppler for evaluation with scan planes either in transverse sections or oblique transverse sections to image the '3 vessel view'. Nevertheless, there were also some interesting similarities in the results from both studies.

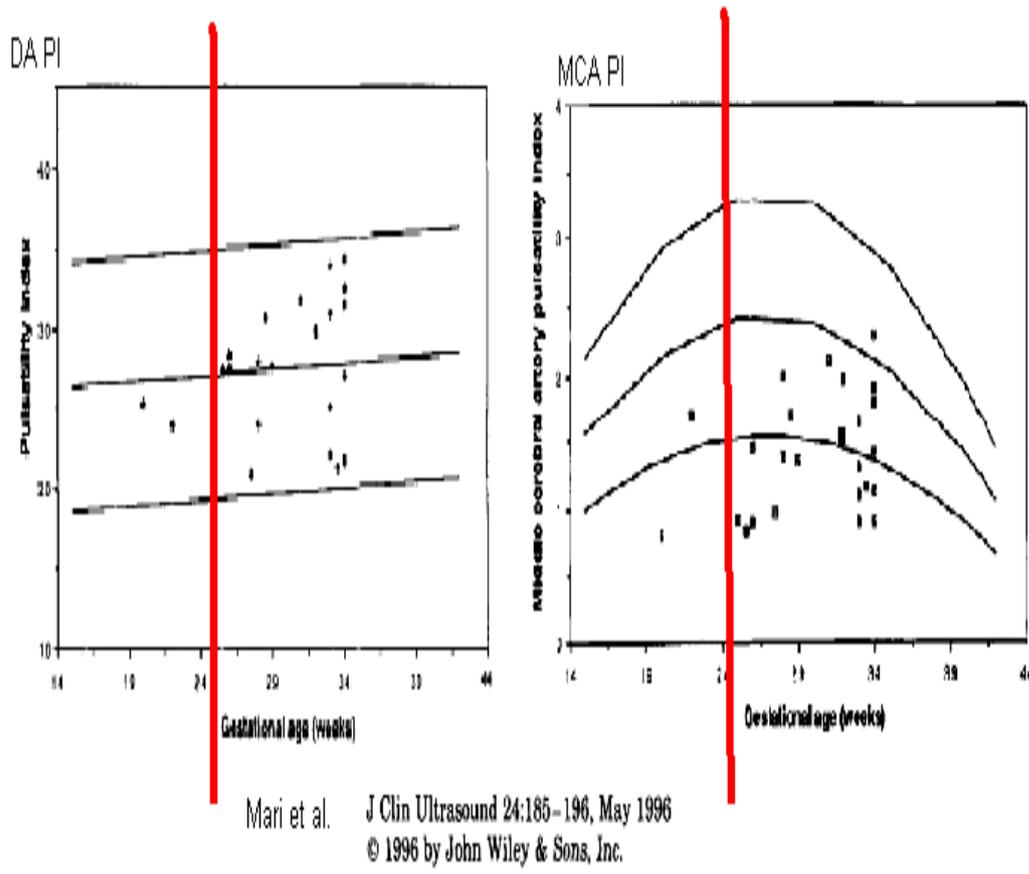


Figure 89 Results of ductus arteriosus PI in 25 SGA fetuses plotted on reference range: a study by Mari and colleagues. [234]

The PI of small for gestational age fetuses from their study demonstrates a ‘normal’ PI in DA PI in association with a reduction in MCA PI, when plotted on the reference ranges they designed in their study (Figure 89). In fact, they reported that the two fetuses with the ‘normal’ PI in DA, associated with a low MCA PI, actually died. We think that this finding is very significant, and in fact, paradoxically supports our own hypothesis of ‘pseudonormalisation’ in DA flows. Mari’s study did not evaluate DA flows in earlier stages of gestation. We interpret their data with normal PI in fetuses with brain sparing in later gestation as ‘pseudonormalisation’, in the light of our own findings. It is probable that the fetuses that died with ‘normal’ DA flows prior to their intra-uterine demise were actually in a severe stage of circulatory compromise. On the basis of findings detailed in this thesis one would have anticipated that had they been evaluated in an earlier stage of compromise in early gestation, an increase in DA PI would have been observed. With progressive severity of fetal compromise, in severe UPI, there is increasing redistribution in favour of cerebral flows. This may also lead to a reversal of flows through the aortic isthmus and may perhaps lead to an increased proportion of flow and pressure through the DA and be reflected as ‘pseudonormalisation’.

A complex, dynamic interplay of different factors involving alterations in the size of the DA, increased viscosity of blood in hypoxia, abnormal loading conditions of the heart, increasing RV shear stress, increasing PA pressure, along with increasing sensitivity to vasomediators, may all contribute towards a reduction in flow velocities and a pseudonormalisation of PI.

PATHOPHYSIOLOGY OF DUCTUS VENOSUS

The ductus venosus has been investigated extensively, and it is now established that increased shunting occurs through DV in fetal compromise[385].

The FO, IVC and DV have been considered to work as a single functional channel [386]. In our study, we did not have any fetus with absent or reversal of 'a' wave, and the decision to deliver was usually based on CTG abnormality. We also did not observe any significant changes in DV indices, at 16 weeks. The changes observed were in pulsatility index DVIV and DV Preload index, at a much later stage in gestation, after intracardiac shunting was seen.

Different mechanisms have been proposed for DV shunting, such as, high velocities owing to the trumpet shape, pressure gradient across liver-IVC, response to vasomediators, and sphincter action of DV valve. It is possible that DV size may be a better indicator of shunting, rather than the pulsatility indices [259, 387]. We did not evaluate DV size in our study, as diameter measurements are fraught with potential overestimation and therefore inaccuracies [256, 388]. However, it is possible that there is an active distension or dilatation of the DV enabling a sustained forward flow, thereby not having any effect on pulsatility indices in early stage of fetal compromise.

Further studies on correlation of DV size with FO flows would be needed, to ascertain whether active shunting is reflected initially in DV. If DV shunting is seen at a later stage, it would suggest a more advanced stage of compromise. On the other hand, if the DV diameter were to be increased before FO or DA flow patterns change, this could imply that liver shunting is actually a protective mechanism, rather than being detrimental to the liver metabolism, in early stages of redistribution and adaptation.

Clinical significance of the study

UPI is associated with adverse pregnancy outcomes such as preeclampsia, IUGR and preterm labour. Currently, in clinical practice, it is possible to identify a compromised fetus with the help of ultrasound and Doppler studies. However, there has been little definitive work that might identify a fetus in early stages of adaptation in fetal compromise.

With the help of data presented in this thesis, we have identified possible tools for fetal monitoring and surveillance, which might be useful in identifying a greater number of fetuses at an earlier gestation affected by UPI. Further prospective clinical studies of this surveillance strategy are warranted before these new strategies can be introduced in clinical care in high-risk pregnancies

Diagnosis of IUGR

We classified IUGR and/or SGA by three different methods, which were on the basis of fetal growth trajectory with ultrasound, birth weight centiles by Australian national centile Roberts and Lancaster charts [283] and customised birth centiles [284]. In comparison with Australian centiles, customised centile charts identified a larger proportion of IUGR fetuses, and also identified the constitutionally small fetuses that had normal outcomes. Currently, this method of identifying IUGR with customised centiles can be employed only after the baby is born; therefore, there is limited usefulness where antenatal management of the IUGR fetus is concerned.

However, our classification of IUGR into CSA (constitutionally small fetuses), mild, moderate and severe IUGR based on observation of fetal growth trajectory, correlated well with severity of adverse outcomes, so we recommend using this method for diagnosing and classifying severity of IUGR. We think that this has a better utility in clinical practice as the high-risk fetuses can be identified earlier and managed appropriately. On the other hand, unnecessary interventions based on a single observation of low estimated fetal weight alone, can be minimised.

Ultrasound parameters in IUGR

Fetal growth monitoring with the help of biometry is widely used in clinical practice. Other current existing methods of monitoring include umbilical artery Doppler, which have been shown to be useful in identification of the more severe adverse outcomes. Biophysical profile assessment has also been proved useful, in combination with cardiotocography (CTG), in identification of an adverse outcome. Venous Doppler, mainly ductus venosus, has been found useful in recognising deteriorating myocardial function. These tests, however, identify the compromised fetus in a later stage of compromise, where circulatory dynamics have already altered.

All these tests have enabled the clinician to try to determine the best time for delivery. However, no test yet exists, where it is possible to identify the high risk fetus, in very early stages of compromise, when the fetus is still able to adapt to the progressing UPI. In addition, there is no test to identify those pregnancies at risk for UPI, where pharmacological interventions, such as antiplatelet agents, maybe useful in minimising adverse pregnancy outcomes. Our study provides a new, potentially useful clinical tool, which might help identify these fetuses and may warrant further clinical investigation.

Central shunts and IUGR

The central shunts (Figure 90) particularly FO could be used in high risk mothers, to identify high risk fetuses and stratify care, as early as 16 weeks. In the unselected population, this could be incorporated in a morphology scan, to identify another proportion of high risk fetuses hitherto unidentified. Serial Doppler evaluation in pregnancy, with high PI in early pregnancy followed by a 'pseudonormalisation' could be ominous and suggest serious compromise and even decompensation and may be a marker as a clinical indication for delivery along with other surveillance methods.

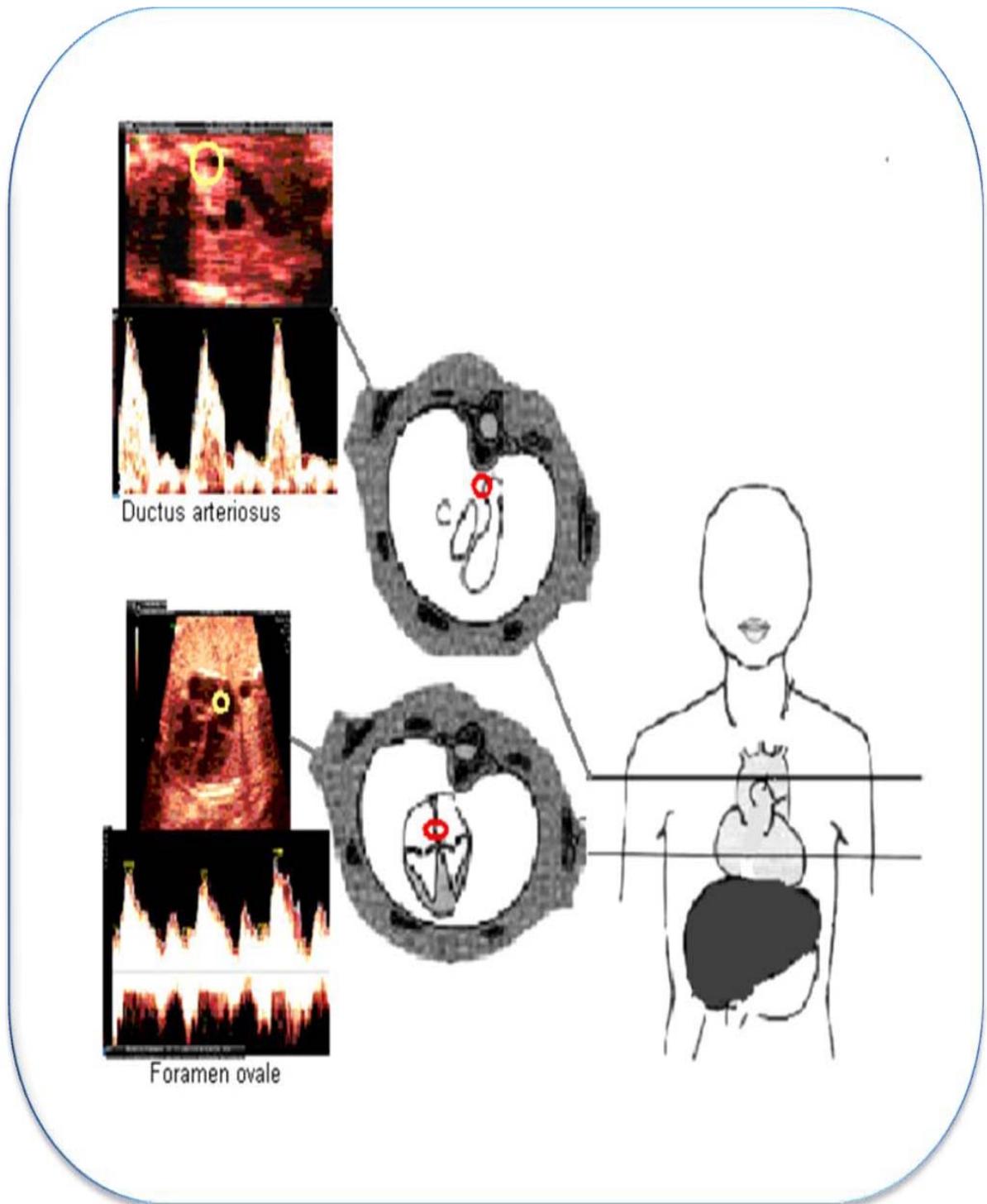


Figure 90 Doppler ultrasound of central fetal shunts

This figure demonstrates the anatomical location and Doppler flow waveforms of the central shunts foramen ovale and ductus arteriosus.

This figure has been adapted from Vinals et al.2002 [389]

Uteroplacental circulation

Uterine artery Doppler has already been found useful in predicting severe early-onset preeclampsia. However, preeclampsia is multifactorial, therefore a small proportion of preeclampsia may still manifest without any demonstrable changes in uterine artery Doppler, which was observed in our study, and is consistent with previously published studies. However, interestingly, the pregnancies which did manifest with adverse outcomes without any changes in uterine artery Doppler, did demonstrate the early changes in FO PI, thus suggesting that irrespective of the cause of UPI, fetal adaption mechanisms exist and can be identified early.

Thus, uterine artery Doppler can be used in combination with FO and DA Doppler, as suggested in the algorithm for surveillance, while acknowledging that FO and DA Doppler flow waveforms are much more sensitive.

Another interesting observation was the increase in umbilical artery PI in earlier gestation, around 16 weeks, where PI was at or above 95th centile. Usually, in clinical practice, umbilical artery RI or S/D ratio are used rather than PI, due to the obvious ease of measuring the indices offline. However, these indices were not so useful in detecting the high risk fetuses, in our study. A change over in practice to the use of umbilical artery PI instead of RI or S/D ratio may be more useful.

Placental thickness

FO PI and DA PI Doppler evaluation necessitates the use of high resolution, higher-end machines with sophisticated technology, which is more expensive. This may not always be feasible. In this scenario, the incorporation of placental thickness may identify a proportion of pregnancies with placental pathology at risk for adverse outcomes. Placental thickness was first described in 1985 [390], but was later abandoned because of the lack of standardisation of the technique. This study has defined strict scanning protocols and has also provided reference ranges, which could be useful in clinical practice.

Therefore, to summarise, this study has attempted to address the significant gaps in knowledge regarding identification and subsequent monitoring of the high-risk pregnancy and fetus. Figure 91 summarises the speculated sequence of changes in different parameters in UPI, useful in monitoring the high-risk fetus.

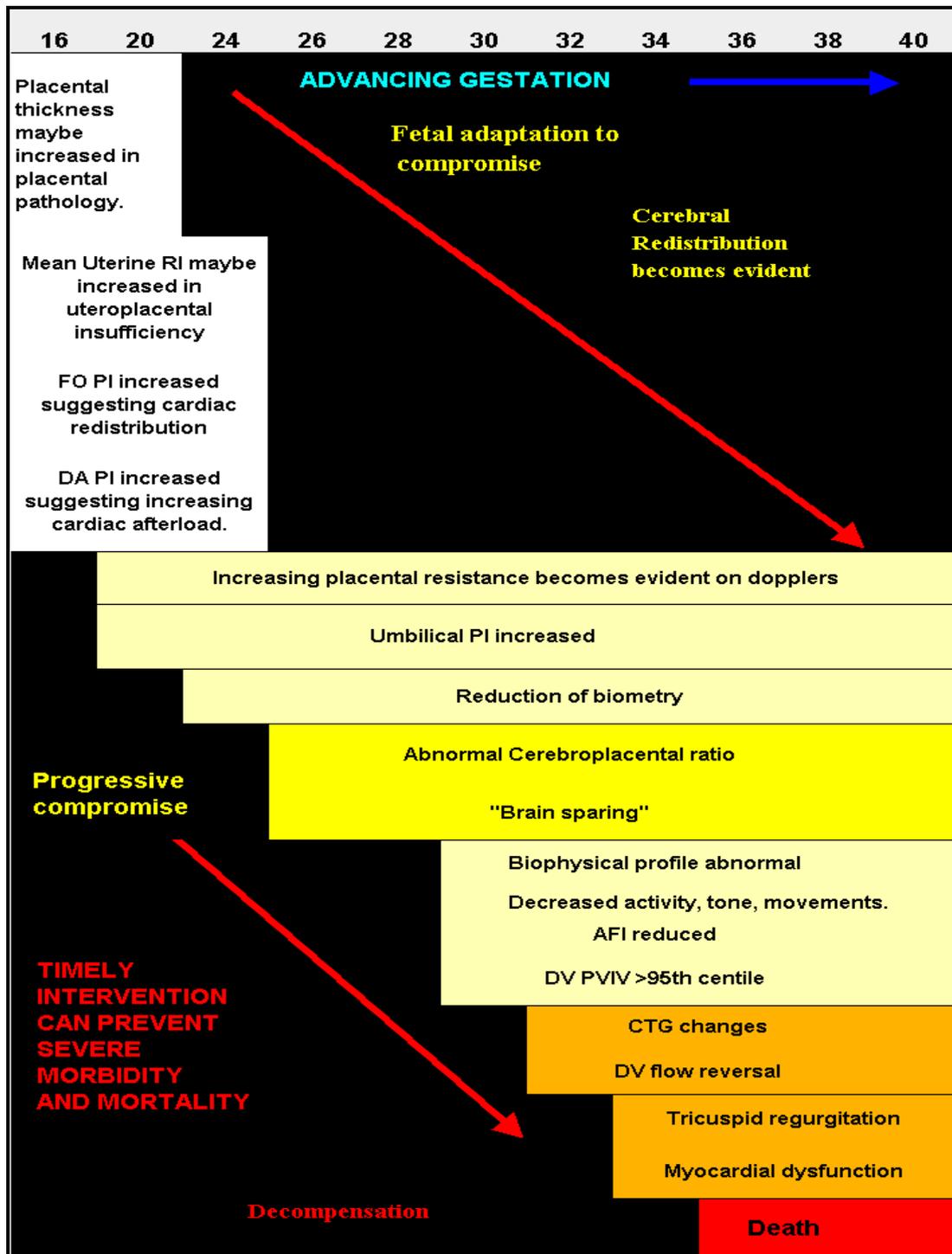


Figure 91 Speculated sequence of events in UPI leading to increasing severity of fetal compromise

This figure has been adapted from Baschat 2005.[160]

We would like to propose an algorithm, (Figure 92) where the new tools have been incorporated in intensive fetal surveillance. This algorithm could be a basis for designing randomised clinical trials or other prospective evaluations to test the usefulness of FO Doppler in early identification of the compromised fetus and in evaluating early pharmacological intervention or fetal monitoring regime.

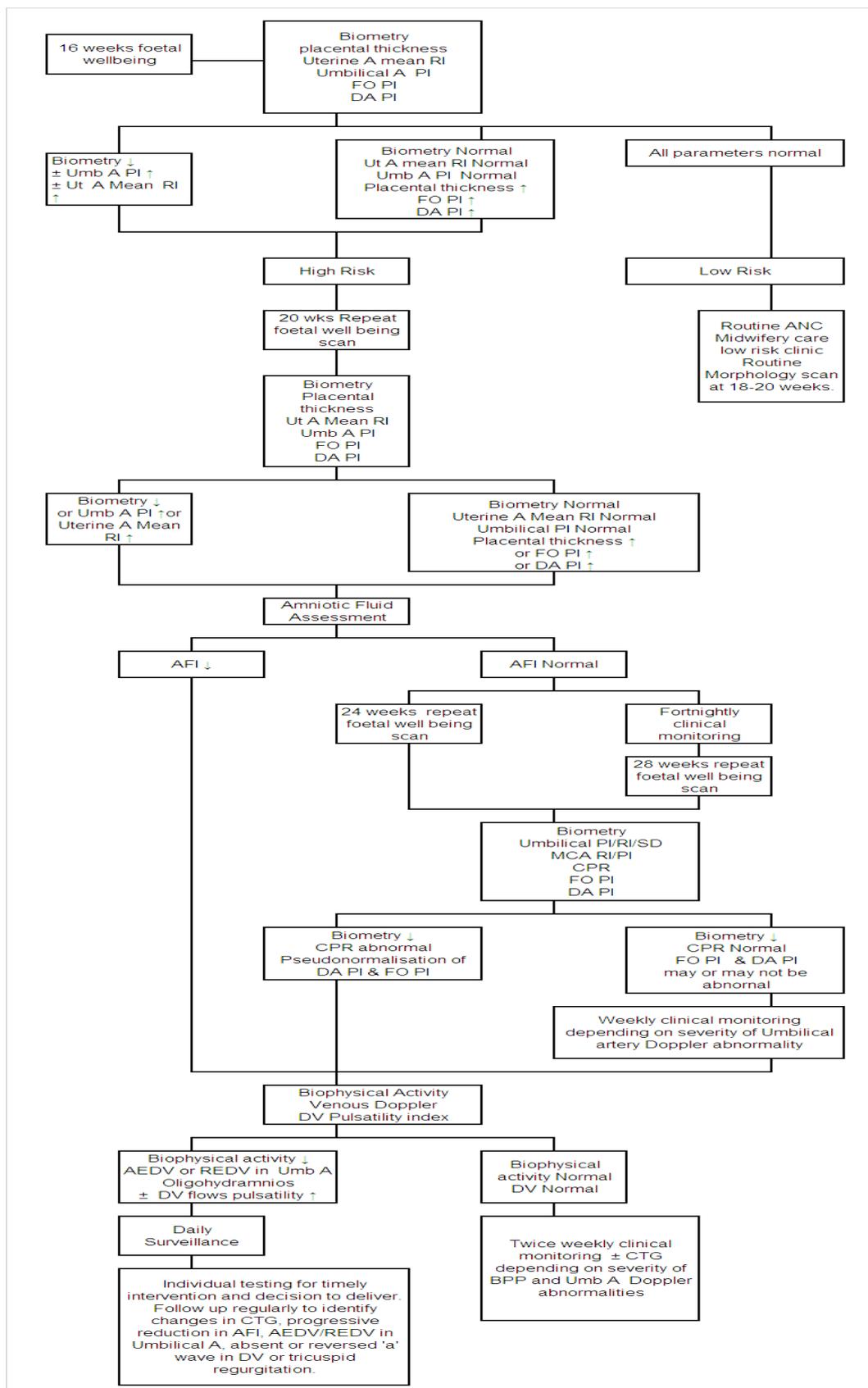


Figure 92 Proposed clinical monitoring algorithm for fetal monitoring for adaptation into clinical trials

“Adapt, Get out or Die” Hypothesis in adverse maternal-fetal outcomes

In this thesis, all the Doppler and ultrasound variables were examined by one experienced specialist examiner in an individual longitudinal sequence for each of the adverse outcomes associated with UPI. Thus, we performed longitudinal analysis on normal, mild IUGR, moderate IUGR, severe IUGR and constitutionally small fetuses with no obvious adverse outcomes. Preeclampsia was also examined under different categories, depending on the gestational age at onset of the disease. We also performed an analysis of preterm births, by categorising them into three separate groups; these categories included preterm associated with UPI, preterm with possible known causes and preterm birth with unexplained aetiology.

We found that the haemodynamic changes in UPI were more pronounced in those fetuses where compromise was less severe, thus enabling a continuation of pregnancy near term or even full-term. This seemed to suggest that a haemodynamic alteration seen at 16 weeks is an indicator of an adaptive mechanism. Preterm fetuses affected by UPI showed similar pattern as described above. However, when the fetuses with UPI were excluded from the analysis and preterm birth with unexplained pathology were examined, we found no differences in haemodynamics as compared to normal fetuses. The only significant difference observed was in uterine artery Doppler flows and birth weight. Fetuses with preterm birth due to unexplained pathology had significantly higher uterine artery RI and lower birth weights than normal fetuses at matched gestational age, although they were not growth restricted.

This suggests that either the fetuses have different adaptive mechanisms or the haemodynamic alteration is not possible, which is why they end up with preterm birth. Thus, a lack of adaptation here could paradoxically be a protective mechanism for the fetus.

This observation has led us to hypothesise that, to ensure a near-normal, full term gestation with less severe adverse outcome, an effective haemodynamic adaptive and redistributive mechanism has to be in place. If this adaptive mechanism fails or is not possible at all, the fetuses have to ‘get out’, i.e. trigger preterm birth to ensure survival, or teleologically, if the severity of compromise is undetected and no intervention occurs, they will die.

Strength and limitations

This thesis has provided evidence that early haemodynamic response mechanisms in fetal hypoxia associated with UPI can be detected noninvasively, with the help of Doppler ultrasound. This is the first large scale study that we are aware of, that simultaneously evaluates all the three intrauterine shunts and compare the flows with cerebral flows, uterine circulation and placental pathology, in high risk pregnancies and compares adverse outcomes with normal outcomes. This is also the first study to observe all these flows simultaneously, within a longitudinal prospective study.

Another strength was that all the observations were performed, on a high resolution machine by a single experienced and qualified examiner. The study included a large sample size and a consequent large number of observations, with rigorous scanning protocols. To improve generalisability, women from different economic, educational and socio-economic backgrounds were recruited.

One of the limitations of our study was that although the clinicians were not aware of the Doppler observations in ductus arteriosus, foramen ovale or in the uterine artery, they were not blinded to the reduction in fetal biometry, growth, amniotic fluid assessment, umbilical Doppler observations, and in case of an abnormality, ductus venosus Doppler observations as well, as this was clinically relevant information. This information about any abnormalities in biometry or umbilical artery/ ductus venosus Doppler observations, might have led to an earlier intervention, either by a change in management, or an earlier delivery, thereby affecting our outcomes. Perhaps as a consequence of this, the severe adverse outcomes were quite low in number. We had one fetal death, and 6 pregnancies out of 233, with severe adverse outcomes, necessitating delivery prior to 30 weeks. We speculate that the ‘flat line’ with ‘no apparent interval growth’ in fetal FL in severe IUGR fetuses (Figure 70) is a reflection of low numbers and must be therefore interpreted with caution.

Another limitation was that our recruitment window was from 16 to 20 weeks; the number of observations was lesser at 16 weeks of gestation in comparison to later gestational ages, as discussed in chapter 3 (Table 10). In addition, it is technically

more difficult to evaluate these shunts at an earlier gestation; therefore we potentially missed some observations at 16 weeks. However, we attempted to address this limitation by using linear mixed models for statistical analysis.

Our study also did not evaluate cardiac contractility and cardiac output or flows across pulmonary and aortic valves. We also did not get information about altered atrioventricular flow-pressure relationship in the fetus. Notwithstanding the above limitations, we have identified a potentially useful tool, which has possible implications for clinical practice, future research as well as clinical education.

Future research and practice

This thesis has provided novel evidence regarding fetal responses in early stages of fetal compromise. In this section, some of the questions left unanswered in this thesis will be briefly discussed and some suggestions for possible future research and clinical practice will be explored.

We have identified that Doppler PI of FO and possibly DA, can be a potentially useful tool for fetal investigation. This provides opportunities for early diagnosis in order to be able to optimise management of high-risk pregnancies. However, the validity and reliability of these parameters have not been investigated yet. Doppler FO PI and DA PI need to be further validated with inter-observer and intra-observer variability studies. Furthermore these novel Doppler flow indices also need to be tested in a tertiary setting before it can be used as a screening tool in an unselected population. This will necessitate further training of obstetric sonographers, sonologists and maternal- fetal medicine specialists. Further training will enhance the technical ease in performing these scans, which can have a significant impact on any application of this technique in clinical practice.

Findings from this thesis add to a significant body of knowledge regarding early redistribution mechanisms in the fetus, and can serve as a basis for designing several longitudinal prospective studies such as evaluation of DV size and FO Doppler, correlation of FO Doppler, FO size and aortic root in high risk versus controls,

correlation of SVC flows with DA flows, correlation of DA and FO flows with cardiac contractility and cardiac output. Randomised control trials for FO PI as a predictor of adverse pregnancy outcome, will determine the usefulness of this finding as a screening tool.

Newer ultrasound technologies such as 4D ultrasound, 4D volume flow assessment and spatiotemporal image correlation can be utilised along with Doppler techniques, to evaluate uteroplacental and fetal cardiac function. . Further studies could also be designed to identify biomarkers for adverse outcomes, using a combination of ultrasound parameters including FO, DA and uterine Doppler flow velocity waveforms along with serum markers, gene typing, genomics and metabolomics.

Conclusion

Based on a thorough examination of the literature, this is the first study to demonstrate evidence of cardiac flow alterations in earlier gestation, in the presence of uteroplacental insufficiency. Doppler indices DA PI and FO PI are increased as early as 16 weeks in IUGR and FO PI is increased in preeclampsia. Figure 91 summarises the sequence of events in uteroplacental insufficiency as observed in this study, in context with previously published literature.

Our study findings have opened up possible new strategies to identify the high-risk fetus early and stratify care. It has also produced further avenues to gain greater insights into fetal cardiovascular pathophysiology and fetal surveillance in high risk pregnancies complicated by uteroplacental insufficiency. These findings have the potential of being useful predictors and screening markers for preeclampsia and IUGR.

Barker and his colleagues proposed that the fetus possibly exhibits compensatory adaptive mechanisms in utero in the presence of a hostile intrauterine environment, which can then lead to cardiovascular disease in adulthood[391]. This thesis may provide further validation of this work as we have demonstrated early circulatory adaptation in the fetus before the disease becomes overtly manifest. Whether these

babies in whom an adaptive circulatory response has been documented also have altered epigenetics and gene expression, needs to be further explored.

Clearly, fetal responses to hypoxia are quite varied, exceedingly complex and thus consequentially long term. Identification of the fetus at risk for demise and neurological injury from hypoxia still remains a challenge. However, our research suggests that a greater portion of these fetuses may be identified earlier by incorporating methods of evaluation of the organ that drives the flow to all organs-the the heart, by adopting a monitoring protocol which includes evaluation of pathophysiology of fetal central shunts. Indeed, the famous ancient philosophy of “Follow your heart and go with the flow” may be an important survival response. Currently we have the advantage of evaluating these fetal responses to cardiovascular stress with Doppler technology.

Contents of the enclosed CD ROM: APPENDIX

All supplementary material for this thesis is stored in the enclosed CD ROM.

Supplementary data has been presented to support the results presented in this thesis, for the reader. These results have been presented in the excel format (EXCEL XP). These excel worksheets include detailed results obtained after analysis for every ultrasound and Doppler variable for each adverse pregnancy outcome as well as adverse placental outcome as defined in chapter 3.

Chapter 6: Supplementary data Worksheet 1

S File 1: File name: SUPPLEMENTARY DATA FOR CHAPTER 6.xls

This file contains 8 worksheets with EMMEANS as discussed in chapter 3. These worksheets have been named as follows:

- Preterm means
- UPI means
- Severe preeclampsia means
- Preeclampsia means
- IUGR Ultrasound means
- IUGR Australian centile means
- IUGR customised centile means
- IUGR severity means

Chapter 7: Supplementary data Worksheet 2

S File 2: File name: SUPPLEMENTARY DATA FOR CHAPTER 7.xls

This file contains 5 worksheets with EMMEANS as discussed in chapter 3. These files have been named as follows:

- All placental outcomes
- Placental pathology means
- Fetoplacental pathology means
- Uteroplacental pathology means
- 'Any' placental pathology means

BIBLIOGRAPHY

1. Laws, P., Abeywardana, S., Walker, J., Sullivan, E.A., *Australia's mothers and babies 2005. Perinatal Statistics Series no. 20.* . Cat. no. PER 40. AIHW National Perinatal Statistics Unit. Sydney., 2007.
2. *Infant wellbeing at 2 years of age in the Growth Restriction Intervention Trial (GRIT): multicentred randomised controlled trial.* The Lancet, 2004. **364**(9433): p. 513-520.
3. *TRUFFLE study(Trial of umbilical and fetal flow in Europe (TRUFFLE): a multicentre randomised study)* <https://trufflestudy.org/truffle/index.htm> Accessed on 9th Feb 2009.
4. NICE, U., *CG62: Clinical guidelines: Antenatal care: Routine care for the healthy pregnant woman.* 2003.
5. RCOG, *Clinical Green Top Guidelines: The Investigation and Management of the Small-for-Gestational-Age Fetus (31).* 2002.
6. Neilson, J.P., *Symphysis-fundal height measurement in pregnancy.* . Cochrane Database of Systematic Reviews, Issue 1. Art. No.: CD000944. DOI: 10.1002/14651858.CD000944, 1998.
7. NICE, U., *National Institute for Clinical Excellence. Updated guidelines. Antenatal care. CG62. Routine care for the healthy pregnant woman.* . . 2008.
8. Mangesi L., H.G.J., *Fetal movement counting for assessment of fetal wellbeing.* Cochrane Database of Systematic Reviews, Issue 1. Art. No.: CD004909. DOI: 10.1002/14651858.CD004909.pub2, 2007.
9. Bricker L., N.J.P., *Routine ultrasound in late pregnancy (after 24 weeks' gestation).* . Cochrane Database of Systematic Reviews, Issue 1. Art. No.: CD001451. DOI: 10.1002/14651858.CD001451.pub2, 2000.
10. Neilson, J.P., *Ultrasound for fetal assessment in early pregnancy.* Cochrane Database of Systematic Reviews, Issue 2. Art. No.: CD000182. DOI: 10.1002/14651858.CD000182, 2001.
11. Statement., R., *C- Obs 3. Antenatal screening tests.* . RANZCOG publications. College Statements., 2006.
12. Lalor J.G, F.B., Alfirevic Z., Devane D., *Biophysical profile for fetal assessment in high risk pregnancies.* Cochrane Database of Systematic

- Reviews, Issue 4. Art. No.: CD000038. DOI: 10.1002/14651858.CD000038.pub2, 2007.
13. Neilson, J.P., Alfirevic Z., *Doppler ultrasound for fetal assessment in high risk pregnancies*. Cochrane Database of Systematic Reviews: Issue 4 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD000073, 1996.
 14. Bricker L., N.J.P., *Routine Doppler ultrasound in pregnancy*. Cochrane Database of Systematic Reviews , Issue 2. Art. No.: CD001450. DOI: 10.1002/14651858.CD001450.pub2, 2000.
 15. Tan K.H., S.A., *Fetal vibroacoustic stimulation for facilitation of tests of fetal wellbeing*. Cochrane Database of Systematic Reviews , Issue 4. Art. No.: CD003396. DOI: 10.1002/14651858.CD003396, 2007.
 16. RANZCOG, *C Obs-17. Intrapartum fetal surveillance clinical guidelines. Second edition*. RANZCOG publications. College Guidelines., 2006.
 17. Alfirevic Z., D.D., Gyte G. M. L., *Continuous cardiotocography (CTG) as a form of electronic fetal monitoring (EFM) for fetal assessment during labour*. Cochrane Database of Systematic Reviews, Issue 3 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD006066, 2006.
 18. Neilson, J.P., *Fetal electrocardiogram (ECG) for fetal monitoring during labour*. Cochrane Database of Systematic Reviews: Reviews Issue 3, John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD000116.pub2, 2006.
 19. Pattison N., M.L., *Cardiotocography for antepartum fetal assessment*. Cochrane Database of Systematic Reviews, Issue 1 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD001068, 1999.
 20. East C.E., C.F.Y., Colditz P.B., Begg L., *Fetal pulse oximetry for fetal assessment in labour*. Cochrane Database of Systematic Reviews, Issue 2 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD004075.pub3, 2007.
 21. ACOG., *Committee Opinion no. 258. Fetal pulse oximetry*. Obstet Gynecol, 2001. **98**: p. 523-524.
 22. RCOG, *The use of electronic fetal monitoring. The use and interpretation of cardiotocography in intrapartum fetal surveillance. Evidence based clinical guideline no 8. . RCOG press., 2001.*

23. SOGC, *Fetal Health Surveillance: Antepartum and Intrapartum Consensus Guideline*. Clinical Practice Guidelines. Journal of Obstetrics and Gynaecology Canada., 2007.
24. Westerhuis M EMH, K.G.M., E v Beek, S M Bijvoet, A P Drogdrop, H P van Geijn, J MM van Lith, B WJ Mol, J G Nijhuis, S G Oei, M M Porath, R JP Rijnders, N WE Schuitemaker, I van der Tweel, G HA Visser, C Willekes, and A Kwee, *A randomised clinical trial on cardiotocography plus fetal blood sampling versus cardiotocography plus ST-analysis of the fetal electrocardiogram (STAN®) for intrapartum monitoring*. BMC Pregnancy Childbirth. , 2007. .
25. East C.E., L., L. R., Colditz P. B., Henshall N. E., *Intrapartum fetal scalp lactate sampling for fetal assessment in the presence of a non-reassuring fetal heart rate trace*. Cochrane Database of Systematic Reviews Protocols , Issue 4 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD006174, 2006.
26. Mozurkewich E., W.F.M., *Near-infrared spectroscopy for fetal assessment during labour*. Cochrane Database of Systematic Reviews: Reviews, Issue 3 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD002254, 2000.
27. Davis, C.H., *Weight in pregnancy; its value as a routine test*. Am. J. Obstet Gynecol 1923. **6**: p. 575-581.
28. Vonnahme, K.A., et al., *Maternal Undernutrition from Early- to Mid-Gestation Leads to Growth Retardation, Cardiac Ventricular Hypertrophy, and Increased Liver Weight in the Fetal Sheep*. Biol Reprod %R 10.1095/biolreprod.102.012120, 2003. **69**(1): p. 133-140.
29. Ford, S.P., et al., *Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring*. J. Anim Sci. %R 10.2527/jas.2005-624, 2007. **85**(5): p. 1285-1294.
30. MacLaughlin, S.M., Walker S.K., Roberts C.T., Kleemann D.O. and McMillen IC, *Periconceptional nutrition and the relationship between maternal body weight changes in the periconceptional period and fetoplacental growth in the sheep*. J Physiol, 2005. **565 (Pt 1)** (111–124).

31. Dawes, M.G., Grudzinskas J.G, *Repeated measurement of maternal weight during pregnancy. Is this a useful practice?* . Br J Obstet Gynaecol 1991. **98**: p. 189-94.
32. Neilson, J.P., *Symphysis-fundal height measurement in pregnancy.* . Cochrane Database of Systematic Reviews, Issue 1. Art. No.: CD000944. DOI: 10.1002/14651858.CD000944, 2000.
33. Gardosi, J. and A. Francis, *Controlled trial of fundal height measurement plotted on customised antenatal growth charts.* Br J Obstet Gynaecol, 1999. **106**(4): p. 309-17.
34. Christensen, F.C., Rayburn W. F., *Fetal movement counts.* . Obstetrics and gynecology clinics of North America 1999. **26**(4): p. 607-621.
35. Baillie, C., Mason G., Hewison J. , *Scanning for pleasure.* BJOG: An International Journal of Obstetrics & Gynaecology, 1997. **104**(11): p. 1223-1224.
36. Reading, A., Cox D.N., Campbell S., *A controlled, prospective evaluation of the acceptability of ultrasound in prenatal care.* . J Psychosom Obstet Gynaecol 1988. **8**(3): p. 191-198.
37. Saari-Kemppainen A, K.O., Ylöstalo P, Heinonen OP, *Ultrasound screening and perinatal mortality: controlled trial of systematic one-stage screening in pregnancy. The Helsinki Ultrasound Trial.* Lancet, 1990. **18**(336 (8712)): p. 387-391.
38. Bakketeig LS, J.G., Brodtkorb CJ, Eriksen BC, Eik-Nes SH, Ulstein MK, et al., *Randomised controlled trial of ultrasonographic screening in pregnancy.* Lancet, 1984. **2**: p. 207-210.
39. S Eik-Nes. et al. *Routine ultrasound fetal examination in pregnancy: the Ålesund randomized controlled trial.* Ultrasound in Obstetrics and Gynecology, 2000. **15**(6): p. 473-478.
40. Waldenström U, A.O., Nilsson S, Eklund G, Fall O, Lindeberg S, Sjödin Y., *Effects of routine one-stage ultrasound screening in pregnancy: a randomised controlled trial.* Lancet, 1988. **2**(8611): p. 585-8.
41. Ewigman, B.G., et al., *Effect of prenatal ultrasound screening on perinatal outcome. RADIUS Study Group.* N Engl J Med, 1993. **329**(12): p. 821-7.

42. Bennet et al. *Predictive value of ultrasound measurement in early pregnancy: a randomized controlled trial*. BJOG: An International Journal of Obstetrics & Gynaecology, 1982. **89**(5): p. 338-341.
43. ASUM, *Policies and statements. D2: Guidelines For The Mid Trimester Obstetric Scan*. ASUM Policies and Statements. Australasian Society for Ultrasound in Medicine. Reaffirmed May 1996, Revised October 1999, July 2005, 1991.
44. ASUM, *D7: Statement On Normal Ultrasonic Fetal Measurements*. ASUM Policies and Statements. Australasian Society for Ultrasound in Medicine. Reaffirmed May 1996, Revised May 2001, 1991.
45. RCOG, *Ultrasound Screening. Supplement to Ultrasound Screening for Fetal Abnormalities. Report of the RCOG Working Party* RCOG press, London, 2000.
46. RANZCOG., *C- Obs 3. Antenatal screening tests*. . RANZCOG publications. College Statements., 2006.
47. Bucher, H.C., Schmidt J.G., *Does routine ultrasound scanning improve outcome in pregnancy? Meta-analysis of various outcome measures*. BMJ, 1993(307): p. 13-17.
48. Dogra, V.S., *Intrauterine Growth Retardation*. EMedicine , WebMD, 2006.
49. Manning, F.A., *Fetal biophysical profile*. Obstet Gynecol Clin North Am, 1999. **26**(4): p. 557-77.
50. Vintzileos, A.M.G., S.E.; Salinger, LM; Kontopoulos, VG; Campbell WA; Nochimson DJ, *The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores*. Am J Obstet Gynecol, 1987. **157**(3): p. 627-31.
51. Palomaki, O., et al., *Intrapartum cardiotocography -- the dilemma of interpretational variation*. J Perinat Med, 2006. **34**(4): p. 298-302.
52. Drogtrop, A.P., R. Ubels, and J.G. Nijhuis, *The association between fetal body movements, eye movements and heart rate patterns in pregnancies between 25 and 30 weeks of gestation*. Early Hum Dev, 1990. **23**(1): p. 67-73.
53. Doppler-e.html, *Christian Andreas Doppler. The Doppler effect as the universal key to movements in space*. <http://www.surveyor.in-berlin.de/himmel/Bios/Doppler-e.html>, c.-.-f.s.h. . Editor. 2006.

54. Divanovic, E. and E.J. Buchmann, *Routine heart and lung auscultation in prenatal care*. Int J Gynaecol Obstet, 1999. **64**(3): p. 247-51.
55. Garcia, J., Corry, M, MacDonald, D, Elbourne, D, Grant A., *Mothers' views of continuous electronic fetal heart monitoring and intermittent auscultation in a randomized controlled trial*. Birth., 1985. **12**: p. 79-86.
56. Steer, P.J., *Has electronic fetal heart rate monitoring made a difference*. Semin Fetal Neonatal Med, 2008. **13**(1): p. 2-7.
57. Devane, D., Lalor, J. G., Daly, S., McGuire, W., *Cardiotocography versus intermittent auscultation of fetal heart on admission to labour ward for assessment of fetal wellbeing*. Cochrane Database of Systematic Reviews Protocols, Issue 1 John Wiley & Sons, Ltd Chichester, UK DOI: 10.1002/14651858.CD005122, 2005.
58. McNamara, H.M. and G.A. Dildy, 3rd, *Continuous intrapartum pH, pO₂, pCO₂, and SpO₂ monitoring*. Obstet Gynecol Clin North Am, 1999. **26**(4): p. 671-93.
59. Engidawork, E., et al., *Effect of perinatal asphyxia on systemic and intracerebral pH and glycolysis metabolism in the rat*. Exp Neurol, 1997. **145**(2 Pt 1): p. 390-6.
60. Allen, R.M., F.G. Bowling, and J.J. Oats, *Determining the fetal scalp lactate level that indicates the need for intervention in labour*. Aust N Z J Obstet Gynaecol, 2004. **44**(6): p. 549-52.
61. Wiberg-Itzel, E., Lipponer, C., Norman, M. et al, *Determination of pH or lactate in fetal scalp blood in management of intrapartum fetal distress: randomised controlled multicentre trial*. BMJ, 2008. ;**336**(7656): p. 1284-7
62. East, C.E., Smyth, R., Leader, L.R., Henshall, N.E., Colditz, P.B., Tan, K.H., *Vibroacoustic stimulation for fetal assessment in labour in the presence of a nonreassuring fetal heart rate trace*. . Cochrane Database of Systematic Reviews 2005, Issue 2. Art. No.: CD004664. DOI: 10.1002/14651858.CD004664.pub2, 2005.
63. Hamilton, R.J., et al., *Intrapartum fetal cerebral near infrared spectroscopy: apparent change in oxygenation demonstrated in a non viable fetus*. Br J Obstet Gynaecol, 1995. **102**(12): p. 1004-7.

64. Adamson, S.L., K.J. Whiteley, and B.L. Langille, *Pulsatile pressure-flow relations and pulse-wave propagation in the umbilical circulation of fetal sheep*. *Circ Res*, 1992. **70**(4): p. 761-772.
65. Adamson, S.L., *Arterial pressure, vascular input impedance, and resistance as determinants of pulsatile blood flow in the umbilical artery*. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 1999. **84**(2): p. 119-125.
66. Barclay, A.E., Franklin, K. J., & Pritchard, M. M. L. , ed. *The Fetal Circulation and Cardiovascular System, and the Changes that They Undergo at Birth*. . ed. O. Blackwell. 1944.
67. Rudolph, A.M et al. *The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output, and organ blood flow*. . *Circ Res* 1967(21): p. 163-184.
68. Dawes, G.S., J.C. Mott, and J.G. Widdicombe, *The circulation of blood in the fetal lamb*. *J Physiol*, 1954. **126**(2): p. 38P.
69. Dawes, G.S., J.C. Mott, and J.G. Widdicombe, *The fetal circulation in the lamb*. *J Physiol*, 1954. **126**(3): p. 563-87.
70. Kiserud, T. and G. Acharya, *The fetal circulation*. *Prenat Diagn*, 2004. **24**(13): p. 1049-59.
71. Kiserud, T., *Physiology of the fetal circulation*. *Semin Fetal Neonatal Med*, 2005. **10**(6): p. 493-503.
72. FitzGerald, D.E., Drumm, J.E., *Non-invasive measurement of human fetal circulation using ultrasound: a new method*. *BMJ*, 1977. **3**(2(6100)): p. 1450-1.
73. Trudinger, B.J., et al., *Umbilical artery flow velocity waveforms in high-risk pregnancy. Randomised controlled trial*. *Lancet*, 1987. **1**(8526): p. 188-90.
74. Karsdorp, V.H., et al., *Clinical significance of absent or reversed end diastolic velocity waveforms in umbilical artery*. *Lancet*, 1994. **344**(8938): p. 1664-8.
75. Yoon, B.H., et al., *Is an abnormal Doppler umbilical artery waveform ratio a risk factor for poor perinatal outcome in the non-small for gestational age fetus?* *Am J Perinatol*, 1993. **10**(3): p. 245-9.
76. Newnham, J.P., et al., *An evaluation of the efficacy of Doppler flow velocity waveform analysis as a screening test in pregnancy*. *Am J Obstet Gynecol*, 1990. **162**(2): p. 403-10.

77. Vergani, P., et al., *Doppler predictors of adverse neonatal outcome in the growth restricted fetus at 34 weeks' gestation or beyond*. Am J Obstet Gynecol, 2003. **189**(4): p. 1007-11.
78. Alfirevic, Z.a.N., J.P., *Doppler ultrasonography in high risk pregnancies. Systematic review with meta-analysis*. Am J Obstet Gynaecol 1995(172): p. 1379-97.
79. Giles, W.B., B.J. Trudinger, and P.J. Baird, *Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation*. Br J Obstet Gynaecol, 1985. **92**(1): p. 31-8.
80. Brosens, I., H.G. Dixon, and W.B. Robertson, *Fetal growth retardation and the arteries of the placental bed*. Br J Obstet Gynaecol, 1977. **84**(9): p. 656-63.
81. Thompson, R.S. and B.J. Trudinger, *Doppler waveform pulsatility index and resistance, pressure and flow in the umbilical placental circulation: an investigation using a mathematical model*. Ultrasound Med Biol, 1990. **16**(5): p. 449-58.
82. Brosens, I.A., *Morphological changes in the utero-placental bed in pregnancy hypertension*. Clin Obstet Gynaecol, 1977. **4**(3): p. 573-93.
83. Assali, N.S., et al., *Measurement of uterine blood flow and uterine metabolism. IV. Results in normal pregnancy*. Am J Obstet Gynecol, 1953. **66**(2): p. 248-53.
84. Ueland, K. and J. Metcalfe, *Circulatory changes in pregnancy*. Clin Obstet Gynecol, 1975. **18**(3): p. 41-50.
85. Iwata, M., et al., *Prenatal detection of ischemic changes in the placenta of the growth-retarded fetus by Doppler flow velocimetry of the maternal uterine artery*. Obstet Gynecol, 1993. **82**(4 Pt 1): p. 494-9.
86. Moffet, A., Lake, C., *Immunology of placentation in eutherian mammals*. Nature. Immunology Reviews., 2006. **6**: p. 584-94.
87. McCowan, L.M., et al., *Uterine artery flow velocity waveforms in normal and growth-retarded pregnancies*. Am J Obstet Gynecol, 1988. **158**(3 Pt 1): p. 499-504.
88. Chien, P.F., et al., *How useful is uterine artery Doppler flow velocimetry in the prediction of pre-eclampsia, intrauterine growth retardation and perinatal death? An overview*. Bjog, 2000. **107**(2): p. 196-208.

89. Papageorghiou, A.T. and N. Roberts, *Uterine artery Doppler screening for adverse pregnancy outcome*. Curr Opin Obstet Gynecol, 2005. **17**(6): p. 584-90.
90. Papageorghiou, A.T. and K. Leslie, *Uterine artery Doppler in the prediction of adverse pregnancy outcome*. Curr Opin Obstet Gynecol, 2007. **19**(2): p. 103-9.
91. Gagnon R, V.D.H.M., *The use of Fetal Doppler in Obstetrics: Clinical Practice Guidelines*. Journal of Obstetrics and Gynecology Canada, 2005. **25**(7): p. 601-7.
92. Figueras, F., Fernandez S., Eixarch, E., Gomez, O., . Martinez, J.M, Puerto B., Gratacos, E., *Middle cerebral artery pulsatility index: reliability at different sampling sites*. Ultrasound in Obstetrics and Gynecology, 2006. **28**(6): p. 809-813.
93. Mari, G. and R.L. Deter, *Middle cerebral artery flow velocity waveforms in normal and small-for-gestational-age fetuses*. Am J Obstet Gynecol, 1992. **166**(4): p. 1262-70.
94. Veille, J.C., R. Hanson, and K. Tatum, *Longitudinal quantitation of middle cerebral artery blood flow in normal human fetuses*. Am J Obstet Gynecol, 1993. **169**(6): p. 1393-8.
95. Al-Ghazali, W., et al., *Evidence of redistribution of cardiac output in asymmetrical growth retardation*. Br J Obstet Gynaecol, 1989. **96**(6): p. 697-704.
96. Sepulveda, W., et al., *Discordant blood flow velocity waveforms in left and right brachial arteries in growth-retarded fetuses*. Obstet Gynecol, 1995. **86**(5): p. 734-8.
97. Sepulveda, W., A.H. Shennan, and M.J. Peek, *Reverse end-diastolic flow in the middle cerebral artery: an agonal pattern in the human fetus*. Am J Obstet Gynecol, 1996. **174**(5): p. 1645-7.
98. Alatas, C., et al., *Prediction of perinatal outcome by middle cerebral artery Doppler velocimetry*. Arch Gynecol Obstet, 1996. **258**(3): p. 141-6.
99. Habek, D., et al., *Doppler cerebro-umbilical ratio and fetal biophysical profile in the assessment of peripartal cardiotocography in growth-retarded fetuses*. Fetal Diagn Ther, 2007. **22**(6): p. 452-6.

100. Signorelli, M., F. Taddei, and T. Frusca, *Reversal of compensatory flow in severe intrauterine growth restriction: middle cerebral artery and intracardiac volume flow modifications*. *Minerva Ginecol*, 2008. **60**(4): p. 287-93.
101. Respondek, M., et al., *Reversal of diastolic flow in the middle cerebral artery of the fetus during the second half of pregnancy*. *Ultrasound Obstet Gynecol*, 1997. **9**(5): p. 324-9.
102. Weiner, Z., et al., *Central and peripheral hemodynamic changes in fetuses with absent end-diastolic velocity in umbilical artery: correlation with computerized fetal heart rate pattern*. *Am J Obstet Gynecol*, 1994. **170**(2): p. 509-15.
103. Dubiel, M., et al., *Middle cerebral artery velocimetry as a predictor of hypoxemia in fetuses with increased resistance to blood flow in the umbilical artery*. *Early Hum Dev*, 1997. **47**(2): p. 177-84.
104. Mari, G., et al., *Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses*. *N Engl J Med*, 2000. **342**(1): p. 9-14.
105. Mari, G., et al., *Gestational Age at Delivery and Doppler Waveforms in Very Preterm Intrauterine Growth-Restricted Fetuses as Predictors of Perinatal Mortality*. *J Ultrasound Med*, 2007. **26**(5): p. 555-559.
106. Mari, G., et al., *Middle cerebral artery peak systolic velocity: a new Doppler parameter in the assessment of growth-restricted fetuses*. *Ultrasound Obstet Gynecol*, 2007. **29**(3): p. 310-6.
107. Forouzan, I. and Z.Y. Tian, *Fetal middle cerebral artery blood flow velocities in pregnancies complicated by intrauterine growth restriction and extreme abnormality in umbilical artery Doppler velocity*. *Am J Perinatol*, 1996. **13**(3): p. 139-42.
108. Van den Wijngaard, J.A.G.W., et al., *Cerebral Doppler ultrasound of the human fetus*. *British Journal of Obstetrics and Gynaecology*, 1989. **96**(7): p. 845-849.
109. Uerpaiojkit, B., et al., *Cerebellar Doppler velocimetry in the appropriate- and small-for-gestational-age fetus*. *Obstetrics and Gynecology*, 1996. **87**(6): p. 989-993.

110. H. Figueroa-Diesel, E.H.-A.R.A.-R.L.C.E.G., *Doppler changes in the main fetal brain arteries at different stages of hemodynamic adaptation in severe intrauterine growth restriction*. *Ultrasound in Obstetrics and Gynecology*, 2007. **30**(3): p. 297-302.
111. Baschat, A.A., et al., *Antenatal prediction of intraventricular hemorrhage in fetal growth restriction: what is the role of Doppler?* *Ultrasound Obstet Gynecol*, 2002. **19**(4): p. 334-9.
112. Scherjon, S.A., et al., *Neurodevelopmental outcome at three years of age after fetal 'brain-sparing'*. *Early Hum Dev*, 1998. **52**(1): p. 67-79.
113. Kirimi, E., et al., *Clinical value of color Doppler ultrasonography measurements of full-term newborns with perinatal asphyxia and hypoxic ischemic encephalopathy in the first 12 hours of life and long-term prognosis*. *Tohoku J Exp Med*, 2002. **197**(1): p. 27-33.
114. Gramellini, D., Folli, M.C., Raboni, S., Vadora, E., Merialdi, A, *Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome*. *Obstet Gynecol*, 1992. **79**: p. 416-20.
115. Akalin-Sel, T., Campbell, S., *Understanding the pathophysiology of intra-uterine growth retardation: the role of the 'lower limb reflex' in redistribution of blood flow*. *Eur J Obstet Gynecol Reprod Biol.*, 1992. **46**(2-3): p. 79-96.
116. Wladimiroff, J.W., et al., *Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies*. *Obstet Gynecol*, 1987. **69**(5): p. 705-9.
117. Arbeille, P., et al., *Assessment of the fetal P2 changes by cerebral and umbilical Doppler on lamb fetuses during acute hypoxia*. *Ultrasound in Medicine & Biology*, 1995. **21**(7): p. 861-870.
118. Harrington, K., et al., *Doppler fetal circulation in pregnancies complicated by pre-eclampsia or delivery of a small for gestational age baby: 2. Longitudinal analysis*
doi:10.1111/j.1471-0528.1999.tb08299.x. *BJOG: An International Journal of Obstetrics and Gynaecology*, 1999. **106**(5): p. 453-466.
119. Hershkovitz, R., et al., *Fetal cerebral blood flow redistribution in late gestation: identification of compromise in small fetuses with normal umbilical artery Doppler*. *Ultrasound Obstet Gynecol*, 2000. **15**(3): p. 209-12.

120. Jain, M., T. Farooq, and R.C. Shukla, *Doppler cerebroplacental ratio for the prediction of adverse perinatal outcome*. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, 2004. **86**(3): p. 384-385.
121. Odibo, A.O., et al., *Cerebroplacental Doppler Ratio and Adverse Perinatal Outcomes in Intrauterine Growth Restriction: Evaluating the Impact of Using Gestational Age-Specific Reference Values*. J Ultrasound Med, 2005. **24**(9): p. 1223-1228.
122. Kutschera, J., et al., *Absent or reversed end-diastolic blood flow in the umbilical artery and abnormal Doppler cerebroplacental ratio--cognitive, neurological and somatic development at 3 to 6 years*. Early Hum Dev, 2002. **69**(1-2): p. 47-56.
123. Groenenberg, I.A., J.W. Wladimiroff, and W.C. Hop, *Fetal cardiac and peripheral arterial flow velocity waveforms in intrauterine growth retardation*. Circulation, 1989. **80**(6): p. 1711-7.
124. Stigter, R.H., et al., *Doppler studies on the fetal renal artery in the severely growth-restricted fetus*. Ultrasound Obstet Gynecol, 2001. **18**(2): p. 141-5.
125. Tekay, A. and P. Jouppila, *Fetal adrenal artery velocimetry measurements in appropriate-for-gestational age and intrauterine growth-restricted fetuses*. Ultrasound Obstet Gynecol, 2000. **16**(5): p. 419-24.
126. Korszun, P., et al., *Fetal superior mesenteric artery blood flow velocimetry in normal and high-risk pregnancy*. J Perinat Med, 2002. **30**(3): p. 235-41.
127. Maruyama, K. and T. Koizumi, *Superior mesenteric artery blood flow velocity in small for gestational age infants of very low birth weight during the early neonatal period*. J Perinat Med, 2001. **29**(1): p. 64-70.
128. Ebbing, C., et al., *Hepatic Artery Hemodynamics Suggest Operation of a Buffer Response in the Human Fetus*. Reproductive Sciences, 2008. **15**(2): p. 166-178.
129. Morales, R., and J. Diaz, G.D., *Study of fetal femoral and umbilical artery blood flow by Doppler ultrasound throughout pregnancy*. Archives of Gynecology and Obstetrics, 1999. **262**(3): p. 127-131.
130. Mari, G., *Arterial blood flow velocity waveforms of the pelvis and lower extremities in normal and growth-retarded fetuses*. Am J Obstet Gynecol, 1991. **165**(1): p. 143-51.

131. Baschat, A.A., *Relationship between placental blood flow resistance and precordial venous Doppler indices*. *Ultrasound Obstet Gynecol*, 2003. **22**(6): p. 561-6.
132. Baschat, A.A., et al., *Doppler and biophysical assessment in growth restricted fetuses: distribution of test results*. *Ultrasound Obstet Gynecol*, 2006. **27**(1): p. 41-7.
133. Hofstaetter, C., S. Gudmundsson, and M. Hansmann, *Venous Doppler velocimetry in the surveillance of severely compromised fetuses*. *Ultrasound Obstet Gynecol*, 2002. **20**(3): p. 233-9.
134. Rizzo, G., et al., *Doppler indices from inferior vena cava and ductus venosus in predicting pH and oxygen tension in umbilical blood at cordocentesis in growth-retarded fetuses*. *Ultrasound Obstet Gynecol*, 1996. **7**(6): p. 401-10.
135. Hecher, K., et al., *Fetal venous, intracardiac, and arterial blood flow measurements in intrauterine growth retardation: relationship with fetal blood gases*. *Am J Obstet Gynecol*, 1995. **173**(1): p. 10-5.
136. Rizzo, G., et al., *The value of fetal arterial, cardiac and venous flows in predicting pH and blood gases measured in umbilical blood at cordocentesis in growth retarded fetuses*. *Br J Obstet Gynaecol*, 1995. **102**(12): p. 963-9.
137. Ferrazzi, E., et al., *Temporal sequence of abnormal Doppler changes in the peripheral and central circulatory systems of the severely growth-restricted fetus*. *Ultrasound Obstet Gynecol*, 2002. **19**(2): p. 140-6.
138. Ozcan, T., et al., *Arterial and venous Doppler velocimetry in the severely growth-restricted fetus and associations with adverse perinatal outcome*. *Ultrasound Obstet Gynecol*, 1998. **12**(1): p. 39-44.
139. Fouron, J.C., et al., *Changes in flow velocity patterns of the superior and inferior venae cavae during placental circulatory insufficiency*. *Ultrasound Obstet Gynecol*, 2003. **21**(1): p. 53-6.
140. Gudmundsson, S., et al., *Venous Doppler velocimetry in relationship to central venous pressure and heart rate during hypoxia in the ovine fetus*. *J Perinat Med*, 1999. **27**(2): p. 81-90.
141. Thornton, J.G., *When to deliver the small, for gestational age, fetus?* *International Congress Series*, 2005. **1279**: p. 321-331.
142. Rizzo, G. and D. Arduini, *Fetal cardiac function in intrauterine growth retardation*. *Am J Obstet Gynecol*, 1991. **165**(4 Pt 1): p. 876-82.

143. Respondek, M.L., et al., *The prevalence and clinical significance of fetal tricuspid valve regurgitation with normal heart anatomy*. Am J Obstet Gynecol, 1994. **171**(5): p. 1265-70.
144. Chaoui, R., *The fetal 'heart-sparing effect' detected by the assessment of coronary blood flow: a further ominous sign of fetal compromise*. Ultrasound Obstet Gynecol, 1996. **7**(1): p. 5-9.
145. Mäkikallio, K., *Is it time to add aortic isthmus evaluation to the repertoire of Doppler investigations for placental insufficiency?* Ultrasound in Obstetrics and Gynecology, 2008. **31**(1): p. 6-9.
146. M. Del Río, J.M.M., F. Figueras, M. Bennasar, A. Olivella, M. Palacio, O. Coll, B. Puerto, E. Gratacós., *Doppler assessment of the aortic isthmus and perinatal outcome in preterm fetuses with severe intrauterine growth restriction*. Ultrasound in Obstetrics and Gynecology, 2008. **31**(1): p. 41-47.
147. Staboulidou, I., et al., *The significance of intracardiac Doppler sonography in terms of fetal growth retardation*. Arch Gynecol Obstet, 2007. **276**(1): p. 35-42.
148. Gardiner, H., et al., *Volume blood flow estimation in the normal and growth-restricted fetus*. Ultrasound Med Biol, 2002. **28**(9): p. 1107-13.
149. Gembruch, U. and J.M. Smrcek, *The prevalence and clinical significance of tricuspid valve regurgitation in normally grown fetuses and those with intrauterine growth retardation*. Ultrasound Obstet Gynecol, 1997. **9**(6): p. 374-82.
150. Reed, K.L., C.F. Anderson, and L. Shenker, *Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow*. Am J Obstet Gynecol, 1987. **157**(3): p. 774-9.
151. Chang, T.C., et al., *Prediction of the small for gestational age infant: which ultrasonic measurement is best?* Obstet Gynecol, 1992. **80**(6): p. 1030-8.
152. de Jong, C.L., et al., *Fetal growth rate and adverse perinatal events*. Ultrasound Obstet Gynecol, 1999. **13**(2): p. 86-9.
153. Chang, T.C., et al., *Identification of fetal growth retardation: comparison of Doppler waveform indices and serial ultrasound measurements of abdominal circumference and fetal weight*. Obstet Gynecol, 1993. **82**(2): p. 230-6.

154. Baschat, A.A., U. Gembruch, and C.R. Harman, *The sequence of changes in Doppler and biophysical parameters as severe fetal growth restriction worsens*. *Ultrasound Obstet Gynecol*, 2001. **18**(6): p. 571-7.
155. Senat, M.V., et al., *Longitudinal changes in the ductus venosus, cerebral transverse sinus and cardiotocogram in fetal growth restriction*. *Ultrasound Obstet Gynecol*, 2000. **16**(1): p. 19-24.
156. Makikallio, K., et al., *Ultrasonographic and biochemical markers of human fetal cardiac dysfunction in placental insufficiency*. *Circulation*, 2002. **105**(17): p. 2058-63.
157. Hecher, K., et al., *Monitoring of fetuses with intrauterine growth restriction: a longitudinal study*
doi:10.1046/j.0960-7692.2001.00590.x. *Ultrasound in Obstetrics and Gynecology*, 2001. **18**(6): p. 564-570.
158. Figueras, F., et al., *Cardiac function monitoring of fetuses with growth restriction*. *Eur J Obstet Gynecol Reprod Biol*, 2003. **110**(2): p. 159-63.
159. Soothill, P.W., et al., *Prediction of morbidity in small and normally grown fetuses by fetal heart rate variability, biophysical profile score and umbilical artery Doppler studies*. *Br J Obstet Gynaecol*, 1993. **100**(8): p. 742-5.
160. Baschat, A.A., *Arterial and venous Doppler in the diagnosis and management of early onset fetal growth restriction*. *Early Hum Dev*, 2005. **81**(11): p. 877-87.
161. AIUM., *Bioeffects and safety of diagnostic ultrasound*. Laurel, MD. *American Institute of Ultrasound in Medicine in AIUM Publications*. 1993.
162. AIUM, *Medical Ultrasound Safety. Part 3. Implementing the ALARA (as low as reasonably possible) principle*. ISBN 1-930047-71-1, ed. O.S.A. publications. 1994; Reapproved 2002.
163. *Safety statement, 2000. International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)*. *Ultrasound Obstet Gynecol*, 2000. **16**(6): p. 594-6.
164. Abramowicz, J.S., et al., *Safety Statement, 2000 (reconfirmed 2003). International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)*. *Ultrasound Obstet Gynecol*, 2003. **21**(1): p. 100.
165. AIUM/NEMA, *Acoustic output measurement standard for diagnostic ultrasound equipment*. Laurel, MD. *American Institute of Ultrasound in*

- Medicine/National Electrical Manufacturers Association*. . AIUM Publications. 1999.
166. BMUS, S., Statement . *Clinical Safety Statement for Diagnostic Ultrasound*, British Medical Ultrasound Society. BMUS publications. 2000.
 167. Barnett, S.B., et al., *International recommendations and guidelines for the safe use of diagnostic ultrasound in medicine*. *Ultrasound Med Biol*, 2000. **26**(3): p. 355-66.
 168. Newnham JP, E.S., Michael CA, Stanley FJ, Landau LI., *Effects of ultrasound during pregnancy: a randomised controlled trial*. *Lancet*, 1993. **342**: p. 887-91.
 169. Newnham, J.P., et al., *Effects of repeated prenatal ultrasound examinations on childhood outcome up to 8 years of age: follow-up of a randomised controlled trial*. *Lancet*, 2004. **364**(9450): p. 2038-44.
 170. Salvesen, K.A. and S.H. Eik-Nes, *Ultrasound during pregnancy and subsequent childhood non-right handedness: a meta-analysis*. *Ultrasound Obstet Gynecol*, 1999. **13**(4): p. 241-6.
 171. Geschwind, N., Galaburda, A.M. , *Cerebral lateralization: biological mechanisms, associations and pathology. A hypothesis and a program for research*. *Arch Neurol*, 1985. **42**: p. 428-59.
 172. Salvesen, K.Å., *Ultrasound and left-handedness: a sinister association?* *Ultrasound in Obstetrics and Gynecology*, 2002. **19**(3): p. 217-221.
 173. Bly Stephen, V.d.H.M., *Obstetrics ultrasound biological effects and safety: SOGC Clinical practice guidelines*. *Journal of Obstetrics and Gynaecology Canada*., 2005. **27**(6): p. 572-5.
 174. ASUM, AI. *Statement On The Safety Of Ultrasound in Grey Scale Imaging In Obstetrics*. ASUM Policies and Statements. Australasian Society for Ultrasound in Medicine., 1998. Reaffirmed 2000.
 175. TOP, G., *Guideline for Ultrasound as a Part of Routine Prenatal Care. Summary of the Alberta Clinical Practice Guideline*.
Clinical Practice Guidelines Toward Optimized Practice. Alberta, 2008.
 176. ACOG., ed. *Routine Ultrasound in Low-Risk Pregnancy, ACOG Practice Patterns: Evidence-Based Guidelines for Clinical Issues. No. 5*. Obstetrics and

- Gynecology 1997: American College of Obstetrics and Gynaecology. Washington, D.C. .
177. Dunn, P.M., *Aristotle (384-322 BC): philosopher and scientist of ancient Greece*. Arch Dis Child Fetal Neonatal Ed, 2006. **91**(1): p. F75-7.
 178. Dunn, P.M., *Andreas Vesalius (1514-1564), Padua, and the fetal "shunts"*. Arch Dis Child Fetal Neonatal Ed, 2003. **88**(2): p. F157-9.
 179. Khan, I.A., S.K. Daya, and R.M. Gowda, *Evolution of the theory of circulation*. Int J Cardiol, 2005. **98**(3): p. 519-21.
 180. Kiserud, T., et al., *Ultrasonographic velocimetry of the fetal ductus venosus*. Lancet, 1991. **338**(8780): p. 1412-4.
 181. Stuart, W.M., *James Jeffray: Observations on the heart and on the peculiarities of the fetus*. Clinical Anatomy, 1999. **12**(1): p. 35-42.
 182. Kiserud, T., *Hemodynamics of the ductus venosus*. Eur J Obstet Gynecol Reprod Biol, 1999. **84**(2): p. 139-47.
 183. Dunn, P.M., *Dr William Hunter (1718-83) and the gravid uterus*. Arch. Dis. Child. Fetal Neonatal Ed., 1999. **80**(1): p. F76-77.
 184. McCulloch, N.A., D. Russell, and S.W. McDonald, *William Hunter's Gravid Uterus: the specimens and plates*. Clin Anat, 2002. **15**(4): p. 253-62.
 185. Amoroso, E.C., et al., *The bifurcation of the posterior caval channel in the eutherian fetal heart*. J Anat, 1942. **76**(Pt 3): p. 240-247.
 186. Michaëlsson, M., Ho, S.Y. , ed. *Chapter 1. Introduction: Normal hearts-a comparison*. Congenital heart malformations in mammals- an illustrated text. available at http://www.worldscibooks.com/medsci/etextbook/p136/p136_chap1.pdf. 2000, Copyright © 2008 World Scientific Publishing Co. .
 187. Perry, S.F., Wilson, R.J.A., Straus, C., Harris, M.B., Remmers, J.E., *Which came first, the lung or the breath?* Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 2001. **129**(1): p. 37-47.
 188. Taylor, E.W., D. Jordan, and J.H. Coote, *Central Control of the Cardiovascular and Respiratory Systems and Their Interactions in Vertebrates*. Physiol. Rev., 1999. **79**(3): p. 855-916.
 189. Axelsson, M., Franklin, C. E., Lofman, C.O., Nilsson, S., Grigg, G.C. , *Dynamic Anatomical Study Of Cardiac Shunting In Crocodiles Using High-*

- Resolution Angioscopy* Journal of Experimental Biology 1999. **199**(2): p. 359-365.
190. Kardong, K.V., ed. *Vertebrates: Comparative Anatomy, Function, Evolution. Third Edition.* . ed. M.-H. pages. 2006.
 191. Bergwerff, M., M.C. DeRuiter, and A.C. Gittenberger-de Groot, *Comparative anatomy and ontogeny of the ductus arteriosus, a vascular outsider.* Anat Embryol (Berl), 1999. **200**(6): p. 559-71.
 192. Anderson, D.F., et al., *Central shunt flows and pressures in the mature fetal lamb.* Am J Physiol, 1981. **241**(1): p. H60-6.
 193. Rudolph, A.M. and M.A. Heymann, *The fetal circulation.* Annu Rev Med, 1968. **19**: p. 195-206.
 194. Rudolph, A.M. and M.A. Heymann, *Circulatory changes during growth in the fetal lamb.* Circ Res, 1970. **26**(3): p. 289-99.
 195. Rudolph, A.M., *Distribution and regulation of blood flow in the fetal and neonatal lamb.* Circ Res, 1985. **57**(6): p. 811-21.
 196. Reed, K.L., *Doppler--the fetal circulation.* Clin Obstet Gynecol, 1997. **40**(4): p. 750-4.
 197. Sansoucie, D.A. and T.A. Cavaliere, *Transition from fetal to extrauterine circulation.* Neonatal Netw, 1997. **16**(2): p. 5-12.
 198. Aaronson PI, R.T., Knock GA, Becker S, Lewis TH, Snetkov V, Ward JP., *Hypoxic pulmonary vasoconstriction: mechanisms and controversies.* J Physiol, 2006. **570**(Pt 1): p. 53-8. Epub 2005.
 199. Hong, Y., *Pulmonary venous flow from fetal to neonatal period.* Early Hum Dev. , 2000. **57**(2): p. 95-103.
 200. Atkins, D.L., Clark, E.B., Marvin, W.J. Jr., *Foramen ovale/atrial septum area ratio: a marker of transatrial blood flow.* Circulation, 1982. **66**(2): p. 281-3.
 201. Feit, L.R., Copel, J. A., Kleinman, C. S., *Foramen ovale size in the normal and abnormal human fetal heart: an indicator of transatrial flow physiology.* Ultrasound in Obstetrics and Gynecology, 1991. **1**(5): p. 313-319.
 202. Berning, R.A., et al., *Reversed shunting across the ductus arteriosus or atrial septum in utero heralds severe congenital heart disease.* J Am Coll Cardiol, 1996. **27**(2): p. 481-6.
 203. Gray, H., *Anatomy of the Human Body.* 1918, Philadelphia: Lea & Febiger.

204. Benson, C.B., et al., *Increasing curvature of the normal fetal ductus arteriosus with advancing gestational age*. *Ultrasound Obstet Gynecol*, 1995. **5**(2): p. 95-7.
205. Castillo, E.H., et al., *Morphometric study of the human fetal heart. I. Arterial segment*. *Clin Anat*, 2005. **18**(4): p. 260-8.
206. Szyszka-Mroz, J. and W. Wozniak, *A histological study of human ductus arteriosus during the last embryonic week*. *Folia Morphol (Warsz)*, 2003. **62**(4): p. 365-7.
207. Hyett, J., G. Moscoso, and K. Nicolaides, *Morphometric analysis of the great vessels in early fetal life*. *Hum Reprod*, 1995. **10**(11): p. 3045-8.
208. Giuriato, L., et al., *Rabbit ductus arteriosus during development: anatomical structure and smooth muscle cell composition*. *Anat Rec*, 1993. **235**(1): p. 95-110.
209. Kajino, H., et al., *Vasa vasorum hypoperfusion is responsible for medial hypoxia and anatomic remodeling in the newborn lamb ductus arteriosus*. *Pediatr Res*, 2002. **51**(2): p. 228-35.
210. Rabinovitch, M., *Cell-extracellular matrix interactions in the ductus arteriosus and perinatal pulmonary circulation*. *Semin Perinatol*, 1996. **20**(6): p. 531-41.
211. Hinek, A., et al., *Impaired elastin fiber assembly related to reduced 67-kD elastin-binding protein in fetal lamb ductus arteriosus and in cultured aortic smooth muscle cells treated with chondroitin sulfate*. *J Clin Invest*, 1991. **88**(6): p. 2083-94.
212. Boyd, D.P., *The nerve supply of the mammalian ductus arteriosus*. *J Anat*, 1941. **75**(Pt 4): p. 457-468.3.
213. Aronson, S., et al., *Innervation and contractile response of the human ductus arteriosus*. *Eur J Pharmacol*, 1970. **11**(2): p. 178-86.
214. Reese, J., Anderson, J.D., Brown, N., Roman, C., Clyman, R.I., *Inhibition of cyclooxygenase isoforms in late- but not midgestation decreases contractility of the ductus arteriosus and prevents postnatal closure in mice*. *Am J Physiol Regul Integr Comp Physiol*, 2006. **91**(6): p. R1717-1723. Epub 2006.
215. Reese, J., *Death, dying, and exhaustion in the ductus arteriosus: prerequisites for permanent closure*. *Am J Physiol Regul Integr Comp Physiol*, 2006. **290**(2): p. R357-8.

216. Lind, J., *Eleventh Edgar Mannheimer Lecture. Human fetal and neonatal circulation. Some structural and functional aspects.* Eur J Cardiol, 1977. **5**(3): p. 265-81.
217. Smith, G.C., et al., *Effect of gestational age, corticosteroids, and birth on expression of prostanoid EP receptor genes in lamb and baboon ductus arteriosus.* J Cardiovasc Pharmacol, 2001. **37**(6): p. 697-704.
218. Smith, G.C.S., *The Pharmacology of the Ductus Arteriosus.* Pharmacol Rev, 1998. **50**(1): p. 35-58.
219. Coceani, F., P.M. Olley, and E. Bodach, *Prostaglandins: a possible regulator of muscle tone in the ductus arteriosus.* Adv Prostaglandin Thromboxane Res, 1976. **1**: p. 417-24.
220. Agren, P., et al., *Ontogeny of chicken ductus arteriosus response to oxygen and vasoconstrictors* 10.1152/ajpregu.00204.2006. Am J Physiol Regul Integr Comp Physiol, 2007. **292**(1): p. R485-496.
221. Smith, G.C. and J.C. McGrath, *Characterisation of the effect of oxygen tension on response of fetal rabbit ductus arteriosus to vasodilators.* Cardiovasc Res, 1993. **27**(12): p. 2205-11.
222. Fay, F.S., P. Nair, and W.J. Whalen, *Mechanism of oxygen induced contraction of ductus arteriosus.* Adv Exp Med Biol, 1977. **78**: p. 123-34.
223. Fay, F.S., *Guinea pig ductus arteriosus. I. Cellular and metabolic basis for oxygen sensitivity.* Am J Physiol, 1971. **221**(2): p. 470-9.
224. Coceani, F., et al., *Cytochrome P 450-linked monooxygenase: involvement in the lamb ductus arteriosus.* Am J Physiol, 1984. **246**(4 Pt 2): p. H640-3.
225. Levin, M., et al., *Postnatal constriction, ATP depletion, and cell death in the mature and immature ductus arteriosus.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(2): p. R359-64.
226. Hong, Z., et al., *Role of Store-Operated Calcium Channels and Calcium Sensitization in Normoxic Contraction of the Ductus Arteriosus* 10.1161/CIRCULATIONAHA.106.641126. Circulation, 2006. **114**(13): p. 1372-1379.
227. Michelakis, E.D., et al., *O₂ sensing in the human ductus arteriosus: regulation of voltage-gated K⁺ channels in smooth muscle cells by a mitochondrial redox sensor.* Circ Res, 2002. **91**(6): p. 478-86.

228. Archer, S.L., et al., *O₂ sensing in the human ductus arteriosus: redox-sensitive K⁺ channels are regulated by mitochondria-derived hydrogen peroxide*. Biol Chem, 2004. **385**(3-4): p. 205-16.
229. Clyman, R.I., et al., *VEGF regulates remodeling during permanent anatomic closure of the ductus arteriosus*. Am J Physiol Regul Integr Comp Physiol, 2002. **282**(1): p. R199-206.
230. Ovadia, B., et al., *Nitric oxide-endothelin-1 interactions after acute ductal constriction in fetal lambs*. Am J Physiol Heart Circ Physiol, 2002. **282**(3): p. H862-71.
231. Momma, K. and M. Toyono, *The role of nitric oxide in dilating the fetal ductus arteriosus in rats*. Pediatr Res, 1999. **46**(3): p. 311-5.
232. Thornburg, K.L., *Fetal response to intrauterine stress*. Ciba Found Symp, 1991. **156**: p. 17-29; discussion 29-37.
233. Rychik, J., *Frontiers in fetal cardiovascular disease*. Pediatr Clin North Am, 2004. **51**(6): p. 1489-502, vii.
234. Mari, G., R.L. Deter, and B. Uerpaiojkit, *Flow velocity waveforms of the ductus arteriosus in appropriate and small-for-gestational-age fetuses*. J Clin Ultrasound, 1996. **24**(4): p. 185-96.
235. Huhta, J.C., et al., *Detection and quantitation of constriction of the fetal ductus arteriosus by Doppler echocardiography*. Circulation, 1987. **75**(2): p. 406-12.
236. T. Kiserud, S.R., *Ultrasound assessment of the fetal foramen ovale*. Ultrasound in Obstetrics and Gynecology, 2001. **17**(2): p. 119-124.
237. Edelstone, D.I. and A.M. Rudolph, *Preferential streaming of ductus venosus blood to the brain and heart in fetal lambs*. Am J Physiol, 1979. **237**(6): p. H724-9.
238. Drighi, I.A., El Mosalami H., Elbadaoui N., Chraibi S., Bennis A., *Patent foramen ovale: A new disease?*
. International Journal of Cardiology., 2007. **122**(1): p. 1-9.
239. Wilson, A.D., P.S. Rao, and S. Aeschlimann, *Normal fetal foramen flap and transatrial Doppler velocity pattern*. J Am Soc Echocardiogr, 1990. **3**(6): p. 491-4.

240. Kiserud T., E.-N.S.H., Blaas, H-G, Hellevik, L.R. , *Foramen ovale: an ultrasonographic study of its relation to the inferior vena cava, ductus venosus and hepatic veins*. *Ultrasound Obstet Gynecol* 1992. **2**: p. 389-96.
241. van Eyck, J., P.A. Stewart, and J.W. Wladimiroff, *Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy*. *Am J Obstet Gynecol*, 1990. **163**(4 Pt 1): p. 1239-42.
242. Kiserud, T., G. Chedid, and S. Rasmussen, *Foramen ovale changes in growth-restricted fetuses*. *Ultrasound Obstet Gynecol*, 2004. **24**(2): p. 141-6.
243. Rudolph, A.M., *Hepatic and ductus venosus blood flows during fetal life*. *Hepatology*, 1983. **3**(2): p. 254-8.
244. Mavrides, E., et al., *The human ductus venosus between 13 and 17 weeks of gestation: histological and morphometric studies*. *Ultrasound Obstet Gynecol*, 2002. **19**(1): p. 39-46.
245. Cocceani, F., et al., *Autonomic mechanisms in the ductus venosus of the lamb*. *Am J Physiol*, 1984. **247**(1 Pt 2): p. H17-24.
246. Adeagbo, A.S., L. Kelsey, and F. Cocceani, *Endothelin-induced constriction of the ductus venosus in fetal sheep: developmental aspects and possible interaction with vasodilatory prostaglandin*. *Br J Pharmacol*, 2004. **142**(4): p. 727-36.
247. Ailamazyan, E.K., et al., *Functional morphology of ductus venosus in human fetus*. *Neuro Endocrinol Lett*, 2003. **24**(1-2): p. 28-32.
248. Tchirikov, M., et al., *Structural evidence for mechanisms to redistribute hepatic and ductus venosus blood flows in nonhuman primate fetuses*. *Am J Obstet Gynecol*, 2005. **192**(4): p. 1146-52.
249. Adeagbo, A.S., et al., *Evidence for a role of prostaglandin I₂ and thromboxane A₂ in the ductus venosus of the lamb*. *Can J Physiol Pharmacol*, 1985. **63**(9): p. 1101-5.
250. Adeagbo, A.S., et al., *Lamb ductus venosus: evidence of a cytochrome P-450 mechanism in its contractile tension*. *J Pharmacol Exp Ther*, 1990. **252**(2): p. 875-9.
251. Richardson, B., et al., *Regional blood flow and the endocrine response to sustained hypoxemia in the preterm ovine fetus*. *Pediatr Res*, 1996. **40**(2): p. 337-43.

252. Paton, J.B. and D.E. Fisher, *Organ blood flows of fetal and infant baboons*. Early Hum Dev, 1984. **10**(1-2): p. 137-47.
253. Boito, S., et al., *Fetal brain/liver volume ratio and umbilical volume flow parameters relative to normal and abnormal human development*. Ultrasound Obstet Gynecol, 2003. **21**(3): p. 256-61.
254. Schmidt, K.G., N.H. Silverman, and A.M. Rudolph, *Assessment of flow events at the ductus venosus-inferior vena cava junction and at the foramen ovale in fetal sheep by use of multimodal ultrasound*. Circulation, 1996. **93**(4): p. 826-33.
255. Kiserud, T., S. Rasmussen, and S. Skulstad, *Blood flow and the degree of shunting through the ductus venosus in the human fetus*. Am J Obstet Gynecol, 2000. **182**(1 Pt 1): p. 147-53.
256. Kiserud, T., et al., *Ductus venosus shunting in growth-restricted fetuses and the effect of umbilical circulatory compromise*. Ultrasound Obstet Gynecol, 2006. **28**(2): p. 143-9.
257. Edelstone, D.I., A.M. Rudolph, and M.A. Heymann, *Effects of hypoxemia and decreasing umbilical flow liver and ductus venosus blood flows in fetal lambs*. Am J Physiol, 1980. **238**(5): p. H656-63.
258. Meyers, R.L., et al., *Cardiovascular responses to acute, severe haemorrhage in fetal sheep*. J Dev Physiol, 1991. **15**(4): p. 189-97.
259. Bellotti, M., et al., *Dilatation of the ductus venosus in human fetuses: ultrasonographic evidence and mathematical modeling*. Am J Physiol, 1998. **275**(5 Pt 2): p. H1759-67.
260. Pennati, G., et al., *Blood flow through the ductus venosus in human fetus: calculation using Doppler velocimetry and computational findings*. Ultrasound Med Biol, 1998. **24**(4): p. 477-87.
261. Tchirikov, M., *Dilation of the ductus venosus by stent implantation increases placental blood perfusion in fetal sheep*. Am J Obstet Gynecol, 2008. **198**(1): p. 138 e1-6.
262. Klabunde, R.E., ed. *Cardiovascular Physiology Concepts. Abbreviated content available at <http://www.cvphysiology.com/>*. 2005, Lippincott Williams & Wilkins. ISBN: 078175030X.
263. Norton, J.M., *REPLY*. Advan. Physiol. Edu., 2003. **27**(2): p. 89-90.

264. Norton, J.M., *Toward consistent definitions for preload and afterload*. Advan. Physiol. Edu., 2001. **25**(1): p. 53-61.
265. Kirkpatrick, S.E., et al., *Frank-Starling relationship as an important determinant of fetal cardiac output*. Am J Physiol, 1976. **231**(2): p. 495-500.
266. Mahoney, L., *Calcium homeostasis and control of contractility in the developing heart*. Semin Perinatol, 1996. **20**: p. 510-519.
267. Romero, T., J. Covell, and W.F. Friedman, *A comparison of pressure-volume relations of the fetal, newborn, and adult heart*. Am J Physiol, 1972. **222**(5): p. 1285-1290.
268. Thornburg, K.L. and M.J. Morton, *Filling and arterial pressures as determinants of left ventricular stroke volume in fetal lambs*. Am J Physiol Heart Circ Physiol, 1986. **251**(5): p. H961-968.
269. Harada, K., et al., *Doppler echocardiographic evaluation of ventricular diastolic filling in fetuses with ductal constriction*. Am J Cardiol, 1997. **79**(4): p. 442-6.
270. Mielke, G. and N. Benda, *Cardiac output and central distribution of blood flow in the human fetus*. Circulation, 2001. **103**(12): p. 1662-8.
271. Rasanen, J., et al., *Role of the pulmonary circulation in the distribution of human fetal cardiac output during the second half of pregnancy*. Circulation, 1996. **94**(5): p. 1068-73.
272. Sutton, M.S., Groves, A., MacNeill, A., Sharland, G., Allan, L., *Assessment of changes in blood flow through the lungs and foramen ovale in the normal human fetus with gestational age: a prospective Doppler echocardiographic study*. Br Heart J, 1994. **71**(3): p. 232-7.
273. Itskovitz, J., E.F. LaGamma, and A.M. Rudolph, *Effects of cord compression on fetal blood flow distribution and O₂ delivery*. Am J Physiol, 1987. **252**(1 Pt 2): p. H100-9.
274. Kiserud, T., et al., *Fetal cardiac output, distribution to the placenta and impact of placental compromise*. Ultrasound Obstet Gynecol, 2006. **28**(2): p. 126-36.
275. Stoddard, M.F., A.J. Labovitz, and A.C. Pearson, *The role of Doppler echocardiography in the assessment of left ventricular diastolic function*. Echocardiography, 1992. **9**(4): p. 387-406.

276. Zielinsky, P., et al., *Alternative parameters for echocardiographic assessment of fetal diastolic function*. Braz J Med Biol Res, 2004. **37**(1): p. 31-6.
277. Vandembroucke, J.P., et al., *Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration*. PLoS Medicine, 2007. **4**(10): p. e297.
278. Huisman, T.W., et al., *Ductus venosus flow velocity waveforms in relation to fetal behavioural states*. Br J Obstet Gynaecol, 1994. **101**(3): p. 220-4.
279. Wladimiroff, J.W., *Behavioural states and cardiovascular dynamics in the human fetus; an overview*. Early Hum Dev, 1994. **37**(3): p. 139-49.
280. Hu, J., et al., *Dependence of aortic pulse wave assessments on behavioural state in normal term fetus*. Early Hum Dev, 1997. **48**(1-2): p. 59-70.
281. Royston, P., Altman, D.G., *Design and analysis of longitudinal studies of fetal size*. Ultrasound Obstet Gynecol. , 1995. **5**: p. 307-12.
282. Brown, M.A., Hague, W.M., Higgins, J., Lowe, S., McCowan, L., Oats, J., Peek, M.J., Rowan, J.A., Walters, B.N.J., *ASSHP Consensus statement. The detection, investigation and management of hypertension in pregnancy: executive summary. Recommendations from the Council of the Australasian Society for the Study of Hypertension in Pregnancy. Endorsed by RANZCOG in 2001.*. 2000.
283. Roberts, C.L., Lancaster, P.A., *Australian national birthweight percentiles by gestational age*. Med J Aust. , 1999. **170**(3): p. 114-8.
284. McCowan, L., Stewart, A.W., Francis, A., Gardosi, J., *A customised birthweight centile calculator developed for a New Zealand population*. Aust N Z J Obstet Gynaecol., 2004. **44**(5): p. 428-31.
285. Ott, W.J., *The diagnosis of altered fetal growth*. . Obstet Gynecol Clin North Am 1988. **15**(237-263).
286. Geert, V., Geert, M. , ed. *Linear mixed models for longitudinal data.*, ed. S.s.i. statistics. 1997.
287. Finucane, M., J. Samet, and N. Horton, *Translational methods in biostatistics: linear mixed effect regression models of alcohol consumption and HIV disease progression over time*. Epidemiologic Perspectives & Innovations, 2007. **4**(1): p. 8.

288. Petkova, E., & Teresi, J., *Some statistical issues in the analyses of data from longitudinal studies of elderly chronic care populations.* . Psychosomatic Medicine., 2002. **64**(3): p. 531-547.
289. Bouchet, C., F. Guillemin, and S. Briançon, *Nonspecific effects in longitudinal studies: Impact on quality of life measures.* Journal of Clinical Epidemiology, 1996. **49**(1): p. 15-20.
290. Murray, M., et al., *The Hawthorne effect in the measurement of adolescent smoking.* J Epidemiol Community Health, 1988. **42**(3): p. 304-306.
291. Bland, J.M., Altman, D.G. , ed. *The statistical analysis of Doppler ultrasound studies.* . in Pearce ed. Doppler Ultrasound In Obstetrics And Gynaecology 1992, Oxford University Press. .
292. Royston, P., Wright, E.M., *How to construct "normal ranges" for fetal variables.* Ultrasound Obstet Gynecol 1998. **11**: p. 30-8.
293. Bahlmann, F., et al., *Reference values of ductus venosus flow velocities and calculated waveform indices.* Prenat Diagn, 2000. **20**(8): p. 623-34.
294. Mielke, G. and N. Benda, *Blood flow velocity waveforms of the fetal pulmonary artery and the ductus arteriosus: reference ranges from 13 weeks to term.* Ultrasound Obstet Gynecol, 2000. **15**(3): p. 213-8.
295. Kurmanavicius, J., Florio, I., Wisser, J., Hebisch, G., Zimmermann, R., Müller, R., Huch, R., Huch, A., *Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24-42 weeks of gestation.* Ultrasound Obstet Gynecol. , 1997. **10**(2): p. 112-20.
296. Ebbing, C., Rasmussen , S., Kiserud , T., *Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements.* Ultrasound in Obstetrics and Gynecology, 2007. **30**(3): p. 287-296.
297. Kessler, J., Rasmussen, S., Hanson, M., Kiserud, T., *Longitudinal reference ranges for ductus venosus flow velocities and waveform indices.* Ultrasound Obstet Gynecol. , 2006. **28**(7): p. 890-8.
298. Royston, P., *Calculation of unconditional and conditional reference intervals for fetal size and growth from longitudinal measurements.* . Stat Med 1995. **14**: p. 1417-1436.
299. Meyberg, G.C., Solomayer, E.F., Grischke, E.M., Bastert, G. , *Does the measurement of four fetal arteries provide more information than the*

- measurement of just two arteries in prenatal Doppler sonography?* Ultrasound Obstet Gynaecol 1999. **13**(407-14).
300. Tulzer, G., et al., *Doppler echocardiography of fetal ductus arteriosus constriction versus increased right ventricular output.* J Am Coll Cardiol, 1991. **18**(2): p. 532-6.
 301. Harrington, K., et al., *Doppler ultrasound of the uterine arteries: The importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby.* Ultrasound in Obstetrics and Gynecology, 1996. **7**(3): p. 182-188.
 302. J. Kurmanavicius, I.F.J.W.G.H.R.Z.R.M.R.H.A.H., *Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24-42 weeks of gestation.* Ultrasound in Obstetrics and Gynecology, 1997. **10**(2): p. 112-120.
 303. Baschat, A.A. and U. Gembruch, *The cerebroplacental Doppler ratio revisited.* Ultrasound Obstet Gynecol, 2003. **21**(2): p. 124-7.
 304. Elchalal, U., et al., *Sonographically thick placenta: a marker for increased perinatal risk--a prospective cross-sectional study.* Placenta, 2000. **21**(2-3): p. 268-72.
 305. Hadlock, F.P., et al., *Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study.* Am J Obstet Gynecol, 1985. **151**(3): p. 333-7.
 306. Westerway, S.C., *Ultrasonic Fetal Measurements* ANZJOG 2000. **40**(3): p. 297-302.
 307. Teixeira, J., et al., *Fetal haemodynamic stress response to invasive procedures.* Lancet, 1996. **347**(9001): p. 624.
 308. Oberhoffer R., G., D., Keckstein, J., Högel, J., Terinde, R., Lang, D., *Cardiac changes in fetuses secondary to immune hemolytic anemia and their relation to hemoglobin and catecholamine concentrations in fetal blood.* Ultrasound in Obstetrics and Gynecology, 1999. **13**(6): p. 396-400.
 309. Gitau, R., N.M. Fisk, and V. Glover, *Human fetal and maternal corticotrophin releasing hormone responses to acute stress.* Arch Dis Child Fetal Neonatal Ed, 2004. **89**(1): p. F29-32.

310. Gitau, R., et al., *Fetal hypothalamic-pituitary-adrenal stress responses to invasive procedures are independent of maternal responses*. J Clin Endocrinol Metab, 2001. **86**(1): p. 104-9.
311. Wladimiroff, J.W., H.M. Tonge, and P.A. Stewart, *Doppler ultrasound assessment of cerebral blood flow in the human fetus*. Br J Obstet Gynaecol, 1986. **93**(5): p. 471-5.
312. Mari, G., *Middle Cerebral Artery Peak Systolic Velocity: Is It the Standard of Care for the Diagnosis of Fetal Anemia?* J Ultrasound Med, 2005. **24**(5): p. 697-702.
313. Mari, G., et al., *Flow velocity waveforms of the vascular system in the anemic fetus before and after intravascular transfusion for severe red blood cell alloimmunization*. Am J Obstet Gynecol, 1990. **162**(4): p. 1060-4.
314. d'Ancona, R.L., et al., *The effect of intravascular blood transfusion on the flow velocity waveform of the portal venous system of the anemic fetus*. Ultrasound Obstet Gynecol, 1997. **10**(5): p. 333-7.
315. Oepkes, D., et al., *Fetal ductus venosus blood flow velocities before and after transfusion in red-cell alloimmunized pregnancies*. Obstet Gynecol, 1993. **82**(2): p. 237-41.
316. Sikkel, E., et al., *Fetal cardiac contractility before and after intrauterine transfusion*. Ultrasound Obstet Gynecol, 2005. **26**(6): p. 611-7.
317. Kiserud, T., *The ductus venosus*. Semin Perinatol, 2001. **25**(1): p. 11-20.
318. Chan, A., Scott, J., Nguyen A.M, Sage, L., *Pregnancy Outcome in South Australia 2006*. Adelaide: Pregnancy Outcome Unit, South Australian Department of Health, 2007. available at <http://www.health.sa.gov.au/pehs/pregnancyoutcome.htm>. 2007.
319. Gardosi J, M.M., Wilcox M, Chang A, Sahota D, Francis A., A. *Gestation Related Optimal Weight program (GROW)*. Software v 6.2., 2007. Gestation Network.
320. Khong, T.Y., De Wolf, F., Robertson, W.B., Brosens, I., *Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants*. Br J Obstet Gynaecol, 1986. **93**(10): p. 1049-59.
321. Khong, T.Y., *The placenta in stillbirth*. Current Diagnostic Pathology, 2006. **12**(3): p. 161-172.

322. van der Veen, F., Fox, H., *The human placenta in idiopathic intrauterine growth retardation: a light and electron microscopic study*. Placenta, 1983. **4**(1): p. 65-77.
323. Las Heras, J., Baskerville, J.C., Harding, P.G., Haust, M.D., *Morphometric studies of fetal placental stem arteries in hypertensive disorders ('toxaemia') of pregnancy*. Placenta., 1985. **6**(3): p. 217-27.
324. Viero, S., Chaddha, V., Alkazaleh, F., Simchen, M.J., Malik, A., Kelly, E., Windrim, R., Kingdom, J.C., *Prognostic value of placental ultrasound in pregnancies complicated by absent end-diastolic flow velocity in the umbilical arteries*. Placenta., 2004. **25**(8-9): p. 735-41.
325. Jauniaux, E., *Placental ultrasonographic measurement: what can we learn and is it worth doing routinely?* Ultrasound Obstet Gynecol. 1992. **2**(4): p. 241-2.
326. Fok, R.Y., Pavlova, Z., Benirschke, K., Paul, R.H., Platt, L.D., *The correlation of arterial lesions with umbilical artery Doppler velocimetry in the placentas of small-for-dates pregnancies*. Obstet Gynecol. , 1990. **75**(4): p. 578-83.
327. Salafia, C.M., Pezzullo, J.C., Minior, V.K., Divon, M.Y., *Placental pathology of absent and reversed end-diastolic flow in growth-restricted fetuses*. Obstet Gynecol., 1997. **90**(5): p. 830-6.
328. Montenegro, N., Laurini, R., Brandão O., Nogueira R., Matias A., Santos F., Barros, H., *Placental Findings in Fetuses with Absent or Reversed End-diastolic Flow in the Umbilical Artery (ARED Flow): A Reappraisal*. Journal of Maternal-Fetal Investigation, 1997. **7**(4): p. 175-179.
329. Adamson, S.L., et al., *Site-dependent effects of increases in placental vascular resistance on the umbilical arterial velocity waveform in fetal sheep*. Ultrasound in Medicine & Biology, 1990. **16**(1): p. 19-27.
330. Karsdorp, V.H., Dirks, B.K., van der Linden, J.C., van Vugt, J.M., Baak, J.P., van Geijn, H.P., *Placenta morphology and absent or reversed end diastolic flow velocities in the umbilical artery: a clinical and morphometrical study*. Placenta, 1996. **17**(7): p. 393-9.
331. Trudinger, B.J., Stevens, D., Connelly, A., Hales, J.R., Alexander, G., Bradley, L., Fawcett, A., Thompson, R.S., *Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation*. Am J Obstet Gynecol, 1987. **157**(6): p. 1443-8.

332. Espinoza, J., et al., *Normal and abnormal transformation of the spiral arteries during pregnancy*. Journal of Perinatal Medicine, 2006. **34**(6): p. 447.
333. Campbell, S., Pearce, J.M., Hackett, G., Cohen-Overbeek, T., Hernandez, C., *Qualitative assessment of uteroplacental blood flow: early screening test for high-risk pregnancies*. Obstet Gynecol, 1986. **68**(5): p. 649-53.
334. Sebire, N.J., Goldin, R.D., Regan, L., *Histomorphological evidence for chronic vasoconstriction of placental stem vessels in pregnancies with intrauterine growth restriction and abnormal umbilical artery Doppler velocimetry indices (Abstract)*. J Pathol 2001. **195**: p. 19A.
335. Langston, C., Kaplan, C., Macpherson, T., Mancini, E., Peevy, K., Clark, B., Murtagh, C., Cox, S., Glenn, G., *Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists*. Arch Pathol Lab Med., 1997. **121**(5): p. 449-76.
336. Gardiner, H., J. brodzki, and K. Marsal, *Ventriculovascular physiology of the growth-restricted fetus doi:10.1046/j.1469-0705.2001.00436.x*. Ultrasound in Obstetrics and Gynecology, 2001. **18**(1): p. 47-53.
337. Harrington, K., et al., *Doppler fetal circulation in pregnancies complicated by pre-eclampsia or delivery of a small for gestational age baby: 2. Longitudinal analysis*. British Journal of Obstetrics and Gynaecology, 1999. **106**(5): p. 453-466.
338. Porter, H.J., *The role of placental examination as a perinatal investigation*. In *The Placenta: Basic Science and Clinical Practice*. J. C. P. Kingdom, E. R. M. Janiaux and P. M. Shaughn O'Brien (Eds). RCOG Study Group. RCOG Press. . 2000.
339. Harrington, K.F., *Making best and appropriate use of fetal biophysical and Doppler ultrasound data in the management of the growth restricted fetus*. Ultrasound in Obstetrics and Gynecology, 2000. **16**(5): p. 399-401.
340. Rychik, J., Ayres N., Cuneo, B., Gotteiner N., and L. Hornberger, Spevak,P.J., Van Der Veld M., *A statement of the Pediatric Council of the American Society of Echocardiography*. American Society of Echocardiography. *Guidelines and Standards for Performance of the Fetal Echocardiogram*. . Journal of the American Society of Echocardiography, 2004.

341. Dean, J.C.S., *Management of Marfan syndrome*, in *Heart* %R 10.1136/heart.88.1.97. 2002. p. 97-103.
342. Patten, B.M., Sommerfield, W.A., Paff, G.H., *Functional limitations of the foramen ovale in the human fetal heart*. *The anatomical record.*, 1929. **44**(2): p. 165-78.
343. Lind, J. and C. Wegelius, *Human fetal circulation: changes in the cardiovascular system at birth and disturbances in the post-natal closure of the foramen ovale and ductus arteriosus*. *Cold Spring Harb Symp Quant Biol*, 1954. **19**: p. 109-25.
344. Anderson, D.F., et al., *Flow through the foramen ovale of the fetal and newborn lamb*. *J Physiol*, 1985. **365**: p. 29-40.
345. Johnson, P., et al., *Intracardiac pressures in the human fetus*. *Heart*, 2000. **84**(1): p. 59-63.
346. Reed, K.L., *Fetal and neonatal cardiac assessment with Doppler*. *Semin Perinatol.*, 1987. **11**(4): p. 347-56.
347. Pinson, C.W., Morton, M.J., Thornburg, K.L., *An anatomic basis for fetal right ventricular dominance and arterial pressure sensitivity*. *J Dev Physiol.*, 1987. **9**(3): p. 253-69.
348. Lohr, J.L., et al., *Atrial myocardial blood flow during acute right ventricular pressure load and adenosine infusion in late gestation fetal sheep*. *Pediatr Res*, 1994. **35**(3): p. 325-8.
349. Thornburg, K.L. and M.D. Reller, *Coronary flow regulation in the fetal sheep*. *Am J Physiol Regul Integr Comp Physiol*, 1999. **277**(5): p. R1249-1260.
350. Belz, G.G., *Elastic properties and Windkessel function of the human aorta*. *Cardiovascular Drugs and Therapy*, 1995. **9**(1): p. 73-83.
351. Wang, J., Jr., et al., *Time-domain representation of ventricular-arterial coupling as a windkessel and wave system*. *Am J Physiol Heart Circ Physiol*, 2003. **284**(4): p. H1358-1368.
352. van den Wijngaard, J.P.H.M., et al., *Abnormal arterial flows by a distributed model of the fetal circulation*. *Am J Physiol Regul Integr Comp Physiol*, 2006. **291**(5): p. R1222-1233.
353. Mori, A., et al., *Stiffness of Systemic Arteries in Appropriate- and Small-for-Gestational-Age Newborn Infants*. *Pediatrics* %R 10.1542/peds.2006-0386, 2006. **118**(3): p. 1035-1041.

354. Mori, A., Iwabuchi, M., Makino, T., *Fetal haemodynamic changes in fetuses during fetal development evaluated by arterial pressure pulse and blood flow velocity waveforms*. BJOG., 2000. **107**(5): p. 669-77.
355. Mori, A., et al., *The fetal aortic pressure pulse waveform in normal and compromised pregnancy*. Br J Obstet Gynaecol, 1997. **104**(11): p. 1255-61.
356. Eicher, J.-C., et al., *Hypoxaemia associated with an enlarged aortic root: a new syndrome?*10.1136/hrt.2003.027839. Heart, 2005. **91**(8): p. 1030-1035.
357. Satoh, S., Fujita Y., Yumoto, Y., Kinukawa, N., Nakano, H., *Changes in aortic distension waveforms in acute hypoxemia and acidosis: fetal lamb study*. Ultrasound Med Biol. , 2007. **33**(5): p. 708-13. .
358. Fujita, Y., Satoh, S., Yumoto Y., Koga T., Kinukawa N., Nakano H., *Fetal aortic distension waveforms for evaluating cardiac function and changes in blood pressure: Fetal lamb validation*. Journal of Obstetrics and Gynaecology Research, 2006. **32**(2): p. 155-161.
359. Song, S.H., Park, H.W., *Development of elastin layers in the aortic wall of human fetuses*. Yonsei Med J., 1992. **33**(4): p. 337-43.
360. Martyn, C.N. and S.E. Greenwald, *Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension*. The Lancet, 1997. **350**(9082): p. 953-955.
361. Myers, L.J. and W.L. Capper, *A transmission line model of the human fetal circulatory system*. Med Eng Phys, 2002. **24**(4): p. 285-94.
362. Capper, W.L., J.G. Cowper, and L.J. Myers, *A transfer function-based mathematical model of the fetal-placental circulation*. Ultrasound in Medicine & Biology, 2002. **28**(11-12): p. 1421-1431.
363. Hill, A.A., et al., *A wave transmission model of the umbilicoplacental circulation based on hemodynamic measurements in sheep*. American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 1995. **269**(5 38-5).
364. Trudinger, B.J., W.B. Giles, and C.M. Cook, *Uteroplacental blood flow velocity-time waveforms in normal and complicated pregnancy*. Br J Obstet Gynaecol, 1985. **92**(1): p. 39-45.

365. Trudinger, B.J., W.B. Giles, and C.M. Cook, *Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulations*. Am J Obstet Gynecol, 1985. **152**(2): p. 155-63.
366. Hecher, K., et al., *Assessment of fetal compromise by Doppler ultrasound investigation of the fetal circulation. Arterial, intracardiac, and venous blood flow velocity studies*. Circulation, 1995. **91**(1): p. 129-38.
367. Areias, J.C., A. Matias, and N. Montenegro, *Venous return and right ventricular diastolic function in ARED flow fetuses*. J Perinat Med, 1998. **26**(3): p. 157-67.
368. Kiserud, T., et al., *Ductus venosus blood velocity and the umbilical circulation in the seriously growth-retarded fetus*. Ultrasound Obstet Gynecol, 1994. **4**(2): p. 109-14.
369. Alvarez, L., et al., *Morphometric data on the arterial duct in the human fetal heart*. Int J Cardiol, 1991. **31**(3): p. 337-44.
370. Brezinka, C., et al., *Anatomical and sonographic correlation of the fetal ductus arteriosus in first and second trimester pregnancy*. Ultrasound Med Biol, 1994. **20**(3): p. 219-24.
371. Szpinda, M., A. Szwesta, and E. Szpinda, *Morphometric study of the ductus arteriosus during human development*. Annals of Anatomy - Anatomischer Anzeiger, 2007. **189**(1): p. 47-52.
372. Ashrafian, H., *The Coanda Effect and Preferential Right Atrial Streaming* 10.1378/chest.130.1.300. Chest, 2006. **130**(1): p. 300-.
373. Chao, K., Moises, V.A., Shandas, R., Elkadi, T., Sahn, D.J., Weintraub, R., *Influence of the Coanda effect on color Doppler jet area and color encoding. In vitro studies using color Doppler flow mapping*. Circulation., 1992. **85**(1): p. 333-41.
374. Kilner, P.J., Yang, G.Z., Wilkes, A.J., Mohiaddin, R.H., Firmin, D.N., Yacoub, M.H., *Asymmetric redirection of flow through the heart*. Nature., 2000. **404**(6779): p. 759-61.
375. Morris, J.A., et al., *Dynamics of blood flow in the ductus arteriosus*. Am J Physiol, 1965. **208**(3): p. 471-476.
376. Morris, J.A., et al., *Dynamics of Blood Flow in the Ductus Arteriosus*. Am J Physiol, 1965. **208**: p. 471-6.

377. Storme, L., et al., *In vivo evidence for a myogenic response in the fetal pulmonary circulation*. *Pediatr Res*, 1999. **45**(3): p. 425-31.
378. Richard, C., et al., *Patency of the preterm fetal ductus arteriosus is regulated by endothelial nitric oxide synthase and is independent of vasa vasorum in the mouse*. *Am J Physiol Regul Integr Comp Physiol*, 2004. **287**(3): p. R652-60.
379. Heymann, M.A., *Prostaglandins and leukotrienes in the perinatal period*. *Clin Perinatol*, 1987. **14**(4): p. 857-80.
380. Clyman, R.I. and M.A. Heymann, *Pharmacology of the ductus arteriosus*. *Pediatr Clin North Am*, 1981. **28**(1): p. 77-93.
381. Clyman, R.I., et al., *The developmental response of the ductus arteriosus to oxygen*. *Biol Neonate*, 1978. **34**(3-4): p. 177-81.
382. Momma, K., et al., *In vivo constriction of the fetal and neonatal ductus arteriosus by a prostanoid EP4-receptor antagonist in rats*. *Pediatr Res*, 2005. **58**(5): p. 971-5.
383. Toyoshima, K., et al., *Constriction of the ductus arteriosus by selective inhibition of cyclooxygenase-1 and -2 in near-term and preterm fetal rats*. *Prostaglandins Other Lipid Mediat*, 2006. **79**(1-2): p. 34-42.
384. Momma, K., T. Nakanishi, and S. Imamura, *Inhibition of in vivo constriction of fetal ductus arteriosus by endothelin receptor blockade in rats*. *Pediatr Res*, 2003. **53**(3): p. 479-85.
385. Baschat, A.A. and C.R. Harman, *Venous Doppler in the assessment of fetal cardiovascular status*. *Curr Opin Obstet Gynecol*, 2006. **18**(2): p. 156-63.
386. Kiserud, T., *Fetal venous circulation--an update on hemodynamics*. *J Perinat Med*, 2000. **28**(2): p. 90-6.
387. M. Bellotti, G.P., E. Ferrazzi., *Re: Ductus venosus shunting in growth-restricted fetuses and the effect of umbilical circulatory compromise*. *Ultrasound in Obstetrics and Gynecology*, 2007. **29**(1): p. 100-101.
388. Kiserud T., R., S. , *How repeat measurements affect the mean diameter of the umbilical vein and the ductus venosus*. *Ultrasound in Obstetrics and Gynecology*, 1998. **11**(6): p. 419-425.
389. Vinals, F., J. Tapia, and A. Giuliano, *Prenatal detection of ductal-dependent congenital heart disease: how can things be made easier?* *Ultrasound Obstet Gynecol*, 2002. **19**(3): p. 246-9.

390. Hoddick, W.K., Mahony, B.S., Callen, P.W., Filly, R.A., *Placental thickness.* J Ultrasound Med., 1985. **4**(9): p. 479-82.
391. Barker, D.J. and M.A. Hanson, *Altered regional blood flow in the fetus: the origins of cardiovascular disease?* Acta Paediatr, 2004. **93**(12): p. 1559-60.