Ultrasound Reference Ranges of the Intra-amniotic Umbilical Cord Vein: Statistical Modelling and Implications for the Detection of Intrauterine Growth Restriction

Doctor of Health Science

Exegesis

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“The vessels join onto the uterus like the roots of plants and through them the embryo receives its nourishment. This is why the embryo remains in the uterus” (Aristotle)

(Needham, 1959, p. 51)
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Certificate of Authorship

I, Jacqueline Spurway, hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the exegesis and portfolio. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

I agree that this thesis be accessible for the purpose of study and research in accordance with normal conditions established by the Executive Director, Library Services, Charles Sturt University or nominee, for the care, loan and reproduction of thesis, subject to confidentiality provisions as approved by the University.

Name: Jacqueline Spurway

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Date: 13 November 2017
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The Greater Western Human Research Ethics Committee granted ethics approval for this research project (HREC/10/GWAHS/34) on the 10 January 2011, for the specific sites of Orange Health Service and Bathurst Health Service (SSA/11GWAHS/6).

The Charles Sturt University Human Research Ethics Committee granted ethics approval for this research project on the 15 February 2011 (2011/020).
Professional Editorial Assistance

Dr Clare Wilding provided paid editorial assistance with the final version of this exegesis. Editorial assistance comprised correction of punctuation and minor changes to formatting to ensure that the presentation conformed to APA 6th guidelines.

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Helen Nicol provided paid review of statistical aspects of the final version of this exegesis.
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List of Publications and Posters

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ii. **Original research publication:**

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iii. **Poster display:**

Presentation Title: Normal reference ranges for the intra-amniotic umbilical cord vein diameter, blood flow and peak velocity in a regional New South Wales population

Venue: The Australasian Society for Ultrasound in Medicine 2016 Annual Scientific Meeting held 28 to 30 October 2016, Brisbane.

Award: Best Poster Presentation at the Australasian Society for Ultrasound in Medicine 46th Annual Scientific Meeting

iv. **Original research publication:**

List of Presentations

i. **Presentation Title:** Blood flow characteristics of the umbilical cord vein and intrauterine growth restriction: the research proposal.
   
   **Audience:** Charles Sturt University Science Symposium, 9 December 2010.

ii. **Presentation Title:** Characteristics of the umbilical cord vein (UCV) in the prediction of intrauterine growth restriction (IUGR): preliminary results and pitfalls.
   
   **Audience:** Charles Sturt University Science Symposium, 6 July 2012.

iii. **Presentation Title:** Reference Ranges for the intra-amniotic umbilical cord vein diameter, peak velocity and blood flow in a regional NSW population.

   **Audiences:** Western NSW Local Health District Sonographers Group (23 March 2017), Orange Health Service Obstetrics and Gynaecology Education Session (4 April 2017) and Charles Sturt University Faculty of Science (27 April 2017).
List of Abbreviations

AC  abdominal circumference
ADF™  advanced dynamic flow
AFI  amniotic fluid index
AGA  appropriate for gestational age
ALARA  as-low-as-reasonably-achievable
ANOVA  analysis of variance
ART  assisted reproductive technology
ASUM  Australasian Society for Ultrasound in Medicine
BHS  Bathurst Health Service
BPD  biparietal diameter
CI  confidence interval
CNGOF  French College of Gynaecologists and Obstetricians
CRL  crown rump length
CTG  cardiotocography
DHEA  dehydroepiandrosterone
DV  ductus venosus
EDD  estimated date of delivery
EFW  estimated fetal weight
FGR  fetal growth restriction
FMF  Fetal Medicine Foundation
GA  gestational age
GIFT  gamete intra-fallopian transfer
HC  head circumference
HELLP  haemolysis, elevated liver enzyme levels and low platelet levels
ICC  intraclass correlation coefficient
ICSI  intracytoplasmic sperm injection
IUGR  intrauterine growth restriction
IUGR/FGR  SGA subgroup defined by birthweight < 10th percentile, EFW ≤ 10th percentile, AC ≤ 5th percentile and UA S/D > 95th percentile
IVC  inferior vena cava
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td>in vitro fertilisation</td>
</tr>
<tr>
<td>LGA</td>
<td>large for gestational age</td>
</tr>
<tr>
<td>LHD</td>
<td>Local Health District</td>
</tr>
<tr>
<td>LMP</td>
<td>last menstrual period</td>
</tr>
<tr>
<td>ln</td>
<td>natural logarithm</td>
</tr>
<tr>
<td>MCA</td>
<td>middle cerebral artery</td>
</tr>
<tr>
<td>MI</td>
<td>mechanical index</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>nAGA</td>
<td>“normal” appropriate for gestational age. Subgroup of the 10th to 90th birthweight category with no confounders for abnormal fetal growth</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>OHS</td>
<td>Orange Health Service</td>
</tr>
<tr>
<td>PACS</td>
<td>picture archive and communication system</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>pregnancy associated plasma protein-A</td>
</tr>
<tr>
<td>PI</td>
<td>pulsatility index</td>
</tr>
<tr>
<td>POP study</td>
<td>Pregnancy Outcome Prediction study</td>
</tr>
<tr>
<td>PRF</td>
<td>pulse repetition frequency</td>
</tr>
<tr>
<td>PV</td>
<td>peak velocity</td>
</tr>
<tr>
<td>Q_{ucv}</td>
<td>intra-amniotic umbilical cord vein blood flow</td>
</tr>
<tr>
<td>Q_{uv}</td>
<td>umbilical vein blood flow – generic term covering both intra-abdominal and intra-amniotic portion unless specified</td>
</tr>
<tr>
<td>RANZCOG</td>
<td>Royal Australian and New Zealand College of Obstetricians and Gynaecologists</td>
</tr>
<tr>
<td>RCOG</td>
<td>Royal College of Obstetricians and Gynaecologists</td>
</tr>
<tr>
<td>S/D ratio</td>
<td>systolic/diastolic ratio</td>
</tr>
<tr>
<td>SOGC</td>
<td>Society of Obstetricians and Gynaecologists of Canada</td>
</tr>
<tr>
<td>SGA</td>
<td>small for gestational age</td>
</tr>
<tr>
<td>SVC</td>
<td>superior vena cava</td>
</tr>
<tr>
<td>T_{amaxv}</td>
<td>time-averaged maximum velocity</td>
</tr>
<tr>
<td>T_{ameanv}</td>
<td>time-averaged mean velocity</td>
</tr>
<tr>
<td>TI</td>
<td>thermal index</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TIB</td>
<td>thermal index for bone</td>
</tr>
<tr>
<td>TIC</td>
<td>thermal index for cranial bone</td>
</tr>
<tr>
<td>TIS</td>
<td>thermal index for soft tissue</td>
</tr>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>UA</td>
<td>umbilical artery</td>
</tr>
<tr>
<td>UCV</td>
<td>intra-amniotic umbilical cord vein</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>maximum velocity</td>
</tr>
<tr>
<td>UV</td>
<td>umbilical vein describing both intra-abdominal and intra-amniotic portions</td>
</tr>
<tr>
<td>$V_{\text{mean}}$</td>
<td>mean velocity</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
Abstract

Background: The umbilical vein transports oxygen and nutrient rich blood from the placenta to the fetus. Vein parameters related to blood flow can be reduced in intrauterine growth restriction.

Aims: The principal research aims were to construct gestational age related reference ranges of the umbilical cord vein diameter, peak velocity and blood flow using a regional New South Wales population and to assess the ability of these ranges to identify intrauterine growth restriction. Secondary aims were to document simple measurement methods and investigate relationships between umbilical cord vein measurements, birthweight categories and gestational age.

Method: This was a quasi-experimental study of low risk, singleton pregnancies between 16 and 42 weeks of gestation. The umbilical cord vein diameter and peak velocity were measured using B-mode and duplex Doppler, respectively, and blood flow was calculated. Intraclass correlation coefficients assessed sonographer reliability. Linear mixed modelling analysed the relationships between the vein parameters, three birthweight categories and gestational age. Reference ranges for umbilical cord vein parameters and ratios were established using quantile regression analysis of data provided by a subgroup of “normal” pregnancies (nAGA group). A small group of moderately growth restricted fetuses (IUGR/FGR group) was used to assess the potential clinical utility of these reference ranges. Sequential plotting of data against advancing gestational age enabled investigation of longitudinal trends and slopes.

Results: Maternal characteristics and pregnancy outcomes were typical of the Australian population. Sonographer reliability showed almost perfect agreement. Birthweight category modelling demonstrated a significant difference between the
categories for all three vein parameters, but overlap of the 95% confidence interval curves at some gestational ages. Reference ranges were developed and all three vein parameters increased with advancing gestational age, with diameter and blood flow exhibiting a decline in the late third trimester. In combination, the 10th percentile of the reference ranges and birthweight categories cut-offs, identified 62.2% of attendances of the IUGR/FRG group for the umbilical cord vein diameter and blood flow, whereas the peak velocity and ratios had poorer rates. The diameter was the only parameter to demonstrate a negative slope on longitudinal plotting.

**Implications for clinical practice:** These reference ranges provide normative data from an Australian sample. The umbilical cord vein diameter is simple to measure, had a negative slope on longitudinal plotting, and can be easily utilised in a broad range of clinical situations. The umbilical cord vein peak velocity had a poor detection rate of growth restricted data and the confidence intervals of the birthweight categories overlapped during a crucial 15 week period of fetal growth. These findings imply that peak velocity has a limited clinical application and may explain the historical neglect of this Doppler measurement. The blood flow in the umbilical cord vein identified more than half of the growth restricted data, but required two measurements and a calculation that incorporated several assumptions. No clinical benefit was identified by analysis of umbilical cord diameter and peak velocity ratios.

**Conclusions:** Reference ranges for the umbilical cord vein diameter, peak velocity and blood flow were developed using quantile regression from a cohort of low risk, singleton pregnancies in Central West New South Wales. The diameter reference range has the most potential to assist in the diagnosis and monitoring of fetal growth restriction in the second half of pregnancy.
SECTION I EXECUTIVE SUMMARY

Chapter 1 Executive Summary

1.1. Aims

The umbilical vein is the solitary conduit supplying oxygen and nutrients to the fetus. The ultrasound measurements of the intra-amniotic umbilical cord vein (UCV) diameter, peak velocity (PV) and blood flow ($Q_{ucv}$) were the focus of this research project. These three features have well documented relationships with advancing gestational age (GA) and have been found to be altered in the presence of reduced fetal growth.

The research reported in this doctoral dissertation addressed two key aims related to the umbilical vein and fetal growth:

i. The construction of reference ranges of the UCV diameter, PV and $Q_{ucv}$ using data collected from a regional New South Wales (NSW) sample.

ii. The examination of these reference ranges in the identification of intrauterine growth restriction (IUGR).

Secondary aims included; documentation of simple measurement methods, the investigation of relationships between UCV parameters, birthweight categories and advancing GA, and the exploration of alternative relationships involving UCV measurements.

1.2 Background

Intrauterine growth restriction has multiple definitions, classifications and causes but it essentially describes a fetus that fails to achieve its growth potential due
to pathological reasons (Detti, Mari, Cheng, & Bahado-Singh, 2004; Royal College of Obstetricians and Gynaecologists, 2014a). Growth restriction complicates approximately 10% of all pregnancies (Lausman et al., 2013; von Beckerath et al., 2013) and has major obstetrical implications, long-term health repercussions and additional financial burdens to the health sector because of increased morbidity and mortality of the fetus, the newborn and the adult. Reliable identification of IUGR could detect fetuses requiring closer monitoring and may avoid neonatal and adult sequelae of growth restriction by implementing preventative, risk reduction and monitoring strategies (Conde-Agudelo, Papageorghiou, Kennedy, & Villar, 2013). The current gold standard for ultrasound monitoring of fetal growth relies on a combination of fetal biometry and Doppler assessment of fetal associated arteries (Kaponis et al., 2011; Ott, 2005; Vayssiere et al., 2015) with scant attention paid to the intra-amniotic umbilical vein.

This research evolved from a desire to develop a deeper knowledge of the UCV and to understand the ultrasound community’s inattention to this vital vessel. In order to encourage examination of the UCV I hoped to document simple measurement protocols, develop local reference ranges for ultrasound features of the umbilical cord vein and ascertain the usefulness of these reference ranges in the identification of IUGR.

1.3 Exegesis and Portfolio Structure

This doctoral dissertation presents two interrelated sections: a portfolio and an exegesis. The portfolio documents my research journey demonstrating personal growth as a researcher and catalogues the sequence of submissions on which the
exegesis was constructed. The portfolio is divided into three sections covering early literature reviews, the groundwork for the research and dissemination of results and information.

The exegesis is divided into six sections containing 18 chapters. Chapter 1 provides an overview of the research and the interconnection of the portfolio and exegesis. Chapter 2 reviews and scrutinises the literature on IUGR, the umbilical cord, the UCV diameter, PV and \( Q_{ucv} \) and their associations with growth restriction. Chapter 3 details the methods and data analysis utilised in this research project. The remaining chapters present the results with associated discussions. Chapters 4 to 7 present and discuss samples sizes, maternal characteristics, pregnancy outcomes and ultrasound indices in relation to two research samples. Chapters 8 and 9 present and discuss sonographer reliability. Presentation and discussion of the UCV diameter, PV and \( Q_{ucv} \) follow in Chapters 8 to 17. Chapter 18 summarises the research results, advantages and limitations of the research methods, contributions to knowledge, implications for clinical practice and areas of future research.

1.4 Research Methods

This was a quasi-experimental, quantitative research project using non-random sample selection and both longitudinal and cross-sectional data collection. The participants were recruited from regional NSW and ultrasound measurements of the UCV diameter and PV were recorded, and the \( Q_{ucv} \) calculated.

Maternal characteristics were collected at recruitment and pregnancy outcomes retrospectively obtained from medical records. Classifying fetuses by Australian birthweight standards, the whole research sample was divided into three
birthweight categories. Linear mixed modelling determined the relationships between the birthweight categories, each of the ultrasound parameters and GA.

A subgroup of the 10th to 90th percentile birthweight fetuses (nAGA group) was constructed by eliminating pregnancies affected by confounders for abnormal fetal growth or fetal structural anomalies. Using the nAGA group data, reference ranges were developed using polynomial quantile regression modelling of the relationships between the UCV diameter, PV, Q
\text{ucv}
 and advancing GA.

A subgroup of fetuses (IUGR/FGR group) with a birthweight < 10th percentile, estimated fetal weight (EFW) ≤ 10th percentile, an abdominal circumference (AC) ≤ 5th percentile and an elevated umbilical artery (UA) ratio defined IUGR for this research project and all reference ranges were assessed for their ability to identify this group.

Quantile regression was also used to model reference ranges for the ratios of the UCV diameter and PV and identification of the IUGR/FRG group was assessed. Lastly, longitudinal trends and slopes of data for multiple attendances were examined.

1.5 Results

The maternal and pregnancy outcome characteristics of the whole study sample and the nAGA group were analysed and compared to local, state and national populations. The whole research sample was typical of the Australian population, except mothers were younger, more mothers smoked at some point in their pregnancy, and neonates were lighter than comparable Australian data. The maternal and pregnancy outcome characteristics of the nAGA group were reasonably typical of the whole study sample. The nAGA group was similar to the broader 2012 Australian
population, except the mothers were younger, parity was higher and fewer male babies were born.

Linear mixed modelling showed a significant difference between the three birthweight categories for each of the UCV features; however, there was overlap of the 95% confidence interval (CI) of the regression curves. The three birthweight curves for the UCV diameter and $Q_{ucv}$ had a similar shape and the CIs overlapped until the late second trimester, whereas UCV PV had differently shaped curves for each birthweight category and the CIs overlapped between 20 weeks and 4 days and 35 weeks and 1 day GA. The points where the CI boundaries ceased overlapping defined the gestational ages when the reference ranges became clinical useful and this information was incorporated into the assessment of the reference ranges’ ability to identify the IUGR/FGR group.

Reference ranges developed from quantile regression demonstrated a curvilinear increase in the UCV diameter with increasing GA up until 37 weeks, followed by a plateau lasting 4 weeks and then a slight decline to 42 weeks GA. There was a positive linear increase in UCV PV with increasing GA and an exponential increase in $Q_{ucv}$ up to a peak at 39 weeks and then a slight decline until 42 weeks GA. Using the 10th percentile and the GA cut-offs established by the birthweight categories curves, the reference ranges correctly identified 15% of IUGR/FGR group attendances for UCV PV and 62.2% for both the UCV diameter and $Q_{ucv}$. Reference ranges for the UCV diameter and PV ratios had curvilinear trends with advancing GA and identified less than 20% of the IUGR/FGR group attendances. The UCV diameter was the only parameter to demonstrate a negative slope on longitudinal plotting.
1.6 Discussion and Implications for Clinical Practice

This research presents the first sonographic UCV reference ranges developed from an Australian based “normal” obstetrics population using quantile regression. The UCV diameter, PV and $Q_{ucv}$ values that were measured or calculated were comparable to previously published values. The reference ranges for all three UCV measurements increased with advancing GA and the associations found in this research have been described previously, some more frequently than others.

The UCV diameter and $Q_{ucv}$ reference ranges had the best rates for the identification of IUGR/FGR group data. In addition, the UCV diameter was the easiest measurement to perform and demonstrated a negative slope with sequential plotting of IUGR/FGR group data. These attributes make the measurement of the UCV diameter and the application of this reference range practical across a wide range of ultrasound machines and a broad range of clinicians. This exegesis demonstrated that UCV PV birthweight category regression curves overlapped during a 15 week period of crucial fetal growth and the reference range had a low identification rate of IUGR/FGR group attendances; these findings may explain the failure of this measurement to be adopted into clinical practice. The $Q_{ucv}$ required an additional calculation and relied on several assumptions making it susceptible to errors and more difficult to adopt into clinical settings. Reference ranges for UCV diameter and PV ratios were developed; however, their detection of IUGR/FGR group data was very low thereby limiting their clinical usefulness.

Statistical analysis was limited in this research due to a sparsity of data between 23 to 28 weeks and after 39 weeks GA, a small IUGR/FGR group sample
comprised of moderately growth restricted fetuses, and the use of completed whole weeks of pregnancy to allocate neonates into birthweight categories.

1.7 Future Research

The main limitation of this research could be overcome by the collection of a larger “normal” sample to obtain an even distribution of data across all gestational ages. The clinical utility of the reference ranges could be further explored by collecting a larger sample of IUGR/FGR fetuses, by including more severely growth compromised fetuses and by undertaking a multicentre, clinical trial involving the broader Australian population. In addition, the possible effects of $Q_{vcv}$ fluctuations over time, the effects of coiling on PV and $Q_{vcv}$ values and comparison of the research measurement methods with automated techniques are areas of possible future research.
SECTION II BACKGROUND AND LITERATURE REVIEW

Chapter 2 Background and Literature Review

2.1 Introduction

The umbilical vein is the solitary supply conduit of gases, molecules and ion rich blood to the fetus. The consequence of poor supply through the umbilical vein is compromised fetal growth. In this literature review I will focus on discussing IUGR, the umbilical cord, ultrasound features of the umbilical vein including their association with IUGR, and ultrasound safety. Lastly, gaps in current knowledge and the aims of this research will be outlined.

The term IUGR will be used throughout this exegesis and portfolio to broadly categorise intrauterine growth that falls below predetermined organisational thresholds with or without abnormal UA Doppler indices or amniotic fluid volume. Use of this term enabled inclusion of publications spanning many years, discussion on placental causes of growth restriction, and a broader discussion of prediction, surveillance and outcomes.

2.2 Intrauterine Growth Restriction

2.2.1 Definition.

IUGR is a descriptive term applied when fetal or neonate measurements do not meet a predetermined threshold or the fetus fails to achieve its growth potential (Mandruzzato et al., 2008; Suhag & Berghella, 2013). The word “growth” is a dynamic feature and refers to size or weight and not the maturity or development of the fetus.
(Shinozuka & Taguchi, 2006). “Restriction” reflects the pathophysiology of adverse intrauterine conditions and implies a reversible, transient condition (Wollmann, 1998).

Authors use a range of terms to describe poor intrauterine growth, including fetal growth restriction (FGR), small for gestational age (SGA), small for dates, light for dates, and low birthweight. There is no international agreement on the definition of IUGR (Sehested & Pedersen, 2014; Unterscheider et al., 2014) and use of these labels as interchangeable and synonymous terms is ambiguous and makes review of the literature exceedingly difficult. Several definitions can be applied to either the antenatal or postnatal period, for example, a birthweight below 2,500 g, EFW or birthweight below the 10th, 5th or 3rd percentile for GA, an AC less than the 10th, 5th or 3rd percentile for GA, reduced ultrasound biometry and abnormal UA and/or middle cerebral artery (MCA) Dopplers, an infant with a low ponderal index, reduced growth velocity on serial ultrasound examinations, or a fetus that fails to achieve its genetic size potential (Bamfo & Odibo, 2011; Catalano et al., 2014; Sehested & Pedersen, 2014; Tate & Mari, 2013). Due to the numerous definitions for IUGR, footnotes have been used throughout this exegesis to describe the IUGR criteria used by specific researchers.

The Society of Obstetricians and Gynaecologists of Canada (SOGC) (Lausman et al., 2013) defined IUGR as an EFW less than the 10th percentile due to a pathological process. A prospective Irish study of 1,116 singleton pregnancies found that an EFW below the 3rd percentile was consistently associated with adverse perinatal outcomes \( (p = 0.0131) \) (Unterscheider et al., 2013a). Unterscheider et al.’s (2013a) finding challenges the clinical usefulness of the 10th percentile EFW cut-off, and this shift in characterisation is supported by the definition proposed by The Royal Australian and

RANZCOG (2015) used the term SGA to describe fetuses with a birthweight less than the 10th percentile, or an EFW or AC on ultrasound less than the 10th percentile; severe fetal growth restriction was defined as SGA less than, or equal to, the 3rd or 5th percentile. The Royal College of Obstetricians and Gynaecologists in the United Kingdom (RCOG) (2014a) referred to SGA as an EFW or AC less than the 10th percentile and severe SGA occurred when these features were less than the 3rd percentile. RCOG (2014a, p. 6) stated that “fetal growth restriction is not synonymous with SGA” and that “growth restriction implies a pathological restriction of the genetic growth potential”. Worton, Sibley, and Heazell (2014, p. 95) stated the term IUGR “may refer to constrained growth of either the fetus, placenta or both” and proposed that FGR was a more specific description of reduced fetal growth. These authors also defined SGA as a birthweight, EFW or AC less than the 10th percentile and reserved the descriptor FGR for instances when there was “pathologically constrained fetal growth” (Worton et al., 2014, p. 96). Recently, the French College of Gynaecologists and Obstetricians (CNGOF) defined SGA as EFW or birthweight below the 10th percentile and severe SGA when these two parameters were below the 3rd percentile (Vayssiere et al., 2015). CNGOF defined FGR or IUGR, as SGA associated with evidence of abnormal growth including abnormal uterine artery Doppler, abnormal UA Doppler or poor longitudinal growth measured three weeks apart, but the EFW may be above the 10th percentile (Vayssiere et al., 2015). They also support the terminology of IUGR when placental, fetal and amniotic fluid are involved and FGA when diagnosis is based on growth restriction of only the fetus (Vayssiere et al., 2015). Albu, Anca, Horhoianu, and Horhoianu (2014, p. 165) summarised the terminology for IUGR and concluded
that “pathological SGA is known as intrauterine growth restriction (IUGR) or fetal growth restriction (FGR)”.

The World Health Organisation (WHO) defined IUGR as a birthweight below the 10th percentile for gender specific GA related birthweight: if GA is not available, a birthweight less than 2,500 g is considered growth restricted and if birthweight is unobtainable a chest measurement less than 30 cm can be used as the indicator of growth restriction (World Health Organization, 1995). In the research described in this exegesis, the Australian birthweight percentiles compiled by Dobbins, Sullivan, Roberts, and Simpson (2012) were used to classify neonates and these authors defined SGA as a birthweight less than the 10th percentile by gender and GA.

A shortcoming of using an arbitrary cut-off to define IUGR is that fetuses that are healthy, but constitutionally small, are mistakenly classified as IUGR. Conversely, fetuses with parameters within the normal ranges, but with the potential to be larger, are not classified as IUGR (Bamfo & Odibo, 2011; Collins, Arulkumaran, Hayes, Jackson, & Impey, 2013; S. Lee & Walker, 2010; Worton et al., 2014).

2.2.2 IUGR classifications.

There are two phenotypical classifications of growth restriction. The original classification describes IUGR on the basis of relative biometry as symmetrical or asymmetrical; whereas, an evolving method based on the GA at onset classifies growth restriction into early and late onset. There is considerable overlap of these two classification systems with the newer system evolving as technology and research provides more methods of diagnosing and monitoring IUGR.
2.2.2.1 Symmetrical and asymmetrical growth restriction.

Symmetrical IUGR constitutes 20% of all growth restricted neonates and the remaining 80% are asymmetrically growth restricted (DeCherney & Nathan, 2003). The features of these classifications are summarised in Table 2.1. Symmetrical, Type I, proportional, harmonious, stunted or hypoplastic growth restriction is an intrinsic growth restriction that presents early in pregnancy with the entire body being proportionally small (Peleg, Kennedy, & Hunter, 1998), due to impaired fetal cellular hyperplasia (Sheridan, 2005). Asymmetrical, Type II, head sparing, disproportional, disharmonious, extrinsic or wasted is a progressive reduction in growth rate. Asymmetrical IUGR usually develops during the third trimester, is often due to placental insufficiency (Peyter et al., 2014), and initially manifests as a reduced AC.

Table 2.1

Characteristics of Symmetrical and Asymmetrical IUGR

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Symmetrical IUGR</th>
<th>Asymmetrical IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aetiopathology</td>
<td>Intrinsic</td>
<td>Extrinsic</td>
</tr>
<tr>
<td>Onset (weeks)</td>
<td>&lt; 28</td>
<td>&gt; 28</td>
</tr>
<tr>
<td>Cell number</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
<tr>
<td>Cell size</td>
<td>Normal</td>
<td>Reduced</td>
</tr>
<tr>
<td>Head circumference</td>
<td>Reduced</td>
<td>Normal</td>
</tr>
<tr>
<td>Malformations</td>
<td>Frequent</td>
<td>Rare</td>
</tr>
<tr>
<td>Catch-up growth</td>
<td>Rare</td>
<td>Frequent</td>
</tr>
</tbody>
</table>


The division of IUGR into symmetrical and asymmetrical is based on a head circumference (HC) to AC (HC/AC) ratio above the 95th percentile, using a nomogram developed by Campbell and Thoms (1977). The division between these two types of IUGR is not absolute as the HC/AC ratio has a unimodal distribution, indicating that the
two types of IUGR are extremes of a continuum of variable presentations of the ratio (Callen, 2000). If the insult that initially induces asymmetrical IUGR is unrelenting, the fetus may lose the ability to compensate and becomes symmetrically small, indicating that the types of IUGR are not exclusive and may change over time (Vandenbosche & Kirchner, 1998).

There is controversy about whether symmetrical or asymmetrical IUGR is more detrimental to long term outcome. Some authors reported there were no differences in clinical outcomes between the two types and advocated that classification using this nomenclature should cease (Mongelli, 2007; Vayssiere et al., 2015). Others found that symmetrical IUGR fetuses were at greater risk of aneuploidy and anatomical defects (Pilu & Nicolaides, 1999). While fetuses with triploidy exhibit extreme asymmetrical IUGR (Pilu & Nicolaides, 1999), and significantly increased adverse intrapartum and neonatal outcomes have been reported among infants delivered with asymmetrical IUGR (Dashe, McIntre, Lucas, & Leveno, 2000). The SOGC advocate that differentiation between symmetrical or asymmetrical IUGR is clinically less important than determining normality of fetal morphology, UA and uterine artery Dopplers (Lausman et al., 2013).

### 2.2.2.2 Early and late onset growth restriction.

Early and late onset growth restriction describes two phenotypes of placental insufficiency and has evolved as a description of timing of onset, association with pre-eclampsia, course of development and the suitability of different Doppler measurements for monitoring affected fetuses (Figueras & Gratacós, 2014; Muresan, Rotar, & Stamatian, 2016; Seravalli & Baschat, 2015). These phenotypes are described in Table 2.2.
Table 2.2

**Early and Late Onset Growth Restriction Comparison**

<table>
<thead>
<tr>
<th>Early onset growth restriction</th>
<th>Late onset growth restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion: 20% to 30%</td>
<td>Proportion: 70% to 80%</td>
</tr>
<tr>
<td>Prevalence: ~ 1%</td>
<td>Prevalence: 3 to 5%</td>
</tr>
<tr>
<td>Onset: before 32 to 34 weeks gestational age</td>
<td>Onset: after 32 to 34 weeks gestational age</td>
</tr>
<tr>
<td>Difficult management</td>
<td>Difficult diagnosis</td>
</tr>
<tr>
<td>Placental disease: severe (UA Doppler abnormal)</td>
<td>Placental disease: mild (UA Doppler normal)</td>
</tr>
<tr>
<td>High association with preeclampsia</td>
<td>Low association with preeclampsia</td>
</tr>
<tr>
<td>Severe hypoxia: systemic cardiovascular changes</td>
<td>Mild hypoxia: central cardiovascular adaption</td>
</tr>
<tr>
<td>Immature fetus: high tolerance to hypoxia</td>
<td>Mature fetus: lower tolerance to hypoxia</td>
</tr>
<tr>
<td>High mortality and morbidity</td>
<td>Lower mortality, (but common cause of late stillbirth) and poor long term outcome</td>
</tr>
</tbody>
</table>


Early onset growth restriction describes an onset at less than 32 or 34 weeks GA (Figueras & Gratacós, 2014), is attributed to suboptimal trophoblastic invasion of the spiral arteries causing poor uteroplacental perfusion, and causes asymmetrical IUGR (Mifsud & Sebire, 2014; Muresan et al., 2016). Early onset growth restriction tends to follow the classic pathway of adaption to hypoxia with vascular deterioration initially exhibited as elevated UA pulsatility index (PI), reduced MCA PI, absent or reversed UA waveforms and then changes in the ductus venosus (DV) waveform; however, this sequence may be altered and rapid (Muresan et al., 2016).

Late onset growth restriction describes an onset after 32 to 34 weeks GA (Figueras & Gratacós, 2014), is often associated with reduced growth velocities, and shows increased HC/AC ratio in a previously normal fetus. This IUGR phenotype is frequently the result of subclinical placental insufficiency with few histological changes.
in the placenta and can present with normal UA Doppler indices (Mifsud & Sebire, 2014; Muresan et al., 2016). The Prospective Observational Trial to Optimize Pediatric Health in Intrauterine Growth Restriction (PORTO) study (Unterscheider et al., 2013a, 2013b) found that fetuses with late onset growth restriction did not follow the classic temporal sequence of deterioration and only 46% of cases showed deterioration in UA Doppler first. In keeping with this finding, surveillance of late onset IUGR fetuses rely on MCA PI and the cerebroplacental ratio (MCA PI:UA PI) measurements; however, the predictive accuracy of this ratio is reduced after 34 weeks GA (Muresan et al., 2016; Seravalli & Baschat, 2015).

2.2.3 Prevalence and incidence of IUGR.

The prevalence of IUGR is the number of existing cases of IUGR within the total population at a given time (Hennekens & Burning, 1987). Given that the working definition of IUGR is fetuses with an EFW below the 10th percentile for GA, then the prevalence of IUGR is 10% of all pregnancies (Lausman et al., 2013). Amongst published research, the prevalence of IUGR for developed countries has varied little in the last decade. Bamfo and Odibo (2011) stated that IUGR affects 5% to 10% of pregnancies, Saleem et al. (2011) and Gardosi (2011) proposed that 10% to 15% of all pregnancies may be compromised by IUGR, von Beckerath et al. (2013) suggested that 7% to 9% of newborns were affected by growth restriction, and more recently Peyter et al. (2014) proposed that approximately 8% of all fetuses may fail to achieve their full growth potential, which is the same prevalence reported by Mandruzzato et al. (2008).

The WHO estimated that 20 to 30 million infants were born with IUGR each year (Cailhol et al., 2009). Saleem et al. (2011) calculated that the rate of IUGR was six times higher in developing countries compared to developed countries and estimated
an international IUGR rate of 23.8%. The prevalence varied dramatically around the world with the highest prevalence rates, as a percentage of live births, seen in India (54.2%), Nepal (36.3%) and Sri Lanka (34%). Lower prevalence rates were found in developed countries such as the United Kingdom (UK) (12.3%) and Argentina (9.7%) (Bale, Stoll, & Lucas, 2003). In 2008, the Organisation for Economic Co-operation and Development (OECD) countries reported the prevalence of low birthweight ranged from 3.8% in Iceland to 11% in Turkey (Australian Institute of Health and Welfare, 2012). In 2009, 18,000 (6.2%) of live born, Australian infants had a birthweight below 2,500 g which was comparable to the 2008 OECD average of 6.7% (Australian Institute of Health and Welfare, 2012). Caution is needed when reviewing international data as weighting scales may not be available or accurately calibrated, babies may not be weighed at birth and true gestational ages are often unknown in developing countries (United Nations Children’s Fund and World Health Organization, 2004).

The incidence of IUGR is the number of newly diagnosed cases of IUGR that develop in the population of pregnant women during a specific time (Hennekens & Burning, 1987). The incidence of IUGR varies with the location, population and definition of IUGR applied (Creasy, Resnik, & Iams, 2004).

2.2.4 Causes and risk factors for IUGR.

IUGR is the result of multiple, interlinked factors including maternal, environmental, fetal and placental factors. The Australian Institute of Health and Welfare (2012) identified first time mothers, Indigenous mothers, female infants, extremes of maternal age, socio-economically disadvantaged mothers, and infants born in remote and very remote areas of Australia as risk factors for Australian babies born with a birthweight less than 2,500 g.
A meta-analysis, undertaken by Kramer (1987), of 895 relevant English and French medical texts published between 1970 and 1984, identified 43 potential causes of low birthweight. In developing countries IUGR was more frequent in black and Indian races and was associated with poor maternal nutrition, low pre-conception weight, short stature and endemic malaria (Kramer, 1987). In developed countries, cigarette smoking, poor maternal nutrition and low pre-conception weight were the most influential factors for IUGR (Kramer, 1987). The maternal, fetal and placental factors that may cause, or increase the risk of, IUGR during pregnancy are summarised in Table 2.3.

Placental insufficiency or dysfunction is a generic term used to describe reduced placental function. The extensive list of risk factors for IUGR that fall under the umbrella classification of “placental insufficiency” make up 60% of the causes of IUGR (Gagnon & Van den Hof, 2003). Placental development relies upon trophoblastic invasion and remodelling of the spiral arterioles to create a low resistance maternofetal vascular interface (Espinoza et al., 2006). Approximately 100 to 150 spiral arteries are involved in this conversion, with roughly a third undergoing change by 18 weeks GA, but full conversion is not achieved until term (Nagtegaal, van Rijswijk, McGavin, & Dekker, 2005). Failure to develop the low resistance, high capacity circulatory interface and reduced surface area of the chorionic villi causes a poor interface for maternofetal circulation and may lead to IUGR caused by nutrient and oxygen deprivation of placental insufficiency (Biswas, Ghosh, & Chhabra, 2008; S. Lee & Walker, 2010; Miller, Turan, & Baschat, 2008). Abnormal placentation may result in reduced placental size, weight and volume (Worton et al., 2014).
Factors Associated with IUGR: (a) Maternal (b) Fetal and (c) Placental

<table>
<thead>
<tr>
<th>(a)</th>
<th>Maternal causes and risk factors for intrauterine growth restriction</th>
</tr>
</thead>
</table>

**Medical complications**
- Hypertension
- Preeclampsia
- Severe chronic infections (malaria)
- Maternal hypoxaemia (asthma, cystic fibrosis, bronchiectasis, heart disease, sickle cell anaemia)
- Other severe diseases (diabetes, malignancy, renal disease)
- Gastrointestinal conditions (Crohn’s disease, ulcerative colitis)
- Systemic lupus erythematosus
- Antiphospholipid antibody syndrome
- Uterine abnormalities (fibroids, bicornuate structure)

**Environmental factors**
- Smoking
- Alcohol abuse
- Greater than 300mg of caffeine per day in the third trimester
- Therapeutic drugs (antimetabolites, anticoagulants, anticonvulsants, folic acid antagonists)
- Narcotics (heroin, cocaine)
- High altitude
- Low socio-economic status
- Malnutrition

**Other conditions**
- Ethnicity
- Short maternal height, low pre-pregnancy weight and low maternal weight gain during pregnancy
- Poor obstetric history (previous low birthweight infant, recurrent miscarriages, previous late fetal demise)
- Extremes of reproductive age
- Nulliparity
- Vigorous daily exercise
- Low fruit consumption before pregnancy
- Pregnancy interval of less than 6 months or greater than 60 months
- PAPP-A less than 0.4MoM at first trimester screening
- Assisted reproductive technologies
- Paternal and maternal IUGR
(b) Fetal causes and risk factors for intrauterine growth restriction

**Genetic**
- Chromosomal abnormalities (aneuploidy, deletions, ring chromosomes)
- Mutations (insulin-like growth factor 1 receptor)
- Metabolism errors (absent pancreas, glycogen storage disorders)
- Gender

**Infection**
- Viral infections (hepatitis B, syphilis, varicella zoster virus, HIV, parvovirus B19, rubella, cytomegalovirus and herpes simplex virus)
- Bacterial infection including syphilis
- Protozoan infection (malaria and toxoplasmosis)

**Malformations**
- Cardiovascular
- Gastrointestinal (omphalocele, gastroschisis)
- Fetal echogenic bowel
- Single umbilical artery
- Genitourinary
- Skeletal

**Multiple gestations**
- Twin to twin transfusion
- Multifetal pregnancies

(c) Placental causes and risk factors for intrauterine growth restriction

**Placental abnormalities**
- Chromosomal mosaicism
- Infarcts
- Threatened miscarriage, placental abruption and unexplained antepartum haemorrhage
- Focal lesions (chorioangioma)
- Abnormal placentation (placenta previa, circumvallate placenta)
- Abnormal cord insertion (velamentous and battledore insertions)
- Reduced chorionic villi surface area

**Metabolism and hormones**
- Growth hormone variant
- Placental lactogen
- Insulin and steroids

The placenta employs a passive to active continuum of mechanisms to mediate and regulate the transfer of gases ($O_2$, $CO_2$), molecules (glucose, fatty acids, amnion acids) and ions (K, Cl, Na) (Figure 2.1). As a consequence of poor placentation, growth restricted fetuses tend to have reduced oxygenation and plasma concentrations of amino acids. The interaction between the endocrine function of the placenta and maternal physiology during an IUGR pregnancy is also altered, with reduced maternal insulin-like growth factor and human placental lactogen (Worton et al., 2014).

**Figure 2.1.** Placenta transfers mechanisms. (a) examples of substrates and mechanisms of transfer and (b) illustration of placental transfer mechanisms within the syncytiotrophoblast. Adapted from “Understanding the placental aetiology of fetal growth restriction; could this lead to personalized management strategies?” by S. Worton, C. Sibley, and A. Heazell, 2014, *Fetal and Maternal Medicine Review*, 25(02), p. 101.
2.2.5 Complications of IUGR.

IUGR has major clinical implications, long-term health repercussions and additional financial burdens to the health sector because of increased morbidity and mortality of the fetus, the newborn and the adult.

2.2.5.1 Fetal complications.

A fetus suffering from IUGR is at risk of hypoxaemia, acidaemia and demise. The expected death rate among IUGR fetuses has been reported to be 60 to 80 per 1,000 (Manning & Hohler, 1991) and the perinatal mortality rate to be 6 to 10 times greater than normally grown fetuses (Collins et al., 2013). The literature reports that between 30% and 52% of stillbirths are growth restricted (Collins et al., 2013; Figueras & Gardosi, 2011). Fetuses compromised by IUGR have altered cardiac function (Cosmi, Fanelli, Visentin, Trevisanuto, & Zanardo, 2011) and are more vulnerable to intrapartum distress, including irregular heart rate patterns and cord compression due to oligohydramnios (American College of Obstetricians and Gynecologists Practice Bulletin, 2001). IUGR increases the risk of preterm delivery (Suhag & Berghella, 2013) and IUGR affected fetuses are more likely to be delivered by caesarean section (Collins et al., 2013; Sehested & Pedersen, 2014).

2.2.5.2 Neonate to adulthood complications.

GA at delivery and the magnitude of placental insufficiency are major contributors to poor pregnancy outcomes with up to 10% of perinatal morbidity a consequence of IUGR (Mandruzzato et al., 2008; Yagel et al., 2010). Growth restricted newborns have a greater risk of death, or requiring resuscitation or prolonged hospitalisation (Australian Institute of Health and Welfare, 2012). Neonates who suffered from IUGR account for approximately 30% of all sudden infant death
syndrome cases (DeCherney & Nathan, 2003). Neonate morbidity associated with IUGR includes hypoglycaemia, dyslipidaemia, polycythaemia, cardiovascular disorders, necrotising enterocolitis, intraventricular haemorrhage (Mandruzzato et al., 2008; Paul, Sciscione, Leef, & Stefano, 2002; Sehested & Pedersen, 2014), periventricular leukomalacia, convulsions (von Beckerath et al., 2013), impaired cognitive function, cerebral palsy (Galan, Ferrazzi, & Hobbins, 2002), meconium aspiration syndrome, hyaline membrane disease and early onset sepsis (Saleem et al., 2011).

IUGR infants have poor long term growth despite catch-up growth (Sayers, Mackerras, & Singh, 2007) and this early rapid catch-up growth has been associated with an increased potential of developing metabolic disorders in childhood (Sehested & Pedersen, 2014). HC catch-up growth is most evident during the first six months of life (Boers et al., 2007) and approximately 90% of height and weight catch-up growth is achieved by 12 months of age (Sehested & Pedersen, 2014). A longitudinal study of Northern Territory Aboriginal children demonstrated that catch-up growth is never complete, as those born with IUGR were 2 cm shorter ($p = 0.10$), 4 kg lighter ($p < 0.01$) and had a 1 cm smaller HC ($p < 0.01$) at a mean age of 11.4 years (Sayers et al., 2007). The researchers re-examined the cohort at a mean age of 18.3 years and those with growth restriction at birth were 3 cm shorter ($p = 0.0026$), 9 kg lighter ($p = 0.0001$) and had a 0.95 cm smaller HC ($p = 0.0008$) than those born without growth restriction (Sayers, Mott, & Singh, 2011). These differences were still evident at 25 years of age (Sayers, Mackerras, & Singh, 2017).

A survey of 4 to 13 year old children in Western Australia showed that IUGR was associated with an increased risk of mental health problems (Zubrick et al., 2000). Severe affected IUGR children were more likely to have poor academic competence,
be withdrawn, suffer from aggression issues and exhibit socialising problems (Zubrick et al., 2000). IUGR is recognised as a risk factor for poor cognitive outcomes in children (Morsing & Marsal, 2014; von Beckerath et al., 2013) and a 2015 Finnish study identified poor intrauterine growth as a risk factor for attention-deficit/hyperactivity disorder (Sucksdorff et al., 2015). Japanese boys with IUGR were also found to have more adverse neurological outcomes compared to normal birthweight boys (Tamaru et al., 2011). Furthermore, an American study identified extremely low birthweight as a risk factor for adolescent boys having behavioural problems, health issues, poor social skills and being bullied more than normal birthweight boys (Yau et al., 2013).

The Barker hypothesis proposed that some adult diseases are a consequence of fetal adaption to malnourishment and oxygen deprivation (Cosmi et al., 2011). This hypothesis has been advanced by the recent proposal that epigenetic modification of regulators of fetal glucose homeostasis, in response to in utero malnourishment, cause maladaptation of multiple organs in adulthood (Devaskar & Chu, 2016). These hypotheses are supported by epidemiological work showing that small babies at birth have increased rates of cardiovascular disease, non-insulin dependent diabetes, metabolic syndrome, obesity, hypertension and hypercholesterolaemia in adulthood (Australian Institute of Health and Welfare, 2012; Barker et al., 1993; Conde-Agudelo et al., 2013; Ross, 2015; Suhag & Berghella, 2013). A review of publications undertaken by Cohen, Wong, Horne, and Yiallourou (2016) found that a large portion of available literature supported an association between IUGR with cardiovascular alterations; however, the progress of these alterations into adulthood required further longitudinal studies.
2.2.6 Fetal growth.

Intrauterine fetal and placental growth initially occurs by cell division followed by an increase in the size of the cells; any disruption to fetal cell growth will result in growth abnormalities (Suhag & Berghella, 2013). In order to detect growth abnormalities, the duration of the pregnancy needs to be correctly established from either the menstrual cycle or ultrasound parameters. Deviations in fetal size can be determined by physical or ultrasound measurements.

2.2.6.1 Gestational age.

The identification of growth restriction begins with the establishment of an accurate GA. German physician, Franz Naegele (1777-1851), calculated the due date by subtracting three months from the first day of a woman's last menstrual period (LMP), adding seven days and adjusting the year if necessary (Fraser & Copper, 2009). Alternatively, the GA can be calculated by adding 280 days or 40 weeks from the last known menses. These methods are notoriously incorrect as they rely on the correct recollection of the date of the first day of the LMP, that a woman has a regular 28 day long menstrual cycle and that conception occurred on day 14 of the cycle (Fraser & Copper, 2009). Determining the GA by pregnancy milestones, such as the date of quickening and auscultation of the fetal heartbeat, are clinically inadequate due to variability in the accurate detection of the onset of these features (Manning & Hohler, 1991).

The ultrasound calculation of GA uses the recognised relationship between fetal age and fetal size, with accuracy decreasing with increasing GA. The accuracy of ultrasound dating at gestational ages 14 to 22 weeks is ± 1 week, from 22 to 28 weeks ± 2 weeks and 28 to 40 weeks ± 4 weeks (Quinton, 2006). Guidelines for assigning
estimated date of delivery (EDD) based on either the LMP or ultrasound dating are summarised in Table 2.4. The EDD should be assigned at the time of the first ultrasound and measurements obtained from subsequent ultrasound examinations should never be used to re-date the pregnancy (Callen, 2000).

Table 2.4

Prediction of EDD Based on LMP and Ultrasound Measurements

<table>
<thead>
<tr>
<th>Gestation at ultrasound (weeks)</th>
<th>Known LMP and regular menstruation (21-35 days)</th>
<th>Unknown LMP or irregular menstruation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMP and ultrasound EDD differ by ≤ 5 days</td>
<td>LMP and ultrasound EDD differ by &gt; 5 days</td>
</tr>
<tr>
<td>6 to 13</td>
<td>NA</td>
<td>LMP</td>
</tr>
<tr>
<td>13 to 24</td>
<td>Ultrasound</td>
<td>LMP</td>
</tr>
<tr>
<td>≥ 24</td>
<td>Ultrasound</td>
<td>LMP</td>
</tr>
</tbody>
</table>

Note. LMP = last menstrual period. EDD = estimated date of delivery. NA = not applicable. Adapted from “Maternity - management of pregnancy beyond 41 weeks gestation,” by New South Wales Government Ministry of Health, 2014, p. 2.

2.2.6.2  Physical measurements.

The age and size of a pregnancy has been traditionally assessed by palpation and visual inspection of the pregnant woman. Abdominal palpation detects only 37% of SGA fetuses in high risk populations (Royal College of Obstetricians and Gynaecologists, 2014a) and performs poorly at predicting fetal weights, especially at the boundaries of normal (Nahum, 2014).

In the late 1970s, the symphyseal-fundal height was introduced as an objective, reproducible and cost effective technique of monitoring changing uterine size during pregnancy. Clinically, the distance in centimetres from the symphysis pubis to the uterine fundus equates to within three weeks of the GA between 20 and 38 weeks. A lag of 3 to 4 cm suggests IUGR (Lausman et al., 2013; Peleg et al., 1998). This method
becomes increasingly inaccurate if the clinical examination is difficult due to uterine pathology, multiparity, abnormal fetal lie, extremes of amniotic fluid, fetal head engagement or maternal obesity (Manning & Hohler, 1991; Royal College of Obstetricians and Gynaecologists, 2014a). The symphyseal-fundal height has a low sensitivity of approximately 26% and specificity of 88% for predicting IUGR (Royal College of Obstetricians and Gynaecologists, 2014a; Vandenbosche & Kirchner, 1998) and a review by Neilson (2000) found inadequate evidence to evaluate the use of this measurement in relation to pregnancy outcomes. However, when serial measurements are performed, the symphyseal-fundal height has been reported as a good surveillance tool (Figueras & Gardosi, 2011).

IUGR can be suspected if maternal weight gain is inadequate or decreasing (Peleg et al., 1998) with the mean maternal weight gain during a normal pregnancy being 14 kg for Australian women (Ash, Fisher, Truswell, Allen, & Irwig, 1989; Goodman, Clarke, & Jehne, 1992). A maternal weight gain for women in the healthy weight range of less than 10 kg (Vandenbosche & Kirchner, 1998), or the loss or gain of less than 5 kg in overweight and obese women is associated with increased risk of IUGR (Catalano et al., 2014).

2.2.6.3 Ultrasound measurements.

In order to isolate a pathologically small fetus from a heterogeneous group of small fetuses, biometric and biophysical measurements need to be undertaken. These measurements include fetal anthropometric measurements, EFW, Doppler examination of various vessels, biophysical profile and amniotic fluid assessment.

Smaller than expected first trimester crown rump length (CRL) has been associated with low birthweights (Bamfo & Odibo, 2011). However, CRL has limited
clinical application as it cannot simultaneously be used to date a pregnancy and determine if the pregnancy is growing normally, unless the exact date of conception is known (Figueras & Gardosi, 2011). A retrospective Danish study involving 3,440 primiparous, spontaneous, singleton pregnancies found that greater than a seven day discrepancy between self-reported and ultrasound GA at the nuchal translucency ultrasound examination increased the risk of a birthweight less than 2,500 g ($p < 0.05$); but, the seven day discrepancy was not an independent predictive factor for low birthweight ($p = 0.0967$) (Bonnesen, Oddgeirsdottir, Naver, Jorgensen, & Nilas, 2016).

The preeminent method for identifying and monitoring IUGR is a combination of Doppler analysis of fetal and umbilical cord vessels, and biometric analysis (Ott, 2005). Both the CNGOF (Vayssiere et al., 2015) and RCOG (2014a) recommended UA Doppler as the principle method of monitoring growth restricted fetuses and the CNGOF recommended the AC as the best screening indicator of growth restriction. This last recommendation was supported by the large United Kingdom Pregnancy Outcome Prediction (POP) study that found that fetuses with an EFW and AC less than the 10th percentile had a relative risk of 17.6, 95% CI [9.2, 34.0], of delivering a SGA neonate with neonatal morbidities of low Apgar score, metabolic acidosis or admission to a neonatal unit (Sovio, White, Dacey, Pasupathy, & Smith, 2015). Seravalli and Baschat (2015) summarised the advantages and disadvantages of different definitions of FGR (Table 2.5).
Table 2.5

*Implications for Diagnostic Cut-offs of FGR*

<table>
<thead>
<tr>
<th>Diagnostic cut-off (percentiles)</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC &lt; 10th</td>
<td>Highest sensitivity for FGR</td>
<td>Lowest specificity for FGR</td>
</tr>
<tr>
<td>EFW &lt; 10th</td>
<td>Acceptable sensitivity for FGR</td>
<td>Unnecessary monitoring of normal fetuses</td>
</tr>
<tr>
<td>EFW &lt; 3rd</td>
<td>Greater specificity for FGR</td>
<td>Less severe FGR is missed</td>
</tr>
<tr>
<td>EFW &lt; 10th and abnormal UA Doppler</td>
<td>Greater specificity for FGR at risk for adverse outcome</td>
<td>Misses term FGR with normal UA Doppler</td>
</tr>
<tr>
<td>EFW &lt; 10th with abnormal UA or MCA</td>
<td>Greater specificity for FGR at risk for adverse outcome across all GA</td>
<td>Requires interpretation of umbilical and cerebral Doppler studies</td>
</tr>
</tbody>
</table>


In 1998, only 30% of IUGR cases in Germany were detected by routine ultrasound examinations (Jahn, Razum, & Berle, 1998). Screening for SGA in France was reported to have a sensitivity of 22% (Vayssiere et al., 2015) whereas, the POP study reported an EFW less than the 10th percentile had a sensitivity of 57% for predicting a birthweight less than the 10th percentile (Sovio et al., 2015). This detection rate was improved to 67%, 95% CI [31, 91], in a recent pilot, randomised, clinical trial in which all uncomplicated pregnancies underwent third trimester scans (Hammad et al., 2016). These low detection rates are partially due to women not undergoing ultrasound examinations, an abundance of reference growth charts, and variation in the definition of IUGR.

The abundance of reference growth charts was addressed by the development of international fetal growth standards by the INTERGROWTH-21st study, which used longitudinal data collected from 4,321 healthy, low risk, singleton pregnancies recruited from eight countries (Papageorghiou et al., 2015). Customised growth potential charts have been reported to improve detection rates of IUGR by...
incorporating maternal weight, maternal height, ethnicity, parity and fetal gender (Bamfo & Odibo, 2011; S. Lee & Walker, 2010). The inclusion of fetal gender specifically improves detection of IUGR as the use of curves, undifferentiated for fetal sex, may lead to a false positive diagnosis of growth restriction in females and a false negative diagnosis in males (Unterscheider et al., 2013b; Vayssiere et al., 2015).

Gestation Related Optimal Weight (GROW) software provides international, web-based customised growth potential charts (https://www.gestation.net/index.htm). The GROW software has incorporated features of the Australian obstetric population, as data collected from 12,420 pregnancies at Nepean Hospital, Sydney, was used in the development of the software (Mongelli, Figueras, Francis, & Gardosi, 2007). The clinical application of the GROW software was assessed in a recent clinical comparison of four EFW models undertaken in Victoria, which found that the detection of growth restriction was not improved by the use of customised fetal growth charts (Baird, Davies-Tuck, Coombs, Knight, & Wallace, 2016).

Diagnostic tests can be used to identify causes of IUGR including: amniocentesis and non-invasive prenatal testing to identify chromosomal causes, infectious workup to exclude miro-organisms, and placental biopsy to identify placental mosaicism (Suhag & Berghella, 2013). With the exclusion of these causes of IUGR, placental insufficiency remains the most common cause of IUGR (Callen, 2000). In placental insufficiency, the AC is the first fetal measurement to undergo reduced growth velocity due to reduced muscle mass, adipose deposition and hepatic size as a consequence of glycogen storage depletion (Alberta Perinatal Health Program, 2008; Callen, 2000). An AC within normal range consistently eliminates a diagnosis of IUGR with a false negative rate of less than 10% (American College of Obstetricians and Gynecologists
Practice Bulletin, 2001). Reduced growth rate of the AC is followed by reduced femur growth rate, then HC and finally reduced growth of the transverse cerebellum diameter, as the severity of IUGR progresses (Pilu & Nicolaides, 1999). In a study involving 41 women, an HC/AC ratio greater than 1.067 was found to be the critical indicator of fetuses born with a birthweight below the 10th percentile and has been found to correctly identify 88% of these fetuses when scanned between 28 and 32 weeks GA (Quinton, Cook, & Peek, 2015).

An interval of two or three weeks between biometry was recommended (Mongelli, Ek, & Tambyrajia, 1998), as the error in biometric measurements is likely to surpass actual fetal growth in a shorter time span (Figueras & Gardosi, 2011). Furthermore, a four or six week interval was shown to be superior in a Scottish study of 274 low risk pregnancies (Owen, Maharaja, Khan, & Howie, 2001). More recent guidelines from RCOG and CNGOF recommend a three week interval between biometry in surveillance of growth restriction (Royal College of Obstetricians and Gynaecologists, 2014a; Vayssiere et al., 2015).

UA Doppler assessment in a normal pregnancy shows that the impedance to flow declines with advancing GA due to the expansion of the number of chorionic villi vessels and intervillous circulation (Nicolaides, Sebrire, & Snijders, 1999). The systolic/diastolic (S/D) ratio and the PI are angle independent indices used to quantify placental impedance. The elimination of small muscular arteries of the chorionic stem villi by vascular sclerosis causes increased placental vascular resistance and elevated S/D ratio and PI (Fleischer, Romero, Manning, Jeanty, & James, 1991). The likelihood of a poor pregnancy outcome increases with diminishing flow in diastole and the indices assume clinical significance when the values exceed two standard deviations above the
mean for GA. No or reversed flow in the UA during diastole is associated with an approximate 45% increase in perinatal mortality rate (Hofer, 2001) and is a consequence of more than half of the placental vessels being obliterated (Figueras & Gardosi, 2011). The extent of UA flow abnormality is directly related to neonatal hypoxia. The temporal sequence of change in the UA waveform in the presence of worsening placental insufficiency is shown in Figure 2.2.

The MCA shows reduced resistance with worsening placental insufficiency, which is reflected in a lower PI, due to vessel vasodilation to compensate for worsening hypoxia (Figure 2.3). The DV waveform loses forward flow in atrial contraction with fetal acidaemia and hypoxia (Figure 2.4). A fetus with absent DV forward flow has a 60% to 100% risk of perinatal mortality with early onset IUGR (Baschat, Gembruch, Weiner, & Harman, 2003). An abnormal DV PI value of less than one has been shown to be the best predictor of poor pregnancy outcomes in severe IUGR (AbdelMaboud & Elsaid, 2015; Baschat et al., 2007).
Figure 2.2. UA Doppler showing the sequence of increased downstream resistance with placental insufficiency. (a) sample gate within the lumen of the UA, (b) normal UA waveform, (c) increased resistance to flow, (d) absent end diastolic flow and (e) reversed end diastolic flow. Adapted from “Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management,” by F. Figueras and J. Gardosi, 2011, American Journal of Obstetrics and Gynecology, 204(4), p. 293.
Figure 2.3. MCA Doppler showing the sequence of vasodilation with placental insufficiency. (a) sample gate placement in the MCA, (b) normal high resistance waveform and (c) low resistance waveform found in fetal hypoxia. Adapted from “Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management,” by F. Figueras and J. Gardosi, 2011, *American Journal of Obstetrics and Gynecology*, 204(4), p. 295.
Figure 2.4. DV Doppler sequence with placental insufficiency. (a) sample gate placement in the DV, (b) normal low resistance waveform, (c) increased resistance to flow, (d) absent forward flow in atrial contraction and (e) reversed flow in atrial contraction. Adapted from “Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management,” by F. Figueras and J. Gardosi, 2011, American Journal of Obstetrics and Gynecology, 204(4), p. 295.
In IUGR caused by placental insufficiency, the amniotic fluid quantity may decrease due to redistribution of blood to vital fetal organs and reduced perfusion of the fetal kidneys causing reduced urine output (Bamfo & Odibo, 2011). The volume of amniotic fluid can be subjectively assessed, the single deepest vertical pool may be measured, or the amniotic fluid index (AFI) can be calculated. A normal AFI, on standard reference charts, implies normal renal perfusion (Hebbar, Rai, Adiga, & Guruvare, 2015). Reduced amniotic fluid volume can be interpreted as: an AFI below the 5th percentile on standard reference ranges (Moore & Cayle, 1990), an AFI of 5 cm or less, or a single deepest pool less than 2 cm (Magann et al., 2015).

The fetal biophysical profile is a group of observations and measurements including the AFI, fetal tone, fetal movement, fetal breathing and heart rate monitoring used to quantify fetal wellbeing. Each component is assigned a score of two if normal, or zero if abnormal, and a total of 8 to 10 is considered normal. The biophysical profile has a false positive rate of 75% for a score of six and 20% for a zero score, and requires at least thirty minutes ultrasound scanning time (Bamfo & Odibo, 2011). Two Cochrane reviews of randomised and quasi-randomised controlled trials concluded that there was insufficient evidence to determine if the biophysical profile was useful in determining fetal wellbeing (Alfirevic & Neilson, 2000; Lalor, Fawole, Alfirevic, & Devane, 2008).

Other ultrasound measurements have been proposed as possible means of identifying IUGR fetuses including cheek to cheek diameter, fetal adipose thickness at different anatomical sites, estimated fetal liver weights or liver lengths, placental volume, three dimensional (3D) limb volumes. None of these measurements has gained clinical acceptance mainly due to the overlap of normal and IUGR reference
ranges (Ott, 2005). An IUGR Index was developed in Australia using three sonographic parameters and two maternal risk factors (Niknafs & Sibbald, 2001). Attempts have been made to categorise and stage IUGR. Mari and Hanif (2008) suggested three categories based on SGA fetuses with normal or abnormal UA and MCA Dopplers or recognisable pathology. In the previous year Mari, Hanif, Drennan, and Kruger (2007) proposed a four stage classification based on EFW less than the 10th percentile, AFI, UA Doppler changes, umbilical vein, MCA, DV and tricuspid flow. These researchers found a significant correlation between the severity at staging and perinatal mortality and mortality. Again, these methods of analysing IUGR have not been widely accepted into clinical practice in Australia.

### 2.2.6.4 Temporal sequence of events.

With worsening IUGR due to placental insufficiency, there are sequential changes in umbilical cord vessel characteristics, in fetal blood profiles and fetal activity. There has been varied opinion on the sequence of initial change in fetal adaption to placental insufficiency with some researchers proposing that arterial Doppler demonstrate changes followed by fetal venous changes (Edwards, 2004; Miller et al., 2008; Quinton, 2006). Others have proposed an initial venous change with decreased umbilical vein blood flow, and then increased resistance in the umbilical cord arteries reflecting placental vessel resistance (Baschat, 2011; Yagel et al., 2010). In either case, there is redistribution of blood flow to major fetal organs due to hypoxia causing a reduction in the AFI and vasodilation of the MCA. As CO₂ removal from the fetus degrades, there is increasing fetal acid/alkali imbalance and changes in the fetal venous flow with pulsations in the umbilical vein waveform and reduced forward flow in the DV (Hecher et al., 2001; Turan et al., 2008). A proposed sequence of alteration
to arterial and venous fetal blood flow in cases of IUGR is shown in Figure 2.5, and if uninterrupted leads to life ending events. The sequence of deterioration is variable in growth restriction (Unterscheider et al., 2013b; Vayssiere et al., 2015) and in IUGR pregnancies complicated by pre-eclampsia, the deterioration may be rapid and the sequence unpredictable (Mari, Hanif, & Kruger, 2008). In preterm IUGR fetuses, there is also variability in the sequences of progression of Doppler abnormalities (Berkley, Chauhan, & Abuhamad, 2012).

If changes in the umbilical vein are one of the first adaptive changes to placental insufficiency, then developing easy to perform measurements and Australian reference ranges of the vein characteristics may provide an IUGR detection method that can be integrated into routine scanning protocols.

2.2.7 Current practices of predicting and managing IUGR.

Reliable prediction of IUGR would identify those fetuses requiring closer monitoring, management in high level obstetric facilities and may avoid neonatal and adult sequelae of IUGR by implementing preventative and risk reduction strategies (Conde-Agudelo et al., 2013). Predicting pregnancies that have the potential to be affected by IUGR begins with collecting a good clinical history identifying all risk factors. Women with a previous history of growth restriction have a 50% increased risk of IUGR in a subsequent pregnancy (Figueras & Gardosi, 2011). In the first trimester, low pregnancy associated plasma protein-A (PAPP-A) or human chorionic gonadotrophin can be associated with IUGR, as is a raised maternal serum alpha-fetoprotein (Bamfo & Odibo, 2011; Lausman et al., 2013). Women with a low first trimester PAPP-A, have an odds ratio of 2.8, 95% CI [2.0, 4.0], of delivering an IUGR fetus compared to women with normal levels (Smith et al., 2006).

Trophoblastic invasion of the maternal spiral arteries causes a reduction in the resistance to blood flow in the uterine arteries, which can be assessed by Doppler ultrasound. Between 19 and 23 weeks GA, uterine artery Doppler with either a raised PI on standard reference ranges or persistent early diastolic notch in one or both arteries, is suggestive of the development of IUGR (Lausman et al., 2013; McLeod, 2008). In a review of 61 studies that assessed uterine artery prediction of IUGR, Cnossen et al. (2008) found that in low risk populations, an elevated uterine artery PI
with notching was the best predictor of GA corrected birthweight less than the 10th percentile (positive likelihood ratio 9.1). Repeating uterine artery Doppler after 24 weeks GA provides no additional information as normalisation and subsequent loss of the early diastolic notch is still associated with an increased risk of SGA (Royal College of Obstetricians and Gynaecologists, 2014a). Velauthar et al. (2014) undertook a meta-analysis of 18 studies and showed that the sensitivity and specificity of abnormal uterine artery waveform during the first trimester in the prediction of early onset IUGR were 39.2%, 95% CI [26.3, 53.8], and 93.1%, 95% CI [90.6, 95.0], respectively. The Fetal Medicine Foundation (2008) First Trimester Screening Program includes software for determining the risk of developing growth restriction based on maternal history, mean maternal arterial pressure, uterine artery Doppler PI and maternal PAPP-A levels.

Recent research has proposed that 3D power Doppler measurement of the placental vascular bed provides an early indication of severe pregnancy problems, one of which was IUGR; however, the technique is heavily software and user dependent and unlikely to be suitable as an independent test (Hafner, Metzenbauer, Stumpflen, & Waldhor, 2013). During the second trimester elevated alpha-fetoprotein, human chorionic gonadotrophin or inhibin A, or low oestriol can be associated with development of IUGR. In a review of 37 novel biomarkers, none were identified as clinically useful for predicting IUGR in women with a singleton gestation (Conde-Agudelo et al., 2013).

There is currently no proficient treatment of IUGR; however, preventive measures to reduce risk factors are possible (Marsál, 2009; Peyter et al., 2014). Preventive methods include altering maternal behaviours, with smoking cessation during pregnancy preventing 17% of low birthweight babies (Suhag & Berghella, 2013).
Other measures include improving nutrition, elimination of illicit drug use, treatment of infections, antimalarial precautions and medical management of maternal diseases. In addition, low dose aspirin from early second trimester through to 36 weeks GA is recommended for women with a previous history of IUGR or with two or more risk factors (Lausman et al., 2013).

IUGR management is based on careful monitoring (Peyter et al., 2014). Provided the fetus has reached a suitable GA, the aim of surveillance in IUGR is to optimise the timing of delivery prior to fetal demise and the occurrence of permanent damage (Kaponis et al., 2011; Marsál, 2009). Timing of delivery is one of the major considerations in management of IUGR, as mortality and morbidity implications of a preterm delivery need to be weighed against the mortality and morbidity of IUGR. At less than 29 weeks, GA was the most important determinant of undamaged survival in a prospective study of preterm, live born, IUGR neonates (Baschat et al., 2007). After 29 weeks, birthweight and DV Doppler were the best predictors of neonatal outcome (Baschat et al., 2007). Several recent clinical trials have attempted to investigate the optimum timing of delivery. The Growth Restriction Intervention Trial (GRIT) encompassed 69 hospitals in 13 European countries between 1996 and 2002 and recruited 548 women who were between 24 and 36 weeks pregnant with an IUGR fetus. The GRIT study showed no difference in motor or intellectual morbidity up to school age regardless of early or delayed delivery (Walker et al., 2011). The Trial of Randomized Umbilical and Fetal Flow in Europe (TRUFFLE) was performed between 2005 and 2010 in 20 European centres. Of the surviving infants, more were free of neurological impairment if they were delivered according to changes in the DV, as opposed to cardiotocography (CTG) changes, but this was accompanied by a non-
significant increase in perinatal and infant mortality (Vora & Chescheir, 2015). Tate and Mari (2013) summarised the results of the Disproportionate Intrauterine Growth Intervention Trial at Term (DIGITAT) from the Netherlands. This trial found maternal and perinatal outcomes were similar for term IUGR fetuses irrespective of whether they were induced or underwent expectant management (Tate & Mari, 2013). Doppler investigation is an efficient method of surveillance in IUGR monitoring, but there remains controversy over which Doppler provides the best predictors of poor outcome. The DV was considered as the strongest Doppler predictor of perinatal mortality in preterm IUGR fetuses (Giuliano et al., 2014; Yagel et al., 2010). If preterm delivery is anticipated, administration of corticosteroids can accelerate fetal lung development (Royal College of Obstetricians and Gynaecologists, 2014a).

When IUGR is diagnosed, serial ultrasound examinations, UA and other fetal Dopplers can be used in fetal monitoring, as previously discussed. In addition, the extent of fetal distress can be detected by the number of gross fetal movements (kick chart) or by analysing CTG fetal heart traces. As the level of hypoxia increases, the compromised fetus will attempt to conserve oxygen by reducing gross body movements, ceasing breathing movements, reducing muscle tone, and can develop a non-reactive CTG heart pattern (Rumack, Wilson, & Charboneau, 2005).

The optimum mode of delivery of IUGR fetuses is controversial, with IUGR fetuses more likely to be delivered by an emergency caesarean section to avoid fetal distress during labour (Marsál, 2009). However, the preferred method of delivery is dependent on the obstetrician, fetal age and the site of delivery (Bamfo & Odibo, 2011; Collins et al., 2013).
2.2.8 Conclusion.

IUGR is a complication of pregnancy with a prevalence of approximately 10%; however, the definitions of IUGR are numerous and diagnostic criteria are controversial. The early identification of IUGR has the potential to reduce complications during pregnancy and adverse consequences throughout life. The sequence of deterioration in a growth restricted fetus suggests that umbilical vein ultrasound characteristics may provide a means of identifying and monitoring fetuses at risk of IUGR.

2.3 The Umbilical Cord

2.3.1 Introduction.

The umbilical cord provides the conduit for unhindered blood transport from the placenta to the fetus and vice versa. Aristotle (384 BC-322 BC) originally identified the umbilical cord as the connection between the mother and unborn child (Gill, Kossoff, Warren, & Garrett, 1984; Needham, 1959). The following description of the development, structure and blood flow within the umbilical cord with specific reference to the fetal venous system formed the basis of a review article published in the Australasian Journal of Ultrasound in Medicine, August 2012 (Appendix A).

In the research described in this exegesis, the term umbilical vein (UV) is a used as a generic term referring to the entire length of the vessel from placenta to within the fetal liver. When the portion within the fetal abdomen is specifically discussed this will be referred to as the intra-abdominal UV. The term umbilical cord vein (UCV) specifically defines the intra-amniotic portion of the vein contained within the umbilical cord.
### 2.3.2 Umbilical vein development.

The fetal venous system transfers blood from the placenta to the fetus and develops from three embryological paired veins (Hofstaetter, Plath, & Hansmann, 2000): the vitelline veins from the yolk sac, the umbilical veins from the chorion and the cardinal veins from the embryo (Fasouliotis, Achiron, Kivilevitch, & Yagel, 2002). Development of the fetal venous system is complete by the end of the first trimester (Fasouliotis et al., 2002) and results in the obliteration of the right umbilical vein and the development of the DV between the persisting left umbilical vein and the inferior vena cava (IVC) (Hofstaetter et al., 2000) (Figure 2.6).

*Figure 2.6. Development of the human venous system and the resulting single left umbilical cord vein (arrow). Adapted from “Prenatal diagnosis of abnormalities of the fetal venous system,” by C. Hofstaetter, H. Plath, and M. Hansmann, 2000, Ultrasound in Obstetrics and Gynecology, 15(3), p. 232.*
2.3.3 Fetal venous circulation.

The persisting left umbilical vein travels from the placenta to enter the fetal abdomen at the umbilicus and courses into the liver (Figure 2.7). In the first half of pregnancy, 32% to 50% of the oxygenated blood passes through the DV to enter the left hepatic vein near its confluence with the IVC, and near to term this reduces to approximately 25% (Baschat, 2006; Vasques et al., 2004). The remaining blood enters the left and right portal veins of the liver and drains into the IVC via the hepatic veins (Vasques et al., 2004).

The blood from the DV enters the IVC along with blood returning from the hepatic veins, lower limbs and abdominal wall and flows into the right atrium. From the right atrium, there are two blood circulation pathways (Figure 2.8):

i. Blood from the IVC combines with blood from the superior vena cava (SVC) and passes through the tricuspid valve into the right ventricle. From the right ventricle, blood enters the pulmonary trunk and the ductus arteriosus to flow into the descending aorta for distribution to the organs of the abdomen, pelvis, lower limbs and return to the placenta by the paired umbilical arteries.

ii. Blood diverted through the DV enters the left atrium via the foramen ovale, and this bypass of the right ventricle occurs due to the higher velocity of the DV blood and valve placement within the IVC. The blood in the left atrium joins blood from the pulmonary veins to enter the ascending aorta for distribution to the heart, head and upper limbs and eventual return to the heart via the SVC (P. L. Williams, 1995).
2.3.4 Umbilical cord structure.

When fully developed the umbilical cord has a length of approximately 50 to 60 cm (Di Naro, Ghezzi, Raio, Franchi, & D'Addario, 2001) and a diameter of 1 to 2 cm (P. L. Williams, 1995). The umbilical cord normally contains two umbilical arteries, one umbilical vein and the remnant of the allantois all embedded in Wharton's jelly, which is surrounded by a single layer of amnion (Figure 2.9) (Bergman, Afifi, & Heidger, 1999; Ferguson & Dodson, 2009). The umbilical arteries and vein are atypical to their counterparts in the remainder of the fetal body as the umbilical vein transports oxygenated blood to the fetal heart, whereas the arteries carry oxygen-depleted blood back to the placenta (Hofstaetter et al., 2000).
The walls of the UA lack an internal and external elastic lamina and the mucous connective tissue replaces the adventitia found in other arteries. The umbilical vein has a thickened muscularis layer with intermingling circular, longitudinal and oblique smooth muscle fibres as well as internal elastic lamina (Bergman et al., 1999). Obliteration of the allantoic duct occurs by the 15th week of gestation and the remains are located between the two arteries. Minute vitelline ducts may also be seen in the cord (Benirschke & Kaufmann, 2000). Wharton’s jelly was named after Thomas Wharton (1614-1673), an English physician who first described the substance. Wharton’s jelly is essentially a mucous connective tissue of interlacing collagen fibres in which the umbilical vessels are embedded (Bergman et al., 1999; Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al., 2001). Wharton’s jelly facilitates the transfer of water.

and metabolites between the amniotic fluid and cord vessels, may provide the contractile function of the absent adventitia, and protects the vessels against compression and bending (Di Naro, Raio, Cromi, & Giocolano, 2012).

A single umbilical cord artery may be the result of atresia, aplasia or agenesis (Figure 2.10a), with the left UA being absent more frequently. Alternatively, there may be multiple arteries (Figure 2.10b) (Predanic, 2005). Located within 3 cm of the cord insertion into the placenta surface (Cunningham et al., 2010), there is a 1.5 to 2 cm long shunt between the umbilical cord arteries, termed the Hyrtl anastomosis (Raio, Ghezzi, Di Naro, Franchi, & Bruhwiler, 1999). The functions of the Hyrtl anastomosis are to equalise pressure between the umbilical arteries before they enter the placenta and to act as a safety valve in case of placental compression or blockage of an UA (Gordon, Eytan, Jaffa, & Elad, 2007).

Figure 2.10. Ultrasound images of the umbilical cord arteries. (a) single UA and (b) three umbilical arteries with in the umbilical cord. De-identified images used with permission of the individuals.
The umbilical arteries and umbilical vein are often arranged in a spiral (Ferguson & Dodson, 2009), which may provide resistance against compression. The umbilical cord spirals are dextral (clockwise) in approximately 90% and sinistral in the remaining (Reynolds, 1978). The umbilical cord may develop up to 40 spirals, with an average being 10 to 11 coils along its length (Di Naro et al., 2012). The spiralling has been attributed to the helical muscle layers in the UA walls (Callen, 2000); however, whether this mechanism is genetic or acquired is still under debate (Di Naro et al., 2012).

In addition to its atypical transportation of oxygenated blood and prominent muscularis layer, the umbilical cord vein has the following distinguishing characteristics:

i. The umbilical cord vein pressure increases from 4.5 mmHg at 18 weeks gestation to 6 mmHg at term (Kiserud, 2005).

ii. The area of the umbilical cord vein is approximately 30% larger than the combined areas of the arteries and as such the velocity in the vein is approximately half as slow as the velocity in either artery (Reynolds, 1978).

2.3.5 Mechanisms of blood flow in the umbilical cord vein.

The fetus can be considered a closed system as the quantity of blood flow from the placenta to the fetus and vice versa are approximately equal (Reynolds, 1978). The placenta delivers oxygenated blood to the umbilical vein, with a residual pressure from the placenta (Kiserud, Eik-Nes, Blaas, Hellevik, & Simensen, 1994), and this blood is transported through the UV to the fetus by the following methods:

i. Blood pressure gradient enables blood movement because blood pressure is higher in the UV than in the fetal IVC (Reynolds, 1978). This gradient is
due to at least two mechanisms. Firstly, normal fetal heart contractions produce a pressure gradient between the atria and ventricles, which in turn diminishes the preload in the venous circulation and allows the blood in the UV to move towards the fetal heart (Fasouliotis et al., 2002). Secondly, changes in fetal abdominal and thoracic cavity pressures occur during fetal breathing movements and these cause a pressure gradient between the UV and the DV (Fasouliotis et al., 2002) such that there is an increase in the velocity of the blood in the UV during inspiration (Vasques et al., 2004).

ii. Passive pressure changes in the UCV occur due to longitudinal distortion of the arteries with each fetal heartbeat. This results in pressure peaks in the UA and UV being out of phase by 180° (Reynolds, 1978) subsequently causing multiple peaks in an additive fashion along the length of the UCV (Di Naro, Ghezzi, Raio, Franchi, D'Addario, et al., 2001).

2.3.6 Conclusion.

The UV is the remnant of embryological venous development that results in the obliteration of the right umbilical vein and the establishment of two pathways through the liver and heart for oxygenated blood travelling from the placenta to the fetus via the persisting left UV. The umbilical cord normally contains two umbilical arteries and an umbilical vein, and it commonly exhibits dextral twisting along its length. Oxygenated blood moves in the UV, from the placenta to the fetus, due to pressure gradients established by fetal heart contractions, fetal breathing and distortions of the umbilical cord arteries.
2.4 Ultrasound Assessment of the Umbilical Vein

2.4.1 History of measurements of the umbilical vein.

In 1884, Cohnstein and Zuntz made the first attempt to examine the circulation within the umbilical cord by placing a stromuhr in the UA of a fetal lamb (Gill et al., 1984). Early investigations into umbilical cord blood flow were hampered by inaccessibility and the risk of infection (Kurjak & Rajhvajn, 1982). In the 1960s and 1970s various in utero and postpartum methods were used to quantify umbilical vein blood flow including xenon isotope injection, plethysmography, electromagnetic flow meters and thermodilution (Eik-Nes, Marsal, Brubakk, Kristofferson, & Ulstein, 1982; Lees, Albaiges, Deane, Parra, & Nicolaides, 1999). In the 1980s, the advancement of ultrasound imaging sparked a cascade of research into the umbilical cord vessels, with UA Doppler parameters dominating research and acceptance into clinical practice. Early research into the umbilical vein was led by Australian researchers (Gill, 1979; Gill et al., 1984) and in recent years several international researchers have published reference ranges for ultrasound B-mode and Doppler measurements of the umbilical vein (Acharya, Wilsgaard, Berntsen, Maltau, & Kiserud, 2005; Barbieri, Cecatti, Surita, Marussi, & Costa, 2012; Flo, Wilsgaard, & Acharya, 2010; Rizzo, Rizzo, Aiello, Allegra, & Arduini, 2016). Clinical practice has been slow to embrace measurements of umbilical vein due to the perceived difficulty in acquiring measurements, relative inaccuracy of results, low reproducibility (Ferrazzi et al., 2000; Kaponis et al., 2011), lack of reference ranges and the absence of clinically proven relevance of such measurements. Recent improvement in image quality, the development of population specific reference ranges, a clinically proven relationship between UV measurements and IUGR, and the suggestion that changes in umbilical venous flow may precede changes in other fetal
measurements in the compromised fetus may overcome this resistance. The development of Australian reference ranges will hopefully accelerate the integration of UV measurements into everyday clinical practice.

2.4.2 Umbilical vein measurement sites.

There are two possible sampling sites for ultrasound measurements of the umbilical vein: the UCV or the intra-abdominal UV. The main advantages of the intra-abdominal UV are the absence of transmitted pulses from the UA (Vasques et al., 2004), the sampling site can be anatomically fixed (Flo, Wilsgaard, & Acharya, 2009; Tchirikov, Strohner, Förster, & Hüneke, 2009) and this portion has a known parabolic flow profile (Flo et al., 2009). The main disadvantages of the intra-abdominal portion include awkward orientation of the fetal abdomen presenting the UV in a plane that is not ideal for accurate diameter or velocity measurements, acoustic shadowing from ossified fetal bones and direct insonation of the fetus.

The main advantages of the UCV include ease of location, sonographer familiarity with Doppler examination of the umbilical cord due to assessment of the UA, easier optimisation of the Doppler angle and reduced insonation of the fetus. The main disadvantages of the UCV are cord mobility, inability to reproduce the sampling site in subsequent examinations (Flo et al., 2009), variability in the spatial velocity profile coefficient along the length of the cord (Acharya et al., 2005) and the potential impact of coiling (Di Naro, Ghezzi, Raio, Franchi, D'Addario, et al., 2001; El Behery, Nouh, Alanwar, & Diab, 2011). In addition W. Li, Ruan, Zhang, and Zeng (2006) reported that the UCV diameter significantly decreased in size ($p < 0.01$) in a segment of cord nearer the fetal end of the cord, and the size reduced from the placental end (3.067 mm) of this segment to the fetal end (2.3 mm) by less than a 1 mm. This trend
has been incorrectly reported in the literature (Figueras, Fernández, Hernández-Andrade, & Gratacós, 2008; Prior, Mullins, Bennett, & Kumar, 2014) due to the confusing nomenclature used by Li and colleagues.

There are persuasive arguments for measurement of either portion of the UV, but the method most easily assimilated into everyday practice is the one that requires the least change in clinical practice, is the easiest to undertake, is always accessible, and that minimises fetal exposure to ultrasound. For these reasons the UCV is favoured in clinical practice as it is easier to locate and measure (Flo et al., 2010).

### 2.4.3 Umbilical vein diameter.

There are numerous techniques for measuring the dimensions of the UV and these include measuring the diameter or the area, vessel wall inclusion or exclusion, and automatic software measurement. In addition, there are variations in the UV diameter depending on the location, fetal behavioural states and GA. The relationship of the UV diameter with GA has been interpreted in several ways, as has the variation of the diameter in the presence of IUGR. This section of the literature review discusses the above aspects and includes information pertaining to both the diameter and area of the umbilical vein, as the area can be derived from diameter measurements and both can be used for calculation of the UV blood flow.

#### 2.4.3.1 Measurement of the umbilical vein diameter.

There are several techniques for measuring the UV diameter. Link, Clark, and Lang (2007) measured the outer to inner diameter in a plane parallel to the longitudinal axis of the UCV three separate times. Acharya et al. (2005) used the average of five measurements of the inner diameter of the intra-abdominal UV. Ferrazzi et al. (2000) and Rigano et al. (2008) used the average of three inner to inner
measurements in the longitudinal plane of the UCV. Sutton et al. (1990) and Barbera et al. (1999) used the transverse plane to measure the UCV. Ghezzi et al. (2005), Togni et al. (2007) and Gerada et al. (2006) calculated the cross-sectional area of the UCV by tracing the circumference of the vein with the ellipse or spline function of the ultrasound machine and more recent research has utilised automated nuchal translucency functions for vein diameter measurements (Rizzo et al., 2016).

In several publications reviewed, researchers indicated that ultrasound data was acquired during fetal quiescence to eliminate irregularities due to fetal breathing, movement, hiccupping, etc. (Acharya et al., 2005; Flo et al., 2009; Rigano et al., 2001). The practice of acquiring data during fetal quiescence was validated by Boito, Ursem, Struijk, Stijnen, and Wladimiroff (2004), who found that in 17 normal pregnancies there was a statistically significant increase of 5.6% in the cross-sectional area of the UCV between a quietly asleep state and a state exhibiting gross fetal and eye movements. Further support for measuring the UCV diameter during fetal quiescence was presented by Nyberg, Johnsen, Rasmussen, and Kiserud (2010), whose research found a 27% increase in UCV area between fetal resting and breathing states.

2.4.3.2. Normal ranges and relationship with gestational age.

Reference ranges have been established for both the diameter and cross-sectional area of the UCV. Both measurements are included in the following discussion to allow a broader analysis of trends.

Moinian, Meyer, and Lind (1969) examined a transverse section of 255 cord specimens collected after normal deliveries that had been double clamped to retain cord blood and fixed in formalin. They determined that the mean UCV luminal diameter was $6.6 \pm 0.2$ mm five seconds after delivery. Although the GA at delivery was
not documented, it is assumed that a normal delivery inferred a term delivery. The validity of pathological studies has been questioned when formaldehyde solution (formalin) was used as the fixing agent, as it causes the umbilical cord and vessels to shrink (Raio et al., 2003).

An early study by Chen, Lu, Cheng, Hsieh, and Liu (1986) using the Octoson ultrasound machine measured the diameter of the intra-abdominal UV on 163 normal pregnancies between 25 and 41 weeks GA. These researchers found a curvilinear relationship between diameter and GA: the UV diameter steadily increased from 4.7 mm at 25 weeks GA to a maximum of 7.8 mm at 38 to 40 weeks GA, followed by a slight decline to 7.6 mm at 41 weeks GA.

The linear regression formula, based on UCV diameter, supplied by Sutton et al. (1990) indicated the UCV diameter increased from 4.3 mm at 19 weeks to 8.7 mm at 40 weeks. Weissman, Jakobi, Bronshtein, and Goldstein (1994) measured the UCV near the entrance to the abdominal wall in the longitudinal plane, using inner to inner diameter, on 368 uncomplicated pregnancies. Their regression formula described a curvilinear relation with GA, with a diameter of 2.6 mm at 16 weeks, 9.1 mm at 40 weeks and a maximum of 9.2 mm at 42 weeks.

Barbera et al. (1999) undertook a study of 70 pregnant women who delivered normally grown fetuses at term. They measured the UCV inner width three times and determined the diameter increased in a linear fashion from 4.1 mm at 20 weeks 8.3 mm at 38 weeks GA. Using 511 longitudinal observations on 130 low risk pregnant women, Acharya et al. (2005) concluded the diameter of the intra-abdominal UV had a curvilinear relation with advancing GA and increased steadily from a median of 2.6 mm at 19 weeks to 6.1 mm at 35 weeks and then slowly increased to 6.8 mm at 41 weeks.
The birthweights of 3% of neonates included in Acharaya et al.’s (2005) study were below the 5th percentile for GA and given that these would be classified as IUGR and assuming that there is a significant relation between UV diameter and IUGR, their inclusion may have reduced the overall diameter measurements. A similar relationship was described in 2012, using a prospective cross-sectional study on 2,310 low risk singleton pregnancies. The UCV adjacent to abdominal wall insertion was measured in the longitudinal plane and a curvilinear relation with GA was described, which showed a rapidly increasing diameter from 12 to 27 weeks and then a slower increase to 9.1 mm at 40 weeks GA (Barbieri et al., 2012).

In a longitudinal prospective study of 53 low risk pregnancies, Flo et al. (2010) established reference percentiles that indicated the UCV diameter had a curvilinear relation with GA and steadily increased from 4.0 mm at 22 weeks to 7.6 mm at 39 weeks. This trend was reflected in a large, prospective, cross-sectional study of 852 low risk pregnancies that used quantile regression for modelling of their intra-abdominal UV data (Rizzo et al., 2016). However, a private communication with Rizzo revealed that the published coefficients for the UV diameter were incorrect; the correct 50th percentile values indicated the intra-abdominal UV diameter increased in a curvilinear trend and ranged from 2.3 mm at 16 weeks to 7.3 mm at 40 weeks.

Togni et al. (2007) published reference ranges of the UCV cross-sectional area for gestational ages ranging from 24 to 39 weeks and showed a statistically significant correlation between the cross-sectional area of the UCV at its insertion into the fetal abdomen and GA. These Brazilian researchers observed that the UCV cross-sectional area increased up to 34 weeks gestation; reached a plateau in the 38th week and then decreased. Togni et al.’s (2007) results supported those published in 2001 that showed
that both the umbilical cord area and diameter increased with GA up to the 34th week of pregnancy and then decreased until delivery (Di Naro, Ghezzi, Raio, Franchi, & D'Addario, 2001). Also using the UCV area, Rostamzadeh, Kalantari, Shahriari, and Shakiba (2015) established reference ranges from a cross-sectional study of 278 low risk pregnancies between 15 and 41 weeks GA. The cross-sectional area was computed using ultrasound machine software and was found to have a curvilinear relationship with advancing GA, as the area steadily increased up to 30 weeks GA and then plateaued.

In summary, the articles reviewed indicate the UV diameter increases with GA, with a diameter of approximately 2.5 mm at 16 weeks, 4 mm at 20 weeks and 8 mm at term. In these articles both linear and curvilinear trends were identified with researchers describing a continued linear increase with GA (Barbera et al., 1999; Sutton et al., 1990), an increase in UV diameter followed by a slower increase towards term (Acharya et al., 2005; Barbieri et al., 2012; Rizzo et al., 2016; Weissman et al., 1994), or an increase in UV diameter followed by a decline towards term (Chen et al., 1986; Di Naro, Ghezzi, Raio, Franchi, & D'Addario, 2001; Togni et al., 2007). The trend of a more rapid increase of the UV diameter earlier in the pregnancy followed by slower growth towards term was reflected in results by Najafzadeh, Jacoby, Mattes, and Dickinson (2016). These Australian researchers, in a prospective longitudinal study of 136 low risk pregnancies, found the UCV diameter had a 107% increase in size between 18 and 26 weeks GA and only a 31% increase between 26 and 34 weeks.

2.4.3.3. Umbilical vein diameter in IUGR.

The diameter of the umbilical vein may be altered in fetuses that are compromised by growth restriction; several theories have been proposed to explain
this change. Ferrazzi and colleagues (2000) studied 37 IUGR fetuses and plotted their UCV diameters against a linear nomogram established by their previous research team (Barbera et al., 1999), and concluded that UCV diameter was not significantly reduced in IUGR fetuses. However, other researchers have found that there was a change in the diameter or area of the UV in growth restricted fetuses. Using computerised morphometry on clamped and fixed umbilical cords, Bruch et al. (1997), found the UCV area of 47 IUGR fetuses with normal UA Dopplers was reduced compared to 63 normal samples. Furthermore, they found that the UCV area tended to be decreased in 32 IUGR samples that had abnormal UA Dopplers compared to the IUGR sample with normal UA Doppler indices (Bruch et al., 1997). Rigano et al. (2001) substantiated Bruch et al.’s (1997) finding; Rigano et al. determined that in fetuses between 23 and 36 weeks of gestation, the UCV diameter (5.09 mm) in 21 IUGR fetuses was significantly less than the 36 age matched control fetuses (6.42 mm). Boito, Struijk, Ursem, Stijnen, and Wladimiroff (2002) also found that the cross-sectional area of the UCV was reduced in 84% of IUGR fetuses.

Raio et al. (2003) found that in a case controlled study of 84 IUGR fetuses, compared to 184 normally growing fetuses, that the prevalence of reduced cross-sectional area of the entire umbilical cord was significantly higher in IUGR fetuses compared to normally grown fetuses. These researchers also found that UCV area decreased significantly with worsening UA Doppler parameters. Supporting evidence by a collaborative, cross-sectional study between Italy and Switzerland involving 1,391

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1 AC < 2 SD below mean and birthweight < 10th %
2 Birthweight < 10th % for GA, normal and abnormal Doppler waveforms
3 AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
4 AC and birthweight < 5th %
5 AC < 5th % and birthweight < 10th %
women, found that the reduction in the UCV cross-sectional area was strongly associated with poor intrauterine growth and neonatal outcomes (Ghezzi et al., 2005).

In a case control study involving 12 singleton IUGR fetuses who died in utero, 14 IUGR fetuses who survived and 22 normal controls, Rigano et al. (2008) demonstrated that the UCV diameter was significantly lower in those fetuses who died, than in viable IUGR and normally grown controls. These researchers proposed that the UCV diameter was prognostically important in cases of early diagnosed IUGR.

Consistent with the previous research, Somprasit, Chanthasenanont, and Nuntakomon (2010) found that the UCV diameter in 70 IUGR fetuses was smaller than the diameter measured in age-matched appropriately grown fetuses. Contradictory to these findings, a 2014 comparison study of 40 patients with pre-eclampsia and/or IUGR with 49 normal controls, found no significant difference ($p = 0.064$) between the two groups for the UCV area measured at the fetal umbilicus; however, there was a correlation between UCV area and birthweight (Arslan, Mendilcioglu, Sanhal, Ozekinci, & Simsek, 2014).

Several hypotheses have been proposed to explain the presence of a narrower UV diameter in fetuses compromised by IUGR. Raio and colleagues (2003) hypothesised that abnormal placentation may cause early reduction in UCV velocity and remodelling of the vein, with subsequently reduced fetal growth and eventually abnormal UA Doppler indices. Bruch and colleagues (1997) proposed two mechanisms for reduction in the vein cross-sectional area: vasoconstriction and vascular

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6 AC < 2 SD below mean, UA PI > 95th % and abnormal uterine artery Dopplers
7 EFW < 10th % for GA
hypoplasia. Vasoconstriction decreases the luminal area by shortening the muscle fibres, whereas vascular hypoplasia results from decreased muscle cell mass. It has also been proposed that the UV is smaller in IUGR fetuses as the vessel size alters as a response to chronic hypovolemia and venous overload (Di Naro et al., 2012).

In summary, the majority of researchers identified a reduction in the diameter or cross-sectional area of the UV when fetuses are compromised by IUGR. This reduction has been found to be more extreme in the presence of abnormal UA Dopplers and poor pregnancy outcomes. Several theories have been proposed to explain the difference in the size of the umbilical vein between normal and growth comprised fetuses, including remodelling, vasoconstriction, vascular hypoplasia and blood volume changes.

2.4.4 Umbilical vein velocity.

Velocity is a vector measurement of the rate and direction of blood movement in the UV. Manual or automatic measurements of the velocity of blood within the UV can be reported in several forms. Calculation of blood flow incorporates the mean velocity which can be measured automatically or computed from the peak velocity. Knowledge of the haemodynamic characteristics of the UV and Doppler angle of insonation is required to correctly measure velocity with Doppler ultrasound. This portion of the literature review discusses the above aspects and also describes the various relationships that have been documented between UV velocity and advancing GA during normal and IUGR pregnancies.
### 2.4.4.1 Measurement of the umbilical vein velocity.

#### 2.4.4.1.1 Measurement methods.

Umbilical vein velocities have been reported using various measurement forms. The time-averaged maximum velocity ($T_{\text{amaxv}}$) is a software measurement equivalent to the maximum ($V_{\text{max}}$) or PV (peak velocity) averaged over several cardiac cycles. The PV or $V_{\text{max}}$ are synonymous terms and will be simply referred to as PV (peak velocity). PV can be measured manually or by automated trace. The time-averaged mean velocity ($T_{\text{ameanv}}$) is a software measurement equivalent to the mean velocity ($V_{\text{mean}}$) averaged over several cardiac cycles. The $V_{\text{mean}}$ can be a software measurement or calculated from $T_{\text{amaxv}}$ or PV and the spatial velocity profile coefficient. Some researchers have reported time-averaged intensity weighted mean velocity, which reflects the spatial mean blood velocity at any given time (Tchirikov, Rybakowski, Huneke, Schoder, & Schroder, 2002), but is not available on all machines (Flo et al., 2009).

The PV is a plainly defined and robust measurement that is resistant to small deviations from a $0^\circ$ insonation angle (Pennati, Bellotti, De Gasperi, & Rognoni, 2004). Automatic measurement of the PV is a software dependent function and will be incorrect if spectral trace thresholds are erroneously set; whereas, manual measurement of the PV is dependent on the subjective choice of the correct peak by the sonographer. The PV can be recorded using a small sample volume compared to the lumen of the vein, ensuring that the venous flow sample is not disturbed by adjacent arterial flow (Boito et al., 2002). Shortcomings in measuring the PV include noise distortion, failure to encompass the PV in the sample gate, and a tendency to overestimate the PV by up to 30% due to geometric spectral broadening as insonation...
angles approach 90° (Boito et al., 2002; Hoskins, 2011; S. Li, Hoskins, Anderson, & McDicken, 1993).

Colour Doppler cineloop analysis was used by researchers to measure PV in a cross-sectional study of 18 uncomplicated, singleton pregnancies (Gerada et al., 2006). Comparisons of PV recorded by both colour Doppler cineloop offline analysis and spectral Doppler showed a significant difference between PV recorded by the two different methods, with the cineloop analysis being approximately 10% higher (Gerada et al., 2006). The cineloop technique is less angle dependent and requires fewer assumptions about the spatial velocity profile than spectral Doppler, but has not been embraced by mainstream ultrasound practice (Gerada et al., 2006). The importance of Gerada et al.’s (2006) research was to emphasise that a strict adherence to protocol is essential in any clinical practice and methods of acquiring numerical data are not always interchangeable.

The mean velocity is required for computation of blood flow and can be automatically measured by encompassing the entire lumen within the sample gate and recording the $T_{mean}$ or $V_{mean}$. Overestimation of automatic measurements of the mean velocity occur if the Doppler sample gate does not encompass the whole vessel and if slower velocities are automatically removed by high pass filters (Fernández et al., 2008; Ferrazzi et al., 2000). This filter is also known as low velocity reject or wall motion filter (Bhide et al., 2013). The accuracy of the $T_{mean}$ is also diminished by noise from vein wall movement and echoes from adjacent vessels (Acharya et al., 2005).

The mean velocity can also be calculated by $0.5 \times T_{max}$ or $0.5 \times PV$, where 0.5 is the commonly used spatial velocity profile coefficient (Acharya et al., 2005). The mean velocity calculated in this way assumes a laminar (Sutton et al., 1990) and
symmetrically parabolic flow profile (Kiserud et al., 1994) (Figure 2.11) through the cord (Fernández et al., 2008; S. Li et al., 1993), throughout the cardiac cycle and a fully developed axial flow, inferring that the velocity profile does not change along the length or axes of the vessel (Hoskins, 2011). Some researchers have modelled the UV blood movement on a helicoidal conduit and assumed an asymmetrical flow profile (Boito et al., 2002). While others found that the flow profiles in the UCV were flat at the placental insertion and developed a more parabolic profile further downstream (Pennati et al., 2004). In Pennati et al.’s (2004) study, involving 10 singleton pregnancies between 26 and 34 weeks gestation, spatial velocity profile coefficients of 0.85 and 0.61 were found to be more suitable for $V_{\text{mean}}$ calculations at the cord insertion site and for the UCV portion cord, respectively.

Figure 2.11. Parabolic and laminar blood flow profile within the internal jugular vein. De-identified image used with permission of the individual.
Researchers (Acharya et al., 2005; Flo et al., 2010) have reported the time-averaged intensity weighted mean velocity. However, this mean velocity measurement is also stated to be highly unstable due to noise, effects of high pass filters (Flo et al., 2009; Pennati et al., 2004), sample gate size, spectral broadening (Figueras et al., 2008), non-uniform insonation, and misalignment of the Doppler beam (S. Li et al., 1993).

2.4.4.1.2 Haemodynamic characteristics.

All UV velocity measurement techniques assume a steady flow; however, pulsations or fluctuations can be present within the UV. Pulsatile flow in the UV is a normal observation until the 12th week of gestation, due to a physiological decrease in ventricular compliance (Hofer, 2001). Pulsatile UV flow can be observed in normal second and third trimester fetuses during fetal breathing, hiccupping and movement (Nicolaides et al., 1999; Zheng, Sampson, & Soper, 1998). Fetal breathing produces changes in intra-abdominal and intra-thoracic pressure causing an increase in UV velocity during inspiration and a decrease during expiration (Vasques et al., 2004).

Pulsatile UV flow is observed in fetuses with severe IUGR, congestive and congenital heart disease, twin-to-twin transfusion syndrome (Hellevik et al., 2000), cord compression, cord knots, or bradycardia (Loughna, 2002). The number and location of the UV pulsations can vary. Pulsations associated with fetal compromise typically occur at the end of diastole and may be single, double or triple (Callen, 2000); whereas, pulsations occurring in systole are associated with cord compression, knots and bradycardia (Loughna, 2002).

The mechanisms producing pulsations in compromised fetuses include increased after-load on the fetal heart as a manifestation of congestive cardiac failure.
and abnormal cardiac filling due to increased placental resistance, sustained peripheral vasoconstriction, and impaired myocardial performance (Callen, 2000; Murta, Moron, & Ávila, 1999). In addition, the IVC and DV have normal cardiac pulsations produced by atrial contractions that are not ordinarily transmitted to the UV due to the ductus sphincter acting as a bulwark preventing back flow (Kiserud, 2000); however, the DV can relax in severe fetal compromise, transmitting double pulsation along the UV (Hofstaetter, Gudmundsson, & Hansmann, 2002).

UV pulsatility can also result from transmitted arterial pulses and external compression from the neighbouring umbilical arteries (Todros et al., 2002; Yagel et al., 2010). The direction of propagation of pulsations in compromised and breathing fetuses is from the fetus to the placenta (Reed & Anderson, 2000), which is in the opposite direction to the UV flow, thus causing a decrement in the velocity waveform (Kiserud, 2003a). Whereas, external pulsation of severely abnormal umbilical arteries is in the same direction as UCV flow and can cause a rise in the velocity (Todros et al., 2002).

2.4.4.1.3 Doppler angle.

In addition to haemodynamic considerations, the Doppler insonation angle is critical in velocity measurements. In measuring velocity, the smaller the angle of insonation the more accurate the Doppler measurement of velocity (Detti et al., 2004). Many researchers use a 0° angle of insonation or apply angle correction if a 0° angle cannot be achieved. Recently researchers have used an insonation angle of less than 20° (Acharya et al., 2005; Flo et al., 2010; Rizzo et al., 2016), and in another study, 15° was used (Najafzadeh et al., 2016). Irrespective of the chosen angle of insonation, the visual error of aligning the Doppler angle to the UV introduces an additional user error
(Yamamoto, Carrillo, Insunza, Mari, & Ville, 2006), but at insonation angles of less than 30° small errors have minimal effect on velocity measurement (Figueras et al., 2008).

2.4.4.2 Normal ranges and relationship with gestational age.

This section of the literature review presents research detailing the range and relationships that researchers have found between UV velocities and advancing GA, during normal pregnancies. To allow comparison between velocities in the following discussions, $V_{\text{mean}}$ values have been converted to PV values by multiplying by 0.5, the commonly used spatial velocity profile coefficient (Acharya et al., 2005).

A pioneer researcher, Gill (1979), used the Octoson ultrasound machine to record the intra-abdominal UV $V_{\text{mean}}$ in 12 patients. The $V_{\text{mean}}$ ranged from 6.1 to 17.0 cm/s, equivalent to a PV range from 12.2 to 34 cm/s, and no clear correlation with GA was demonstrated (Gill, 1979). Using the same ultrasound equipment and method as Gill (1979), Chen et al. (1986) recorded an intra-abdominal UV $V_{\text{mean}}$ of 13.6±2.7 cm/s, which is equivalent to a PV of 27.2 cm/s; Chen et al. (1986) found that the velocity remained relatively stable after 25 weeks GA.

Sutton et al. (1990) described the UCV PV in 74 normal fetuses between 19 and 42 weeks GA as having a linear relation with GA and ranging from 7 to 23 cm/s. In a cross-sectional study of 129 singleton, healthy pregnancies between 23 and 33 weeks GA, Lees et al. (1999) found that UCV $T_{\text{amaxv}}$ increased in a linear fashion from 8.16±2.24 cm/s at 23 weeks to 10.79±2.25 cm/s at 33 weeks. Barbera and colleagues (1999) reported a linear relation between velocity and GA in a cross-sectional study and found that the UCV $V_{\text{mean}}$ increased by only 20% from 8 cm/s at 20 weeks to 10 cm/s at 38 weeks GA.
In a reference sonography manual, Hofer (2001) indicated that UV velocity was 10 to 15 cm/s without reference to GA or UV location. Di Naro, Ghezzi, Raio, Franchi, D’Addarin, et al.’s (2001) study of 104 women who delivered at term (mean GA 39.5 weeks) with normal, entire umbilical cord cross-section areas found the $V_{\text{mean}}$ to be 9.0±3.6 cm/s, giving a PV of 18 cm/s at term. Boito et al. (2002) showed a curvilinear relation between $T_{\text{amaxV}}$ and advancing GA in a cross-sectional study of 100 normal singleton pregnancies and concluded that the UCV $T_{\text{amaxV}}$ did not have a statistically significant increase from 5.3 cm/s at 20 weeks to 7.1 cm/s at 36 weeks GA.

A larger longitudinal study completed in 2005, followed 130 low risk singleton pregnancies between 19 and 41 weeks gestation, and gathered 511 observations that were used for the construction of reference ranges (Acharya et al., 2005). The authors found that the intra-abdominal UV PV had a curvilinear relation with GA and ranged from 16.2 cm/s at 19 weeks to a maximum of 24.7 cm/s at 32 and 33 weeks GA, before declining to 22.3 cm/s at 40 weeks GA (Acharya et al., 2005). Acharya et al.’s (2005) study provided reference ranges developed from longitudinal study data, in contrast to earlier cross-sectional research by Barbera et al. (1999). The perceived benefit of longitudinal studies is that they better reflect the clinical practice of serial measurements that are undertaken for fetal monitoring (Acharya et al., 2005; Flo et al., 2010); however, recent changes in statistical modelling, such as linear mixed modelling and quantile regression, can accommodate variance within and between participants thus negating the need for exclusive longitudinal or cross-sectional data collection for reference range modelling (Littell, Pendergast, & Natarajan, 2000).

Recently developed UV reference ranges have described a linear relationship between UV $T_{\text{amaxV}}$ and GA. A longitudinal study, with a sample of 53 low risk
pregnancies, showed the UCV T_{\text{amax}} had a linear relation with GA and ranged from
13.6 cm/s at 22 weeks to 17.8 cm/s at 40 weeks GA (Flo et al., 2010). Women in Flo et al.’s (2010) study were assumed to be normal as they had no prior poor obstetric
described the relation between intra-abdominal UV T_{\text{amax}} and GA, with
the calculated 50th percentile value increasing from 8.8 cm/s at 14 weeks to 18.5 cm/s
136 low risk pregnancies measured the UCV T_{\text{amax}} at three specific gestational ages
(Najafzadeh et al., 2016); the study recorded a non-linear relationship, with the
average T_{\text{amax}} being 14.7 cm/s at 18 weeks, then a 14% increase to 16.7 cm/s at 26
weeks and a further 17% increase to 19.5 cm/s at 34 weeks’ gestation.
In summary, there appears to be three opinions regarding UV velocity
throughout normal pregnancies: some authors assumed a relatively constant velocity
for the duration (Jouppila & Kirkinen, 1984; Link et al., 2007; Sutton et al., 1990);
others demonstrated an increase in PV with advancing GA in either a linear (Barbera et
al., 1999; Flo et al., 2010; Lees et al., 1999; Rizzo et al., 2016) or curvilinear relationship
(Boito et al., 2002; Kiserud, Rasmussen, & Skulstad, 2000); and the third opinion was a
mid-third trimester peak followed by a decline towards term (Acharya et al., 2005). In
this review of articles spanning more than 30 years of research the UV velocity values
have consistently ranged from 8 to 20 cm/s between the second and third trimesters.

2.4.4.3 Umbilical vein velocity in IUGR.

Reduced velocity of the blood travelling in the UV has been found in fetuses
compromised by growth restriction and several authors have proposed plausible
causes of this change in velocity. In 1984, Finnish researchers, Jouppila and Kirkinen (1984) examined a small sample of 11 growth restricted\(^8\) pregnancies and found that in eight patients the final measurement of the intra-abdominal \(V_{\text{mean}}\) was below the 10th percentile, using reference ranges they established in 1981. In sampling the velocity, a fixed Doppler angle of 50° was employed (Jouppila & Kirkinen, 1984), which is larger than that used in more recent research. This factor may have contributed to underestimation of the velocity readings; however, the same protocol was employed for establishing the reference ranges and therefore the growth restricted results are valid.

In a cross-sectional study of 37 IUGR\(^9\) fetuses, Ferrazzi et al. (2000) found that the UCV \(V_{\text{mean}}\) was significantly \((p < 0.001)\) reduced compared to normally growing fetuses. This research group undertook a longitudinal study in 2001 to consolidate their findings and targeted 21 IUGR\(^10\) fetuses with GA ranging from 23 to 36 weeks (Rigano et al., 2001). Rigano et al. (2001) showed that the UCV \(V_{\text{mean}}\) was significantly higher \((p < 0.001)\) in the control group \((0.90 \pm 0.16 \text{ cm/s})\) than in the IUGR group \((0.50 \pm 0.16 \text{ cm/s})\). The values quoted by Rigano and colleagues for the UCV \(V_{\text{mean}}\) were a factor of ten less than those cited by other researchers. A possible explanation is that Rigano et al. (2001) measured velocity over a 10 s time period, in which case their denominator was a unit of 10 s instead of one second, converting control PV to 18 cm/s and IUGR to 10 cm/s.

Reduced UV velocity in growth restricted fetuses was also documented in two other studies of singleton pregnancies and one study involving monochoronic twins.

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\(^8\) Birthweight < 10th %, reduced fetal growth, Apgar < 7 at 1 and 5 minutes, pathological CTG

\(^9\) AC < 2 SD below mean and birthweight < 10th %

\(^10\) AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
Boito et al. (2002) found the UCV $T_{\text{amaxv}}$ was reduced in 66% of IUGR$^{11}$ fetuses when compared to normal reference ranges. While, Spurway’s (2006)$^{12}$ comparison of the UCV PV between 108 normal singleton and 10 IUGR$^{13}$ fetuses aged between 26 and 41 weeks showed a statistically significant association ($p < 0.001$) between IUGR and reduced UCV PV. Liao, Nomura, Liao, Francisco, and Zugaib (2014) conducted a study of 18 monochoronic twin pregnancies compromised by placental insufficiency and measured the $T_{\text{amaxv}}$ in the straight portion of the intra-abdominal UV. A low UV $T_{\text{amaxv}}$ was found to be a predictor of fetal demise and birth acidaemia. These researchers used the reference range developed by Acharya et al. (2005) to plot serial UV $T_{\text{amaxv}}$ for a set of twins to visually demonstrate that the twin compromised by placental insufficiency consistently plotted lower than the uncompromised twin.

Two studies involving younger fetuses demonstrated that reduced UCV PV was present in the second trimester in IUGR affected fetuses: the first of these studies was a small, retrospective study (Rigano et al., 2008). The researchers matched by GA 12 singleton IUGR$^{14}$ fetuses who died in utero with a GA less than 26 weeks and a fetal weight below 600 g with 14 singleton IUGR fetuses who were born alive after 26 weeks gestation, and 22 normal fetuses who delivered at term. Both IUGR groups showed lower UCV PV when compared to the controls, with a significant reduction in UCV diameter seen in the IUGR fetuses that died in utero (Rigano et al., 2008). The researchers proposed that a small UCV diameter multiplies the effect of low velocity and that larger studies were needed to determine if UCV size was an important prognostic tool for early onset IUGR (Rigano et al., 2008). The second study, published

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$^{11}$ AC and birthweight < 5th %
$^{12}$ Unpublished Master of Health Science thesis
$^{13}$ EFW < 10th % or AC < 5th %
$^{14}$ AC < 2 SD below mean, UA PI > 95th % and abnormal uterine artery
data on 181 pregnancies with GA ranging from 17 to 41 weeks (Tchirikov et al., 2009). The researchers classified the pregnancies in relation to outcome: a poor outcome was based on cord blood pH, Apgar score, birthweight percentile, GA, requirement of respiratory support, and transfer of the newborn to a specialist ward. In the normal group the intra-abdominal $V_{\text{mean}}$ (15.95 cm/min) was significantly higher ($p = 0.0033$) than in the group with poor outcomes (12.58 cm/min) (Tchirikov et al., 2009). It appears that there was a typographical error in Tchirikov et al.’s (2009) report because velocity were given in units of centimetres per minute instead of the more commonly used measurement of centimetres per second, and conversion to centimetres per second results in unusually small values. Tchirikov et al.’s (2009) study supports the theory that UV velocity has a prognostic potential for predicting IUGR, as fetuses in the early second trimester were included in the data collection and reduced UV velocities were present in IUGR fetuses across a range of gestational ages.

Other researchers identified an association between lean umbilical cords, reduced UV velocity, and IUGR. An examination of 116 consecutive pregnancies, within 24 hours of delivery at term, found that 10.3% had lean umbilical cords (Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al., 2001). Fetuses with lean umbilical cords had a significantly reduced UCV $V_{\text{mean}}$ (6.6 cm/s) compared to the group of fetuses with normal sized cords (9.0 cm/s), and significantly more fetuses with lean cords had FGR\textsuperscript{15} (Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al., 2001). Although Di Naro and colleagues (2001) confirmed the reduction in UCV $V_{\text{mean}}$ in pregnancies affected by IUGR, the results have no prognostic value as the data collection occurred within 24 hours of delivery. Several years after Di Naro et al.’s (2001) study, an Egyptian research

\textsuperscript{15} Birthweight < 10th % for GA
group examined the association between lean umbilical cords, IUGR and reduced velocity (El Behery et al., 2011). These researchers examined 224 women carrying fetuses of greater than 24 weeks gestation and found that 15.5% had a lean umbilical cord. El Behery et al. (2011) reported that a lean umbilical cord was associated with IUGR and fetal distress during labour. In addition, UCV $V_{\text{mean}}$ was significantly lower in the fetuses with lean cords (8.6 cm/s) compared to normal umbilical cords (12.1 cm/s) (El Behery et al., 2011).

The cause of reduced UV velocity in IUGR pregnancies has been addressed by two research groups. Ferrazzi and colleagues (2000) proposed that reduced velocity in IUGR fetuses was a result of a decline in the pressure between the placental and fetal ends of the umbilical cord. While Detti et al. (2004) proposed that changes in resistance due to dilation, vasospasm, constriction or obliteration in downstream vessels influence the velocity of blood flowing into the UV and as such it was the status of both the fetus and placenta that influenced the UV velocity.

In the publications that I reviewed, the UV velocity was persistently lower in IUGR fetuses. This conclusion was evident in both longitudinal and cross-sectional data collection methods, in all sample sizes, in both intra-abdominal and intra-amniotic sampling sites and across a range of gestational ages. Thus, there is evidence that UV velocity has prognostic and surveillance roles in growth compromised pregnancies. Changes in pressure and resistance were proposed as causes of reduced UV velocity in growth restricted fetuses.

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16 Birthweight < 10th %
2.4.5 Umbilical vein blood flow.

Blood flow is the volume of blood moving in the umbilical vein and “determines the amount of nutrients, energy and gas exchange available to the developing fetus” (Rigano et al., 2008, p. 908). In the research described in this exegesis, the term umbilical vein blood flow \( Q_{uv} \) is used as a generic term referring to blood flow within the entire length of the vessel from placenta to within the fetal liver. When the portion within the fetal abdomen is specifically discussed, it will be referred to as the intra-abdominal \( Q_{uv} \). The term umbilical cord vein blood flow \( Q_{ucv} \) specifically defines blood flow within the intra-amniotic portion of the vein contained within the umbilical cord.

In this section of the literature review I will present the various methods available for measuring and calculating \( Q_{uv} \). I will identify the normal range of \( Q_{uv} \) and describe the different relationships that have been documented between \( Q_{uv} \) and increasing GA during normal and IUGR pregnancies. Included in these discussions are the proposed influences of UV diameter and velocity on \( Q_{uv} \). I will also outline the ways in which researchers have analysed \( Q_{uv} \) to account for variations in fetal sizes.

2.4.5.1 Measurement or calculation of the umbilical vein blood flow.

Blood flow can be defined as the “volume of fluid that passes a given cross-sectional area per unit time” (Figueras et al., 2008, p. 588). Blood flow is automatically calculated by ultrasound machine software or it can be computed from UV measurements.

Ultrasound software calculation of blood flow is accepted in some clinical areas, such as when assessing haemodialysis arteriovenous fistulas (Tirinescu et al., 2016; Zamboli, Fiorini, D’Amelio, Fatuzzo, & Granata, 2014), but it has not been embraced in obstetrics ultrasound. The lack of acceptance of software calculated \( Q_{uv} \) in
obstetric ultrasound may be due to the technical difficulty of performing the function, slow velocities in the UV, and dependence on software programs thereby making the calculation difficult to standardise and assimilate into routine obstetric practice.

The most commonly published formula for the calculation of UV blood flow uses measurements of the UV diameter and velocity (Acharya et al., 2005; Flo et al., 2010; Najafzadeh et al., 2016; Parra-Saavedra et al., 2014; Rigano et al., 2008; Rizzo et al., 2016). The formula is:

\[
Q_{uv}(ml/min) = \pi(UV \text{ diameter} \times 0.5)^2 \times UV \text{ V}_{\text{mean}} \times 60 \\
= \pi(UV \text{ diameter} \times 0.5)^2 \times (UV \text{ PV} \times 0.5) \times 60.
\]

The formula assumes a circular vein and uses a UV spatial velocity profile coefficient of 0.5 to calculate the mean velocity from the peak velocity, and this coefficient value assumes a parabolic flow profile. However, other authors have proposed the use of higher coefficient values, for example 0.62 (Flo et al., 2010) and 0.61 (Pennati et al., 2004), which suggest a blunted parabolic profile. Some researchers used the time-average intensity weight mean velocity (Acharya et al., 2005; Flo et al., 2010), and Lees et al. (1999) used the time averaged mean velocity (\(T_{\text{mean}}\)), for the mean velocity values.

Some researchers do not include the multiplication by 60 to convert seconds to minutes, but still indicated their blood flow as millilitres per minute (Boito et al., 2002; Rizzo et al., 2016). Other researchers measured the diameter in millimetres, without conversion to centimetres, and expressed the formula as (Prior et al., 2014):

\[
UV \text{ blood flow (ml/min)} = \pi(UV \text{ radius})^2 \times (UV \text{ maximum velocity}) \times 0.3.
\]

Whereas, Link et al. (2007) expressed the formula as:

\[
UV \text{ blood flow (ml/min)} = UV \text{ mean velocity} \times \text{diameter}^2 \times \pi \times 0.15.
\]
Acharya et al. (2005) found little difference between the intra-abdominal $Q_{uv}$ calculated from $0.5 \times PV$ and intensity weighted mean velocity. S. Li et al. (1993) found little difference in calculations using either PV or $V_{mean}$. However, both Flo et al. (2010) and Pennati et al. (2004) found that the blood flow calculated from $T_{amaxv}$ was just under 20% lower than that calculated using time average weighted mean velocity.

### 2.4.5.2 Normal ranges and relationship with gestational age

This section of the literature review presents research detailing the range and relationships that researchers have found between $Q_{uv}$ and advancing GA, during normal pregnancies. Fetal requirements for oxygen and nutrients increase in parallel with fetal growth (Link et al., 2007) and researchers have recommended that $Q_{uv}$ be related to a tissue mass, otherwise a smaller mass will have a smaller flow without carrying any implication of perfusion (Ferrazzi et al., 2000; Hebbar, Rubeena, Adiga, & Rai, 2015; Rigano et al., 2008). $Q_{uv}$ is related to the size and metabolic state of the object supplied, with normal, younger fetuses having less flow compared to older fetuses, but both have similar perfusion per weight unit (Hebbar, Rubeena, et al., 2015; Rigano et al., 2008). Normalising for fetal weight takes into account perfusion of the entire fetus (Hebbar, Rubeena, et al., 2015; Rigano et al., 2008), but introduces the innate error of the EFW formula, the error involved in biometric measurements incorporated into the formula (Ferrazzi et al., 2000), and introduces an inconsistency from the use of different EFW formulae. For example, Sutton et al. (1990) used an EFW algorithm that incorporated biparietal diameter (BPD) and AC for their normalisation of $Q_{uv}$ to fetal weight; whereas, Hebbar, Rubeena, et al. (2015) used four anthropometric measurements incorporated into the Hadlock formula. Other researchers advocate normalising for AC as this is the first parameter reduced in IUGR
(Rigano et al., 2001) or for HC (Di Naro et al., 2002; Ferrazzi et al., 2000). The scope of this literature review, describing blood flow trends in normal and IUGR pregnancies, has been broaden to include normalisation of $Q_{uv}$ for EFW and biometric measurements.

Two Australian studies using the obsolete Octoson multi-transducer, water-coupled B scanner, forged research into the umbilical vein blood flow. Gill, Trudinger, Garrett, Kossoff, and Warren (1981) found the intra-abdominal $Q_{uv}$ increased from 100 ml/min at 22 weeks to 300 ml/min at 38 weeks. In a later study of normal pregnancies conducted by Gill and colleagues, intra-abdominal $Q_{uv}$ was reported to steadily increase with GA up until 37 to 38 weeks, after which the blood flow reduced (Gill et al., 1984). Gill et al. (1984, p. 353) also calculated the rate of flow per kilogram of fetal weight “to account for variations in fetal size”. In doing so, the researchers introduced a large unknown quantity, as fetal weights at this time were estimated from locally derived birthweight charts incorporating preterm deliveries. The flow per kilogram was relatively constant at 120 ml/min/kg until 35 weeks and then declined to 90 ml/min/kg at 40 weeks (Gill et al., 1984). Gill and colleagues summarised the data collected by their predecessors and favourably compared their data to that collected by various means in the 1970s and 1980s, with the exception of 1965 postpartum data. Gill et al.’s (1984) research was also comparable to an intra-abdominal $Q_{uv}$ of 115 ml/min/kg published by Eik-Nes et al. (1982) from Sweden and Norway.

Contrasting Gill and colleagues’ (1984) results, a 1986 Taiwanese publication involving 163 normal pregnancies and using the equipment and methods described by Gill et al., showed a linear increase in intra-abdominal $Q_{uv}$ from $115.5 \pm 14.8$ ml/min at 25 weeks GA to $450.0 \pm 77.0$ ml/min at 41 weeks GA with no decrease approaching term
(Chen et al., 1986). Chen et al. (1986) normalised the blood flow to the abdominal area and found that the value remained relatively constant after 28 weeks GA.

Three subsequent Northern Hemisphere studies also described increasing $Q_{uv}$ with advancing GA. American researchers, Gerson et al. (1987), used a cross-sectional study to analyse the intra-abdominal $Q_{uv}$ results of 209 normal pregnant women between 20 and 40 weeks GA. These results showed a linear increase in intra-abdominal $Q_{uv}$ with GA (Gerson et al., 1987). In addition, flow normalised per kilogram steadily declined with advancing GA, which conflicts with the findings of Gill et al. (1984). Supporting Gearson’s results was another American study (Sutton et al., 1990) that used a sample of 74 uncomplicated pregnancies and found the $Q_{uv}$ increased exponentially from 19 to 40 weeks. Blood flow normalised for EFW showed a slight decline with increasing GA (Sutton et al., 1990). A British study (Lees et al., 1999) involving 129 healthy pregnant women between 23 and 33 weeks gestation, calculated the $Q_{ucv}$ and found a linear relationship between GA and $Q_{ucv}$ with $Q_{ucv}$ significantly increasing from 95.04±64.8 ml/min at 23 weeks to 303.28±63.8 ml/min at 33 weeks (Lees et al., 1999). Lees et al. (1999) identified that the high pass filter may have elevated their $V_{mean}$; they demonstrated that the increase in flow with advancing GA was more dependent on increasing vessel cross-sectional area than on the $V_{mean}$, and they suggested that M-mode could be used to measure the diameter of the vessel. Lees and colleagues (1999) also demonstrated that there was a close correlation between the flow in the UA and UV during the 23 to 33 week gestational period, which supports the theory that the fetus is a closed system.

A collaborative study by Barbera et al. (1999) confirmed fetal $Q_{uv}$ data by comparing their ultrasound values to those in obtained in ewes. Barbera et al. (1999)
in a cross-sectional study of 74 fetuses found there was an exponential increase in $Q_{ucv}$, rising from 97.3 ml/min at 20 weeks to 529.1 ml/min at 38 weeks. Barbera et al. (1999) determined that the increase in $Q_{ucv}$ was a result of growth in the area of the UV and not the increase in $V_{mean}$, as the UV cross-sectional area had a fourfold increase, whereas the velocity increased by only 20%. Barbera et al. (1999, p. 178) also reported that flow per kilogram of EFW showed a non-significant linear reduction and that “expression of blood flow per kilogram of estimated fetal weight introduces unnecessary errors and may obscure underlying pathophysiologic characteristics”. Barbera et al. (1999) validated their technique by repeating their ultrasound protocol on seven ewes and comparing their outcomes to previous data obtained from steady-state diffusion techniques in 34 ewes matched for weight and pregnancy stage; the results between the two groups showed no difference. Barbera et al. (1999) suggested that the results would be more binding if the same ewes were used for both blood flow measurements.

Validation of Doppler techniques using pregnant ewes has been a common practice; however, there should be some hesitation when comparing ovine results to human fetal data. Problems with using ovine results as models for human outcomes include:

i. **Ovine and human placentae differ in their structure** (Figure 2.12). Sheep have a combined epitheliochorial placenta in which fetal chorion is in contact with the uterine epithelium and a syndesmochorial placenta where the chorion is in contact with the maternal connective tissue. Humans develop haemochorial placentae in which free maternal blood bathes the chorionic cells (Noden & De Lahunta, 1985).
Figure 2.12. Classification of placentae according to the number of maternal cell layers present. Adapted from “The embryology of domestic animals. Development mechanisms and malformations,” by D. Noden and A. De Lahunta, 1985, Baltimore, MD: Williams & Wilkins, p. 51.

ii. Ovine fetuses have two umbilical veins as opposed to the single vein in the human cord. Barbera et al. (1999) did consider this fact by doubling the ovine flow data obtained so that comparison could be made with human data.

iii. Compared to human fetuses, ovine fetuses have a longer intrathoracic IVC, a smaller brain, a higher temperature, a different haemoglobin level, a faster growth rate, a shorter gestation (Kiserud, 2005; Kiserud & Acharya,
2004), lower pulmonary flow, and higher foramen ovale flow (Kiserud et al., 2000).

Barbera et al. (1999) noted that the length of time spent acquiring blood flow data on each patient was 3±1 mins. Rigano et al. (2001) also reported that it is common to spend only 2 to 4 mins per patient measuring $Q_{uv}$, which supports the assertion that collecting $Q_{uv}$ data is not time consuming.

Two further studies identified a linear relationship between $Q_{ucv}$ and GA, and a decline when $Q_{ucv}$ was normalised for EFW. The first study, by Boito et al. (2002), showed a seven fold increase in $Q_{ucv}$ from 33.2 ml/min at 20 weeks GA to 221 ml/min at 36 weeks GA according to their linear regression formula derived from a cross-sectional study of 100 normal, singleton pregnancies. Boito et al. (2002) recorded lower flow per kilogram of fetal weight than others, recording 117.5 ml/min/kg at 20 weeks and 78.3 ml/min/kg at 36 weeks gestation, but the discrepancy was attributed to time-averaged velocity calculations. At the time of Boito et al.’s (2002) research, images were stored and measured from videotape, and due to poor image reproduction quality may have led to measurement inaccuracies. Current ultrasound machines permit greater accuracy as measurements are performed on high resolution ultrasound monitors with digital storage. The second study, by Flo et al. (2010), was a longitudinal study of 53 singleton, low risk pregnancies measuring $T_{amaxv}$ in a free-floating portion of cord. Flo et al. (2010) recorded a linear increase in $Q_{ucv}$ from 53 ml/min at 22 weeks to maximum of 250 ml/min during the 39th week of pregnancy, compared to 66 ml/min and 313 ml/min using time-averaged intensity-weighted mean velocity over the same GA range. When blood flow was normalised for EFW, using the same parameters over the same GA range, there was a decrease from 110 ml/kg/min
to 68 ml/kg/min and 134 ml/kg/min to 85 ml/kg/min, respectively. Flo et al. (2010) provided an online supplement containing their reference values, which was a concise and practical method for providing a large amount of data.

In contrast to the previous two studies, other research groups choose to describe the relationship between the $Q_{ucv}$ with GA as curvilinear or non-linear. Acharya et al. (2005) demonstrated a steady curvilinear increase in the intra-abdominal $Q_{uv}$ throughout pregnancy from a minimum calculated from PV of 27.6 ml/min at 19 weeks to a maximum of 271.1 ml/min at 41 weeks. Acharya et al. (2005) obtained similar values using time-averaged intensity-weighted mean velocity. Acharya et al. (2005) reported that $Q_{uv}$ normalised for EFW decreased after 25 weeks GA, but when normalised for AC showed a continual increase from 19 to 41 weeks GA. Very recently, in a cross-sectional study, Rizzo et al. (2016) found that the intra-abdominal $Q_{uv}$ had a curvilinear increase throughout pregnancy from 27.7 ml/min at 16 weeks to 424.8 ml/min at 40 weeks GA. These authors found both the diameter and velocity contribute to increased blood flow. Rizzo et al. (2016) normalised the blood flow against AC, as they concluded that AC growth rate best reflects intrauterine growth, and showed a slight increase with GA from 0.2506 ml/min/mm at 16 weeks GA to 1.1458 ml/min/mm at 40 weeks GA. Supporting the non-linear relationship described by Rizzo et al. (2016) and Acharya et al. (2005), Najafzadeh et al. (2016) found a threefold increase from 18 weeks (28.7 ml/min) to 26 weeks (131.5 ml/min), followed by doubling from 26 to 34 weeks (269 ml/min).\(^{17}\)

In recent years obstetric ultrasound has embraced 3D imaging, including 3D fetal blood flow. Three dimensional $Q_{ucv}$ was measured in seven singleton and one

\(^{17}\) Incorrect unit of cm/s quoted (Najafzadeh et al., 2016, p. 102)
twin pregnancy between 17.9 and 36.3 weeks GA (Pinter et al., 2012). The $Q_{ucv}$ results compared favourably with those published by Tchirikov et al. (2002). Pinter et al. (2012) overcame many of the problems faced in traditional Doppler measurements including angle of insonation, flow profiles in the vessels, non-circular vessels and coiling, by calculating $Q_{ucv}$ from “power weighted surface integration of velocity vectors” (Pinter et al., 2012, p. 1931). A major limitation of this technique was the eight minute data acquisition time during which fetal acquiescence was necessary and also the lack of appropriate nomograms (Pinter et al., 2012). Similarly, Scholbach, Heien, and Eggebø (2016) calculated serial 3D $Q_{ucv}$ for 43 low risk, singleton pregnancies between 17 and 20 weeks GA. The readings were recorded every 30 seconds over a 5 min period. Scholbach et al. (2016) found the mean $Q_{ucv}$ ranged from 1.01 to 2.60 ml/s, which were slightly higher than recorded from traditional blood flow methods at comparable gestational ages. Interestingly, Scholbach et al. (2016) showed a variation in $Q_{ucv}$ over the 5 min period and vasomotion of the UCV. Scholbach et al. (2016) proposed that these changes might reflect changes in metabolic requirements of the fetus due to growth, movement and brain activity. Scholbach et al.’s (2016) findings confirm the need to repeat readings and ensure fetal quiescence when undertaking ultrasound assessment of the UCV.

In summary, during normal pregnancies $Q_{uv}$ has a linear (Boito et al., 2002; Chen et al., 1986; Flo et al., 2010; Gerson et al., 1987; Lees et al., 1999) or curvilinear (Acharya et al., 2005; Barbera et al., 1999; Rizzo et al., 2016; Sutton et al., 1990) increase with advancing GA and a decrease in flow as term approaches has been described (Gill et al., 1984). When $Q_{uv}$ was normalised for EFW, the commonest relationship described by researchers was an overall decline with advancing GA. When
blood flow was normalised for AC, a general increase in blood flow with advancing GA was described. Given the variation in measurement sites and method of calculation the volume, there is a general consensus that \( Q_{uv} \) increases from approximately 30 ml/min at 19 weeks, to 95 ml/min at 22 weeks and 300 ml/min near term. Both the blood velocity and the size of the vein have been found to influence \( Q_{uv} \).

### 2.4.5.3 Umbilical vein blood flow in IUGR.

In this section, research that details the relationships between \( Q_{uv} \) and advancing GA in IUGR pregnancies, including research where \( Q_{uv} \) is normalised for fetal weight and other parameters will be discussed. Explanations for \( Q_{uv} \) changes in IUGR pregnancies will also be presented.

In 1984, Australian research led to an eminent ultrasound publication whose findings sparked ongoing research about \( Q_{uv} \) and IUGR. This research involved 124 complicated pregnancies\(^{18}\) and 118 normal pregnancies, with GA ranging from 22 weeks to term. Gill et al. (1984) reported that low \( Q_{uv} \) was detected up to three weeks before reduced growth was detected by biometry. Gill et al. (1984) classified no detectable flow in the intra-abdominal UV as “low flow” and this classification may have erroneously elevated the number of low flow cases. Further research in 1984, by Jouppila and Kirkinen, examined a sample of 11 growth restricted fetuses\(^{19}\) between 30 and 38 weeks GA. Jouppila and Kirkinen (1984) showed all fetuses had an intra-abdominal \( Q_{uv} \) normalised for EFW, below the 10th percentile on Finnish reference ranges, at the last ultrasound examination prior to delivery. They reported an association between IUGR and reduced intra-abdominal UV diameter and proposed

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\(^{18}\) Poor obstetric history or hospitalisation for preeclampsia, diabetes, suspected growth restriction, etc.

\(^{19}\) Birthweight < 10th %, reduced fetal growth, Apgar < 7 at 1 and 5 minutes, pathological CTG
that reduced diameter was secondary to reduced flow rather than the cause of the reduced flow.

Three sequential studies supported Gill et al.’s (1984) proposal that decreased $Q_{uv}$ was an early change in the IUGR temporal sequence of events. The first, was a study by Kiserud et al. (1994) of 38 fetuses$^{20}$ of which seven died in utero and four postnatally. Kiserud et al. (1994) recorded the intra-abdominal $Q_{uv}$ for 33 cases and found that 25 of these had a reduced flow. Kiserud et al. (1994) also found that a raised UA PI was more common (19 of the 25) in the group with reduced blood flow. In addition, Kiserud et al. (1994) found that reduced intra-abdominal $Q_{uv}$ was more frequent before 31 weeks GA, and they proposed that at this may reflect a more serious manifestation of IUGR, as it occurred during a more vulnerable phase of fetal development when fetal blood pressure was lower. Kiserud et al. (1994) demonstrated that there was an inter-relationship between the umbilical arteries and umbilical vein by showing the association between increased placental impedance (increasing UA Doppler) and reduced UV blood flow in severe IUGR. The second was a cross-sectional study of 37 IUGR$^{21}$ fetuses matched against normal controls by Ferrazzi et al. (2000). Ferrazzi et al. (2000, p. 434) showed that $Q_{ucv}$ “in IUGR pregnancy was significantly lower ($p < 0.001$) at any given gestational age in comparison to normal pregnancies”. Ferrazzi et al. (2000) showed that three IUGR fetuses with normal UA PI values had reduced $Q_{ucv}$. This finding supported earlier research by Gill et al. (1984), who reported that decreased UV flow pre-empted other characteristic changes of IUGR. The third study was undertaken by another research team that included Ferrazzi (Rigano et al., 2001). This study built upon Ferrazzi et al.’s (2000) research by conducting a

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$^{20}$ BPD, abdominal diameter and birthweight < 2.5th %
$^{21}$ AC < 2 SD below mean and birthweight < 10th %
longitudinal study of 21 IUGR\textsuperscript{22} fetuses with GA ranging from 23 to 36 weeks; Rigano et al. (2001) found that the Q\textsubscript{ucv} normalised for EFW or AC was reduced. In addition, this reduction was not a “transient phenomenon” (Rigano et al., 2001, p. 838) but was present throughout the gestation, thus suggesting that abnormally low Q\textsubscript{uv} may be useful as a predictor of IUGR. Rigano and colleagues (2001) showed that IUGR fetuses had persistent reduced Q\textsubscript{ucv} and that this reduction was mainly due to reduced velocity and not diameter. Rigano et al. (2001) speculated that a reduction in blood flow was not a late occurrence in the sequence of events in an IUGR pregnancy. Rigano and colleagues (2001) concluded that in clinical practice a decrease in Q\textsubscript{ucv} should allow earlier differentiation of constitutionally small fetuses from IUGR affected fetuses.

Two publications from 2002 provide supporting evidence that IUGR was associated with reduced Q\textsubscript{ucv}. Boito et al. (2002) showed the mean Q\textsubscript{ucv} was below the 5th percentile on their graphs for 32 of 33 (97%) IUGR\textsuperscript{23} fetuses and was mainly determined by reduction in UCV cross-sectional area. While longitudinal research involving 15 IUGR\textsuperscript{24} fetuses and 30 normally growing fetuses by Di Naro et al. (2002), showed that Q\textsubscript{ucv} was less in IUGR fetuses than in appropriately growing fetuses. Di Naro et al. also showed that the Q\textsubscript{ucv} normalised for AC and HC was lower in IUGR fetuses. Di Naro et al.’s (2002) study showed that IUGR fetuses had a reduction of Q\textsubscript{ucv} over a two week period, even in the presence of normal UA Doppler indices. Di Naro et al.’s (2002) research utilised reference ranges established by Barbera et al. (1999); however, Kiserud (2003b) argued that Barbera et al.’s reference ranges were derived from cross-sectional data, which were not ideal for Di Naro’s longitudinal data.

\textsuperscript{22} AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
\textsuperscript{23} AC and birthweight < 5th %
\textsuperscript{24} AC < 5th % and birthweight < 10th %
Di Naro et al. (2002) proposed that blood flow was reduced in IUGR, partly due to both blood viscosity and velocity; they cited Poiseuille’s law for their explanation of reduced flow. Poiseuille’s law assumes Newtonian fluid characteristics and defines blood flow as:

\[ Q_{uv} = \pi (\text{pressure difference}) (r^4) / 8 (\text{viscosity}) (\text{length}). \]

In citing Poiseuille’s law, Di Naro et al. failed to identify the conditions in which Poiseuille’s law can apply to blood. Blood does not normally act as a Newtonian fluid as its viscosity is velocity dependent (Hobbie & Roth, 2015). Blood is composed of cells that change in shape and movement during velocity changes leading to variable viscosity and non-Newtonian behaviour (Tu, Inthavong, & Wong, 2015). However, there are situations in which non-Newtonian liquids can act in a Newtonian manner with independent viscosities and velocities (Hobbie & Roth, 2015). Viscosity can be assumed to be constant when velocity is constant as in vessels with venous flow (Sochi, 2014). In vessels with diameters greater than 1 mm, when shear stresses are negligible, blood can also be considered to have Newtonian properties (Sochi, 2014). Therefore, during fetal quiescence, blood in the umbilical vein may behave in a Newtonian manner and Poiseuille’s law may be applicable (Tu et al., 2015). Similarly, Jouppila, Kirkinen, and Puukka (1986) found that UV blood viscosity and haematocrit had a significant positive correlation and they proposed that an increase in haematocrit may cause increased blood viscosity, which in turn reduces blood flow. Blood viscosity is a result of the cohesive forces between molecules and the viscosity of blood is dependent on the haematocrit which is the ratio of the volume of red blood cells to the total blood volume (Hobbie & Roth, 2015).
The association between IUGR and reduced $Q_{ucv}$ has been described in several more recent publications; for example, Bellotti et al. (2004) reported that there was a statistically significant reduction in $Q_{ucv}$ and $Q_{ucv}$ normalised for EFW in growth restricted fetuses, in research involving 56 compromised fetuses\(^{25}\) compared to 137 normal fetuses. Rigano et al. (2008) reported the $Q_{ucv}$ per kilogram was significantly lower in the fetuses that died in utero in a small retrospective study involving 12 IUGR\(^{26}\) fetuses less than 26 weeks GA that died in utero matched against 14 IUGR fetuses born alive after 26 weeks gestation. The differences between the IUGR groups were more dramatic when $Q_{ucv}$ was expressed per unit of AC (Rigano et al., 2008). The association between IUGR and reduced $Q_{uv}$ was also reported in a recent conference presentation where $Q_{uv}$ was reduced in 108 pregnancies complicated by IUGR\(^{27}\) compared to 31 controls (Di Martino et al., 2016). These researchers found that the $Q_{uv}$ was reduced irrespective of the severity of fetal compromise. Di Martino et al.’s (2016) finding adds supporting evidence for the clinical application of blood flow, as many previous studies have used extremely compromised fetuses.

The association between IUGR and low $Q_{uv}$ was supported by a Spanish study of placental specimens from singleton pregnancies greater than 34 weeks GA with confirmed birthweights below the 10th percentile (Parra-Saavedra et al., 2014). There were 44 normal placentae and 51 placentae that exhibited histological findings consistent with placental under-perfusion (Parra-Saavedra et al., 2014). There was a significant reduction in the $Q_{ucv}$ ($p = 0.003$) and $Q_{ucv}$ normalised for EFW ($p < 0.001$) associated with the under-perfused placentae (Parra-Saavedra et al., 2014). The

\(^{25}\) AC and birthweight < 10th %, abnormal uterine and umbilical artery indices

\(^{26}\) AC < 2 SD below mean, UA PI > 95th % and abnormal uterine artery Dopplers

\(^{27}\) Fetal growth < 10th % for Italian standards
researchers proposed that $Q_{ucv}$ may be a proxy for placental under-perfusion providing a marker for less efficient placenta function and thereby the quantity of oxygen and nutrients available to the fetus, especially in cases of late onset IUGR (Parra-Saavedra et al., 2014)

Poor pregnancy outcomes were found to be associated with low intra-abdominal $Q_{uv}$ normalised for fetal weight by Tchirikov et al. (2009) in a sample of 181 fetuses of which 33 had poor pregnancy outcomes. Tchirikov et al. demonstrated that normalised $Q_{uv}$ had a sensitivity of 54.5%, a specificity of 95.6%, a positive predictive value of 75%, and a negative predictive value of 90.4%, for predicting poor fetal outcome. These values compare adequately with the UA PI, which is the current gold standard for assessing a fetus compromised by placental insufficiency (Tchirikov et al., 2009). The reference ranges developed by Tchirikov et al. were utilised in a 3D study of blood flow which demonstrated that a fetus with IUGR and a pregnancy that later developed pre-eclampsia, both had low intra-abdominal $Q_{uv}$ (Pinter et al., 2012). Another 3D blood flow study on fetuses aged between 23 and 40 weeks demonstrated a reduced $Q_{ucv}$ in IUGR (141 ml/kg/min) fetuses compared to small (253 ml/kg/min) and normal (226 ml/kg/min) fetuses (Scholbach, Fersis, & Stolle, 2012). Scholbach et al.’s (2012) data was used in a subsequent study that proposed IUGR might be the consequence of low $Q_{ucv}$ and restricted vasomotion of the UCV due to fetal signal molecules causing “autonomous venous contractions” (Scholbach et al., 2016, p. 628).

Adverse pregnancy outcomes and low $Q_{uv}$ have also been described in other publications. For example, a study in London examined 589 women with

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28 Poor outcome based on the cord blood pH, Apgar score, birthweight percentile, length of pregnancy, requirement of respiratory support and transfer of the newborn to a specialist ward

29 No definition documented
uncomplicated pregnancies (Prior et al., 2014). Prior et al. (2014) demonstrated that fetuses with the lowest $Q_{ucv}$, both uncorrected and corrected for birthweight, had the highest rates of delivery by emergency caesarean section or instrumental delivery. The researchers proposed that low $Q_{ucv}$ was significantly associated with intrapartum distress and that $Q_{ucv}$ could contribute to pregnancy risk assessment prior to labour (Prior et al., 2014). Also, in a study of 103 women within two weeks of delivery, Hebbar, Rubeena, et al. (2015) found that a lower intra-abdominal $Q_{uv}$ normalised for EFW was associated with IUGR, reduced birthweight and complications, including preterm delivery and higher admission rates to neonatal intensive care.

An association between lean umbilical cords, IUGR and low $Q_{uv}$ was found in 116 women who underwent ultrasound examinations within 24 hours of delivery (Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al., 2001). $Q_{ucv}$ normalised for EFW was lower in cases of a lean cord (93.7 ml/kg per minute) versus a normal cord (126.7 ml/kg per minute). Di Naro et al. (2001) also found a significant correlation between $Q_{ucv}$ and birthweight. In addition, El Behery et al. (2011) found $Q_{ucv}$ normalised for EFW was significantly reduced in fetuses with lean cords (83.4 ml/kg/min) compared to fetuses with a normal cord (131 ml/kg/min). The association of lean umbilical cords and IUGR was corroborated by Proctor et al. (2013) in a study of 497 post-delivery cords collected from pregnancies with a GA range from 18 to 41 weeks. Proctor et al. attributed thin cords to a significant reduction in Wharton’s jelly and found that thin cords were associated with a birthweight less than the 10th percentile based on Canadian standards for gender and GA.

Ovine studies have shown that the transportation of oxygen and nutrients to the fetus is not affected until the $Q_{uv}$ is reduced by roughly 50% (Battaglia, 2003). This
haemodynamic cause of IUGR has been supported by a human magnetic resonance imaging (MRI) study that found IUGR fetuses have reduced oxygen delivery and consumption (Zhu et al., 2016). In this prospective case controlled study involving 14 IUGR and 26 normally grown fetuses between 32 and 42 weeks GA, MRI showed that IUGR fetuses had lower intra-abdominal $Q_{uv}$ ($p = 0.04$) and pulmonary ($p = 0.01$) blood flow, high SVC flow ($p < 0.0001$), and smaller brains ($p < 0.0001$) (Zhu et al., 2016). MRI assessment of fetal blood flow is compromised by fetal movement, small vessel size, high cost and the limited number of machines (Zhu et al., 2016). Confirmation of ultrasound findings by MRI endorses the need to pursue the development of robust methods of ultrasound measurement of $Q_{uv}$.

In summary, $Q_{uv}$ has been shown to be reduced in fetuses compromised by IUGR and in some instances has been shown to be reduced prior to changes in UA indices. Reduced $Q_{uv}$ has been associated with poor pregnancy outcomes and lean umbilical cords. Both EFW and AC normalised $Q_{uv}$ were found reduced in IUGR fetuses when compared to normal controls. The cause of reduced $Q_{uv}$ in IUGR fetuses has been attributed to both a smaller UV diameter and reduced velocity, with viscosity being identified as a contributing factor to the latter. $Q_{uv}$ analysis is complex to perform and is dependent on the angle of insonation, determination of vein dimensions, and application of mathematical formula. All these factors increase the likelihood of errors; however, there is still potential to identify those fetuses that may develop IUGR. Therefore the development of Australian based reference ranges which can be incorporated into routine ultrasound examinations is supported.

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30 A score of ≥ 2 of the 4 following parameters defined IUGR: birthweight ≤ 3rd % or ≥ 20% drop in the centile in EFW, cerebroplacental ratio after 30 weeks < 5th %, ponderal index < 2.2 and placental histology
2.5 Ultrasound Safety during Obstetric Examinations

In this section of the literature review current standards and opinions on ultrasound safety during obstetric ultrasound examinations will be explored. This review formed the basis of a Local Operating Protocol which is contained in Portfolio Appendix H.

Diagnostic ultrasound has been used for at least four decades with no substantial evidence of serious adverse effects (Callen, 2000; Sande & Kiserud, 2013; World Federation for Ultrasound in Medicine and Biology, 2013) and there has been no causal link established between antenatal exposure to ultrasound and adverse health outcomes (Australasian Society for Ultrasound in Medicine, 2008a; Safety Group of the British Medical Ultrasound Society, 2010). However, several bioeffects have been described with human and non-human exposure to ultrasound. The physical effects of ultrasound exposure include streaming in fluids, stress at tissue interfaces, heating of tissues by absorption, and cavitation due to the formation of gas bubbles.

The Output Display Standard is a screen display of the mechanical index (MI) and the thermal index (TI), and both are estimates of bioeffects. The MI is an indicator of the mechanical or non-thermal bioeffects and relates to peak rarefaction pressure (Beirne, Westerway, & Ng, 2016). Current guidelines (Table 2.6) recommend an MI less than 0.4 when gaseous bodies are present and up to 1.9 in the absence of gaseous bodies (Moderiano, McEvoy, & Childs, 2015; T. R. Nelson, Fowlkes, Abramowicz, & Church, 2009). A report prepared by the Safety Group of the British Medical Ultrasound Society (2010) considered that there was no reason to restrict obstetric scanning to an MI of less than 0.3 as no gaseous bodies are present, but indicated that there was a theoretical risk of cavitation at an MI of greater than 0.7.
The TI indicates the potential to increase tissue temperature with exposure to ultrasound. A TI value of one indicates that the output settings are capable of raising the temperature by 1°C (Sande & Kiserud, 2013). There are three thermal indices:

i. Thermal index for soft tissue (TIS) is used when there is no insonation of bone and applies to obstetric ultrasounds less than 10 weeks GA.

ii. Thermal index for bone (TIB) is used when there is insonation of structures containing bone and applies to obstetric ultrasounds of greater than 10 weeks GA.

iii. Thermal index for cranial bone (TIC) is used when scanning very close to bone and does not apply to obstetric examinations.

During obstetric imaging a TI of less than 0.7 is recommended; higher values are acceptable but only with time limits (Table 2.6), and a TI of greater than 3.0 is not recommended for obstetric scanning (Moderiano et al., 2015; T. R. Nelson et al., 2009; Royal Australian and New Zealand College of Radiologists, 2014; Safety Group of the British Medical Ultrasound Society, 2010; World Federation for Ultrasound in Medicine and Biology, 2013). International and American guidelines recommend that when using Doppler in the first trimester, a TI of 1.0 is not exceeded and scanning time limited (American Institute of Ultrasound in Medicine, 2016; Bhide et al., 2013). The New Zealand and Maternal Fetal Medicine Network (2015) specified that by reducing the acoustic output power, a TIB less than 1.0 should be maintained when using Doppler in obstetric examinations.
Table 2.6

*Thermal and Mechanical Indices Guidelines*

<table>
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<th>Index level</th>
<th>Obstetrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TI &lt; 0.7</td>
<td>Always used unless otherwise required</td>
</tr>
<tr>
<td>0.7 &lt; TI ≤ 1.0</td>
<td>60 min</td>
</tr>
<tr>
<td>1.0 &lt; TI ≤ 1.5</td>
<td>30 min</td>
</tr>
<tr>
<td>1.5 &lt; TI ≤ 2.0</td>
<td>15 min</td>
</tr>
<tr>
<td>2.0 &lt; TI ≤ 2.5</td>
<td>4 min</td>
</tr>
<tr>
<td>2.5 &lt; TI ≤ 3.0</td>
<td>1 min</td>
</tr>
<tr>
<td>3.0 &lt; TI ≤ 4.0</td>
<td>Not recommended</td>
</tr>
<tr>
<td>4.0 &lt; TI ≤ 5.0</td>
<td>Not recommended</td>
</tr>
<tr>
<td>5.0 &lt; TI ≤ 6.0</td>
<td>Not recommended</td>
</tr>
<tr>
<td>MI &lt; 0.4</td>
<td>When gaseous bodies are present</td>
</tr>
<tr>
<td>MI &lt; 1.9</td>
<td>When gaseous bodies are absent</td>
</tr>
</tbody>
</table>


The bioeffects of ultrasound exposure on in vitro and non-human subjects were summarised by Bello and Ekele (2012) and included altered differentiation of in vitro mesenchymal stem cells, increased haemolysis of in vitro whole human blood samples due to gas-based contrast agents, increased membrane damage in phagocytes, reduced birthweight in mice, induced lung haemorrhage in small rodents, and increased brain temperature in guinea pigs and sheep. Other studies using current machines have shown disturbances to the life cycle of liver cells, gene expression and neural migration in rats (Sande & Kiserud, 2013).

Sande and Kiserud (2013) summarised the finding of several studies examining human prenatal ultrasound exposure and the increased risk of low birthweight and neurological disorders. Sande and Kiserud (2013) concluded that the increase of non-right-handedness was the only bioeffect that was substantiated by both randomised control trials and epidemiological studies. The fetal eyes have been identified as being extremely susceptible to thermal hazards (Safety Group of the British Medical
Ultrasound Society, 2010). However, a prospective Western Australian study investigating the impact of in utero ultrasound exposure on ocular biometry and visual acuity found “no detrimental consequence of multiple ultrasound scans on ocular health” at a 20 year follow-up (Forward et al., 2014, p. 169). Webb et al. (2016) recently raised concerns that the diversity of autism spectrum disorder may be the consequence of early pregnancy ultrasound exposure. However, the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) Bioeffects and Safety Committee (Salvesen et al., 2016) found no scientifically substantiated evidence to support the claim. A review by McLennan (2016) corroborated the Committee’s findings and questioned the bias and statistical accuracy of Webb et al.’s study.

To minimise any possible detrimental effects of diagnostic ultrasound examinations, both B-mode and Doppler imaging should apply the ALARA principle (as-low-as-reasonably-achievable). In keeping with this principle, the acoustic power should be as low as diagnostically possible, overall examination times should be limited, and dwell times shortened (Australasian Society for Ultrasound in Medicine, 2008a, 2008b, 2008c). Even though the use of ultrasound appears to have no detrimental effects, ultrasound should be judiciously used by competent, qualified health professionals using well maintained units and should not be used without medical value (McLennan, 2016; World Federation for Ultrasound in Medicine and Biology, 2013).

There are three areas that warrant further investigation when assessing the validity of low bioeffect risks from in utero ultrasound exposure: changes to maximal power output, sensitivity of fetuses, and cumulative exposure. The maximum output of ultrasound machines was increased from 94 mWcm$^{-2}$ to 720 mWcm$^{-2}$ in 1991, and a
majority of studies that found no detrimental effects from in utero ultrasound exposure where completed prior to this output change (Sande & Kiserud, 2013; Thoirs & Lee, 2016). There has also been an assumption that all fetuses are equally vulnerable to in utero ultrasound exposure (Bello & Ekele, 2012), whereas it is more likely that a continuum exists from low to high tolerance. The third area of investigation relates to cumulative exposure and dose. With the increase in machine maximal output and frequent use of ultrasound during pregnancy, researchers need to fully investigate the impact of cumulative dosage and associated exacerbation of bioeffects (Bello & Ekele, 2012). It is evident that further and ongoing investigation, using current technology, is warranted.

2.6 Gaps in Current Knowledge and Research Aims

After an extensive review of the literature relating to ultrasound measurements of the umbilical vein diameter, PV and blood flow, I have identified several gaps within the current body of knowledge including:

i. A majority of investigations have been carried out in the Northern Hemisphere. The three most recent reference ranges published have been developed from data collected in Norway and Italy. However, there is no contemporary Australian data on the diameter, peak velocity or blood flow of the intra-amniotic portion of the umbilical cord vein.

ii. The incorporation of UCV measurements into the diagnosis and surveillance regimes of fetal wellbeing has been poor. The main difficulties have been dependence on software and use of advanced imaging
techniques. There is a lack of simple protocols that can be embraced by all
sonographers, irrespective of their scanning expertise.

iii. The ability of UCV diameter, velocity and blood flow measurements to
identify growth compromised fetuses has been demonstrated by several
investigators; however, a majority of the research articles have
concentrated on preterm fetuses with grossly abnormal UA Doppler
indices, leaving the assessment of the moderately-compromised fetuses as
an under-investigated area.

iv. Research has indicated that the UCV diameter and velocity both contribute
by varying degrees to reduced blood flow in IUGR. Research investigating
other possible ways of examining the interconnection of UCV diameter and
velocity has not been published; such investigation may provide an easier
method of assessing the interplay of diameter and velocity, without using a
complicated Quv formula and its inherent errors.

v. Many researchers have used birthweight to confirm normality of their
control groups and to confirm IUGR, but no research has investigated the
interplay between birthweight percentiles, GA and ultrasound features of
the UV. Assessment of in utero UV measurements of fetuses born with
different birthweight percentiles may identify unknown relationships.

Arising from these gaps in current knowledge, the following research aims have
been determined:

i. Construct population specific reference ranges (nomograms) for the
ultrasound measurements of the UCV diameter, PV and Qucv.
ii. Develop, document and publish a simple protocol for the measurement of the UCV diameter and PV, and calculation of the $Q_{ucv}$.

iii. Ascertain if the nomograms developed for the UCV diameter, PV or $Q_{ucv}$ have a clinical application in identifying fetuses with mild to moderate growth compromise.

iv. Determine if there is a significant difference between the UCV diameter, PV and $Q_{ucv}$, depending on birthweight percentiles and GA. Determine if this information is useful in the clinical application of the reference ranges.

v. Investigate other relationships between UCV diameter and PV.
SECTION III MATERIALS AND METHODS

Chapter 3 Materials and Methods

3.1 Introduction

The overarching aims of this research project were to develop Australian specific, GA related reference ranges for the UCV diameter, PV and $Q_{ucv}$ and to determine if any of these measurements were useful in identification of growth compromised fetuses. Secondary aims were to develop technically easy measurements procedures, investigate interrelations between UCV measurements, birthweight and advancing GA, and explore alternative relationships between UCV measurements.

The quasi-experimental design of this quantitative research project employed both prospective and retrospective data collection. Participants, sonographers, ultrasound machines and computer software were the materials utilised. Research approval, the processes involved in recruitment, and the means of obtaining maternal characteristics, pregnancy outcomes and all ultrasound data will be detailed in this chapter. The methods of constructing birthweight categories and other subgroups, plus the statistical methods employed in this research will also be described.

3.2 Study Design

This quantitative research project was quasi-experimental in design using non-random sample selection from a naturally formed group of potential participants. The study sample was non-random due to automatic exclusion of part of the population based on maternal and gestational age. Furthermore, potential participants self-selected. Of all hospitals and private practices within the study location, potential
participants were only drawn from among the women who chose to attend either Orange Health Service (OHS) or Bathurst Health Service (BHS) Medical Imaging Departments.

Descriptive patient characteristics, as discrete or categorical quantitative data, were collected using a prospective questionnaire. Pregnancy outcomes as continuous, discrete and categorical quantitative data were collected postpartum. UCV continuous quantitative data were collected prospectively, using both cross-sectional and longitudinal sampling.

3.3 Ethics and Research Approval

The Greater Western Human Research Ethics Committee (HREC) granted ethics approval for this research project (HREC/10/GWAHS/34, Appendix B) on 25 January 2011, for the specific sites of OHS and BHS (SSA/11GWAHS/6, Appendix C). The National Ethics Application Form and the Site Specific Assessment Form submitted to the Greater Western HREC are included in the portfolio along with my responses to issues that were raised by the Committee (Portfolio Appendices B2, B4 and B6).

Charles Sturt University Board of Graduate Studies approved the research proposal on 8 February 2011 (Appendix D), and a copy of the proposal is included in the portfolio (Portfolio Appendix B13). The Charles Sturt University HREC granted ethics approval for the research on 15 February 2011 (2011/020, Appendix E).

3.4 Recruitment

An invitation to participate in the research was extended to eligible clients who had been referred to OHS and BHS for obstetric ultrasounds between 5 April 2011 and 22 March 2013. General Practitioners, obstetricians, staff medical officers and
midwives referred clients to the Medical Imaging Departments. There was no intended selection bias towards public or private health insurance status, ethnicity or complexity of the pregnancy.

Participation was voluntary and no client was coerced into enrolling into the research. A participant could withdraw from the research at any stage, as outlined in the Participant Information Sheet (Appendix F), without influencing the professional diligence with which their ultrasound examinations were performed.

Participant confidentiality was guaranteed, as the final data were summarised in non-identifiable data blocks used for statistical analysis. No individual could be identified by any characteristic in the results or any subsequent publications. Access to the data was restricted and all data were stored in a secure cabinet in the Medical Imaging Department at OHS.

3.5 Informed Consent

At the initial contact with the Medical Imaging Departments, eligible clients were offered a Participant Information Sheet and Consent Form (Appendix G). Providing information prior to their ultrasound examination allowed time for clients to read the information and make an informed choice about participation without coercion from sonographers. If the client had difficulty understanding the Participant Information Sheet and Consent Form due to language or literacy issues, the sonographer offered assistance or sourced help through the Aboriginal Liaison Officer or The Department of Immigration and Citizenship Translating and Interpreting Service.
If the client agreed to participate in the research, the signed Consent Form was collected and any questions raised by the client were answered by the sonographer prior to the commencement of the ultrasound examination. The Information Sheet remained in the participant’s possession and the signed Consent Form was stored in a secure cabinet in the Medical Imaging Department at OHS.

3.6 Study Sample

3.6.1 Research population.

The research population consisted of women who were greater than 16 weeks pregnant with a single fetus and aged between 16 years 0 days and 55 years and 0 days. This age range covered a large proportion of the female reproductive years. The minimum age of 16 years adhered to the legal age for consensual sex in NSW according to the Crimes Act 1900 Section 66C (Australian Institute of Family Studies, 2013; New South Wales Government, 2015) and with the NSW Ministry of Health policy directive regarding the age at which an individual can consent to medical treatment (New South Wales Government, 2005a).

A minimum GA of 16 weeks allowed the UCV to be reliably visualised on transabdominal ultrasound imaging as the accurate measurement of the umbilical vessels in early pregnancy is difficult unless a transvaginal examination is undertaken (Barbieri et al., 2012). A lower limit of 16 weeks GA is in keeping with other published UV works, although recently, some researchers opted for an earlier GA of 14 weeks (Rizzo et al., 2016) whereas others utilised a latter GA of 19 to 22 weeks (Acharya et al., 2005; Barbera et al., 1999; Flo et al., 2010).
3.6.2 Inclusion and exclusion criteria.

In the initial sample, women were not excluded due to the presence of fetal or cord abnormalities, parity, outcomes of previous pregnancies, ethnicity or maternal medical conditions. Women were excluded if the EDD was not established and confirmed by ultrasound prior to 14 weeks gestation. Women in labour were excluded from participation to eliminate the effects of uterine contractions on umbilical cord blood flow. Multifetal pregnancies were excluded so that data were not compromised by potential vascular malformations between placentae. In the initial research design participants were to be excluded if the EFW was above the 90th percentile or if accelerated fetal growth velocities were observed. This exclusion was removed prior to the start of data collection when actual birthweight, not ultrasound EFW, was chosen as the parameter for pregnancy categorisation.

The inclusion and exclusion criteria were available to the sonographer on the Participant Characteristics Sheet (Appendix H). Eligibility was assessed during routine history taking prior to the ultrasound examination, or if there was insufficient data at that time, eligibility was determined during the ultrasound examination as the GA and fetal number were established. The inclusion and exclusion criteria were applied by the sonographer completing the examination. Assessment of inclusion and exclusion criteria occurred primarily in the ultrasound examination room, but it also occurred at the time of booking if the client provided information that indicated her ineligibility to participate in the research.

At the time of data entry into the re-identifiable, coded, aggregated data spreadsheet, I reviewed all documents and images on the Picture Archive and Communication System (PACS) ensuring strict adherence to the inclusion and exclusion
criteria. Further revisions of the exclusion criteria occurred during the course of the study:

i. Participants were removed from the study if the pregnancy outcomes were unobtainable.

ii. Stillbirths were not excluded if the death occurred during labour. If the fetus died prior to the onset of labour the participant was excluded. This criterion was decided as the three fetuses that died during labour were appropriately grown and their deaths were a consequence of antepartum haemorrhages and not as a result of any undetermined adverse intrauterine events prior to labour.

iii. Participants were excluded if the pregnancy was terminated.

iv. Attendances in which ultrasound measurements did not comply with protocol were omitted.

3.6.3 Sample size.

Utilising research by Di Naro et al. (2002) and Lees et al. (1999), the Quantitative Consulting Unit at Charles Sturt University estimated the minimum sample size that was required for this research. Using a 95% confidence level and power of 80%, the Quantitative Consulting Unit calculated that a sample size of at least 160 women was needed to detect differences between normally growing and IUGR fetuses at the 5% significance level. A copy of the calculations is included in the Appendix I. Allowing for a 10% dropout rate, a minimum of 176 women were needed to be recruited to the research.
The study sample was recruited from an estimated 4,810 eligible ultrasound examinations undertaken at the Medical Imaging Departments of OHS and BHS during the recruitment period. The rationales for the sample location were:

i. The principal researcher was a fulltime sonographer at OHS.

ii. OHS and BHS ultrasound equipment, PACS, medical records and staff were accessible to the principal researcher.

iii. Both sites had comparable ultrasound machines that were serviced by the same engineer, thereby limiting reproducibility errors due to machine and calibration variation.

iv. Both sites were located in similar sized regional towns servicing a low to moderate risk obstetric population.

There were 661 clients who volunteered to participate in this research project and 36 were excluded, which left a sample size of 625.

3.7 Maternal Characteristics

After informed consent had been given, the sonographer completed the Participant Characteristics Sheet (Appendix H) by questioning the participant, or reviewing and confirming information provided on the referral form. The following maternal characteristics were collected: ethnicity, maternal age at conception, conception, parity, maternal diseases and smoking during pregnancy.

3.7.1 Ethnicity.

Maternal ethnicity categories were modified from The Fetal Medicine Foundation (FMF) groups, who had devised categories to reflect differences in fetal nasal bone and maternal serum biochemistry depending on maternal ethnicity.
The FMF maternal ethnicity categories were utilised in this research as all research sonographers were familiar with the category definitions of the original groups. The FMF categories were modified with the addition of Indigenous and Southeast Asian categories (Table 3.1) to tailor the classifications to the Australian population.

Table 3.1

Maternal Ethnicity Categories Based on Nationality or Geographical Origin

<table>
<thead>
<tr>
<th>Maternal ethnicity</th>
<th>Nationality or geographical origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous</td>
<td>Australian Aboriginal and Torres Strait Islander</td>
</tr>
<tr>
<td>White</td>
<td>Australian, European, Middle Eastern, North African, Canadian, American, South American and Hispanic</td>
</tr>
<tr>
<td>Black</td>
<td>African, Caribbean, and African American</td>
</tr>
<tr>
<td>East Asian</td>
<td>Chinese, Japanese and Korean</td>
</tr>
<tr>
<td>South Asian</td>
<td>Indian, Pakistani, Bangladeshi and Sri Lankan</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>Philippines, Indonesia, Papua New Guinean, Bruneian and Malaysian</td>
</tr>
<tr>
<td>Mixed</td>
<td>Any combination of the above groups</td>
</tr>
</tbody>
</table>


3.7.2 Maternal age at conception.

The maternal age at conception was defined as the age of the participant 38 weeks prior to her EDD or two weeks after her known or calculated first day of her LMP. In the majority of cases the maternal age was established by questioning the patient. Alternatively, the maternal age was deduced with the aid of the ultrasound machine calculation packages.
3.7.3 Conception.

Spontaneous conception was defined as a pregnancy that occurred naturally without any medical assistance. Assisted conception was defined as a pregnancy that occurred following medical assistance and was divided into two broad categories:

i. Assisted reproductive technologies (ART) including conceptions aided by techniques such as in vitro fertilisation (IVF), gamete intra-fallopian transfer (GIFT), intracytoplasmic sperm injection (ICSI) and artificial insemination.

ii. Hormonal therapy to improve successful ovulation and implantation included pharmaceuticals such as clomiphene citrate (Clomid®), metformin, letrozole, dehydroepiandrosterone (DHEA), etc. In these cases the pregnancy was assisted, but conception was spontaneous.

3.7.4 Parity.

Parity was defined as the number of births after 20 weeks GA, regardless of pregnancy termination, or whether pregnancy resulted in a live or demised neonate (Z. Li, Zeki, Hilder, & Sullivan, 2013).

3.7.5 Maternal diseases.

An overview of participants’ general wellbeing was documented by recording the first disorder reported by participants when asked about their health. There was no interrogation about the severity of the disorder or priority of multiple disorders as this was a broad, non-specific question. The first stated disorder was documented and categorised into the broad groups shown in Table 3.2. Hypertension and diabetes were not combined into broader categories due to their known association with birthweight.
diversities. Although substance abuse is a known risk factor for IUGR, it was grouped in
the miscellaneous category as only two participants disclosed their drug use.

Table 3.2

*Maternal Diseases Categories*

<table>
<thead>
<tr>
<th>Maternal disease category</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No maternal diseases</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Gestational diabetes mellitus, pregestational diabetes</td>
</tr>
<tr>
<td>Bowel disorders</td>
<td>Coeliac disease, Crohn’s disease, ulcerative colitis</td>
</tr>
<tr>
<td>Blood disorders</td>
<td>Thalassemia, thrombocytopenia, Factor V Leiden, anaemia, maternal antibodies</td>
</tr>
<tr>
<td>Gynaecological problems</td>
<td>Endometriosis, polycystic ovarian syndrome</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Thyroid disorders</td>
<td>Hypothyroid, hyperthyroid, Graves’ disease, goitre</td>
</tr>
<tr>
<td>Other complications of pregnancy</td>
<td>Cholestasis, previous HELLP syndrome</td>
</tr>
<tr>
<td>Chronic disorders</td>
<td>Mild asthma, chronic fatigue, renal disease, rheumatoid arthritis, personality disorders, vitamin deficiency, hypotension, osteoporosis, epilepsy, allergies</td>
</tr>
<tr>
<td>Viral diseases</td>
<td>Shingles, hepatitis C</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Migraine, neurological pain, substance abuse</td>
</tr>
</tbody>
</table>

*Note.* HELLP = haemolysis, elevated liver enzyme levels and low platelet levels.

### 3.7.6 Smoking during pregnancy.

At recruitment each participant was required to nominate her status as either
smoker or non-smoker. Smoking during any stage of the pregnancy was documented
as smoker. If participants did not smoke or had ceased smoking prior to falling
pregnant they were classified as non-smokers.

The rationale for this strict demarcation was that in 2011 only 3.3% of
Australian pregnant women reported cessation of smoking during the second half of
pregnancy (Z. Li et al., 2013) and as such it was irrelevant at what GA the client was recruited as there was only a small chance that she would cease smoking later in the pregnancy. In addition, Shipton et al. (2009) asserted that self-reported smoking status is underestimated by approximately 25% of pregnant women because of social stigma, making the true smoking status of the study sample potentially inaccurate. Hence a simple categorisation of the participants made their status easier to define, eliminated subcategories of pregnancy duration when smoking ceased, and removed any perceived judgemental opinion, which encouraged participants to be honest about their smoking status when questioned by the sonographer.

3.8 Birthweight Percentiles and Pregnancy Outcomes

3.8.1 Birthweight percentiles and categories.

In the initial research proposal the fetus was assigned as SGA, appropriate for gestational age (AGA), or large for gestational age (LGA) based on ultrasound biometry (Table 3.3). An AFI below the 5th percentile, in the absence of ruptured membranes or fetal renal tract malformations, was supportive evidence for the classification of SGA.

Table 3.3

<table>
<thead>
<tr>
<th>Classification</th>
<th>Estimated fetal weight at known GA</th>
<th>Growth velocity (14 day intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small for GA</td>
<td>≤ 10th percentile</td>
<td>Reduced fetal growth velocities of at least one parameter</td>
</tr>
<tr>
<td>Appropriate for GA</td>
<td>&gt; 10th and ≤ 90th percentiles</td>
<td>Normal growth velocities</td>
</tr>
<tr>
<td>Large for GA</td>
<td>&gt; 90th</td>
<td>Abdominal circumference &gt; 95th percentile or accelerated growth velocities</td>
</tr>
</tbody>
</table>

Note. GA = gestational age.
Pregnancy outcome data highlighted a discrepancy between the ultrasound classification and actual birthweight classification of SGA, AGA or LGA. This inconsistency was most obvious when a fetus had only one scan in the second trimester and ultrasound information was then extrapolated to give a term classification. This was not an unexpected outcome given that experienced sonographers have an accepted error rate of between 15% and 20% (Quinton, 2006) in ultrasonically estimating fetal weight. In addition, in a review article of ultrasonic fetal weight estimation, Dudley (2013, p. 186) argued that “any trial where subjects are selected on the basis of EFW will have serious limitations owing to the accuracy, sensitivity and specificity of the technique”. Given these opinions and irregularities it was appropriate to use actual birthweight to retrospectively classify fetal growth in this research. This modification to research design allowed fetuses who only had an early ultrasound to be classified using a known quantifiable endpoint, and not an extrapolation based on an early ultrasound examination.

Birthweights were divided into categories using Australian national birthweight percentiles for singleton infants, developed by Dobbins et al. (2012). These percentiles covered gestational ages from 20 to 44 weeks with the GA at delivery rounded to whole weeks and birthweight rounded to the nearest 5 g. The percentiles developed by Dobbins et al. allowed the use of robust, current, Australian data collected from 2.53 million live births over a 10 year period from 1998. In addition, these percentiles allowed classification to be gender specific, as the average Australian birthweight of live born females is typically less than males (Z. Li et al., 2013).

When the birthweights, sex and GA at delivery of the fetuses was retrieved from participants’ medical records, the information was used to assign the fetuses into
percentile bands according to Dobbins et al. (2012). These percentiles were summarised into three categories: SGA (< 10th percentile), AGA (10th to 90th percentiles) and LGA (> 90th percentiles). These classifications formed the basis of comparison and statistical analyses for the remainder of the research with participants defined by the birthweight category of their offspring.

3.8.2 Pregnancy outcomes.

Pregnancy outcomes were retrieved from handwritten documents or through password restricted, electronic medical records (InfoMedix – Clinical Patient Folder). If these avenues were unfruitful, but the delivery hospital could be identified, a list of required outcomes and the signed Consent Form giving permission for retrieval of this information was faxed to the appropriate site. These sites included Nepean, Westmead, Parkes, Forbes, and Cowra hospitals. The following six outcomes were collected and entered into a re-identifiable spreadsheet:

i. **Birthweight.** The birthweight documented by the midwife at the time of delivery was recorded as a continuous variable rounded to the nearest 5 g.

ii. **Perinatal and infant deaths.** Fetal deaths were defined as death before birth after a GA of at least 20 weeks and a birthweight of more than 400 g, neonatal deaths up to 27 completed days after birth, and infant death as death occurring at less than one year of age (Hilder, Zhichao, Parker, Jahan, & Chambers, 2014).

iii. **Gestational age at delivery.** The date of delivery was retrieved from the participants’ medical records and the GA at delivery, expressed in weeks and days, was calculated using the web-based Auckland District Health Board newborn clinical guidelines package (Auckland District Health Board,
2009). This package automatically adjusted for the 2012 leap year.

Allocation of birthweight categories was by completed whole weeks and statistical analyses were undertaken using GA age in days. Statistical analyses used three categories of GA at delivery: preterm (< 37 weeks 0 days), term (between 37 weeks 0 days and 41 weeks and 6 days) and post term (> 41 weeks and 6 days).

iv. *Sex of the newborn*. The sex of the neonate was classified into discrete categories of male or female.

v. *Mode of delivery*. The mode of delivery was categorised as either normal vaginal, instrumental including forceps or vacuum extraction, or caesarean section.

vi. *Apgar scores at one and five minutes after birth*. The Apgar score was recorded as a value between 0 and 10 reflecting the baby’s neurological and cardiorespiratory rank at one and five minutes after birth (DeCherney, Nathan, Goodwin, & Laufer, 2007). The midwife assigned a value between 0 and 2 for each of the following features: skin colour, pulse rate, reflex, muscle tone and breathing. The sum of the five values gave a score out of 10; with a score of 7 to 10 considered normal, 4 to 7 required some resuscitation, and 3 or less required immediate resuscitation.

Spreadsheet data was fully de-identified once all outcome data for each pregnancy was included.
3.9 Construction of the “normal” AGA (nAGA) Group

In an uncomplicated pregnancy women are usually referred for only one ultrasound examination after the first trimester, which is the morphological assessment at 18 to 20 weeks GA. At this examination women can be recruited, but the difficulty lies in rescanning or recruiting these “normal” participants later in the pregnancy as they have no medical reason to undergo another ultrasound examination and are therefore not available for follow-up or recruitment. Recruiting non-referred “normal” pregnant women would attract challenging ethical issues and financial constraints beyond the capacity of this research. This limitation has been acknowledged in previous hospital based research (Proctor et al., 2013).

The participants in the AGA birthweight category had already been identified as delivering a neonate within the normal birthweight range (10th to 90th percentile) based on Australian standards and were from a low risk population by virtue of attending regional hospitals that did not offer high risk obstetric management. In order to include only “normal” pregnancies in the data set used to develop the UCV nomograms, the AGA birthweight category was further refined by the exclusion of participants who were identified as having obstetric or ultrasound characteristics that may cause, or indicate, abnormal fetal growth. These exclusion characteristics (Table 3.4) included non-term delivery, stillbirths, assisted conception, abnormal AFI or UA Doppler measurements, fetal structural anomalies and significant maternal risk factors for abnormal fetal growth. The refinement of the AGA birthweight category was based on responses from participants at recruitment, the clinical history provided by the referring medical officer, medical records and ultrasound results. The remaining sample of “normal” AGA birthweight participants was identified as the nAGA group.
Table 3.4

*Exclusion and Inclusion Criteria for the nAGA Group*

<table>
<thead>
<tr>
<th>Exclusion</th>
<th>Exclusion comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birthweight</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Outside the 10th and 90th percentiles</td>
</tr>
<tr>
<td><strong>Pre or post term delivery</strong></td>
<td>Delivery &lt; 259 days or &gt; 293 days</td>
</tr>
<tr>
<td><strong>Stillbirths</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Assisted conception</strong></td>
<td>In vitro fertilisation, intra-cytoplasmic sperm injection</td>
</tr>
<tr>
<td><strong>Elevated umbilical artery indices</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Reduced AFI</strong></td>
<td>Persistent, or as a single event in association with an increased umbilical artery ratio</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>Smokers eliminated based on response at recruitment</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>Gestational diabetes mellitus and pre-gestational diabetes</td>
</tr>
<tr>
<td><strong>Bowel disorders</strong></td>
<td>Coeliac disease, Crohn’s disease and ulcerative colitis</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>Hypertension and previous HELLP syndrome</td>
</tr>
<tr>
<td><strong>Fetal and cord anomalies</strong></td>
<td>Heart defects or two vessel umbilical cord</td>
</tr>
<tr>
<td><strong>Viral diseases</strong></td>
<td>Shingles, hepatitis C</td>
</tr>
<tr>
<td><strong>Chronic disorders</strong></td>
<td>Renal disease</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>Substance abuse</td>
</tr>
<tr>
<td><strong>Inclusion</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Birthweight</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Between the 10th and 90th percentiles</td>
</tr>
<tr>
<td><strong>Term delivery</strong></td>
<td>Delivery between ≥ 259 days and ≤ 293 days</td>
</tr>
<tr>
<td><strong>Live births</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Spontaneous conceptions</strong></td>
<td>Spontaneous conceptions</td>
</tr>
<tr>
<td><strong>Normal umbilical artery indices</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Normal AFI</strong></td>
<td>Reduced AFI not eliminated if associated with SROM or post dates</td>
</tr>
<tr>
<td><strong>Non smoker</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Chronic disorders</strong></td>
<td>Mild asthma, chronic fatigue, rheumatoid arthritis, personality disorders, vitamin deficiency, hypotension, osteoporosis, epilepsy, allergies</td>
</tr>
<tr>
<td><strong>Gynaecological problems</strong></td>
<td>Endometriosis, polycystic ovarian syndrome</td>
</tr>
<tr>
<td><strong>Thyroid disorders</strong></td>
<td>Hypothyroid, hyperthyroid, Graves’ disease, goitre</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>Migraine, neurological pain</td>
</tr>
<tr>
<td><strong>Blood disorders</strong></td>
<td>Thalassemia, thrombocytopenia, anaemia, maternal antibodies (A, D, Duff)</td>
</tr>
</tbody>
</table>

Notes. AFI = amniotic fluid index. SROM = spontaneous rupture of membranes.

<sup>a</sup>Birthweight percentiles according to Dobbins et al. (2012).
The nAGA participants were women who delivered a live, term neonate with term defined as the GA range from 37 completed weeks (≥ 259 days) until less than 42 weeks (< 294 days) (Hilder et al., 2014). Stillbirths were excluded from the nAGA subgroup as a fetal death may reflect placental disorders, IUGR, congenital abnormality or maternal hypertension (Collins et al., 2013). Altman and Chitty (1994) advocated the inclusion of fetal deaths in data used to develop nomograms; however, they were excluded from this research as this was perceived as an abnormal outcome.

IVF singleton pregnancies are considered to be at higher risk of IUGR compared to spontaneously conceived fetuses (McDonald et al., 2009; Suhag & Berghella, 2013). However, there was evidence that when confounders such as maternal age, parity, and fetal number are considered, the outcomes of IVF pregnancies are comparable to unassisted conceptions (Kondapalli & Perales-Puchalt, 2013). Due to a lack of consensus, ICSI and IVF conceptions were eliminated and only spontaneous conceptions remained in the nAGA sample.

Participants were excluded if the umbilical cord artery S/D ratio or PI were above the 95th percentile on standard nomograms. If the indices were elevated at a single attendance the participants were excluded as they may have been subsequently administered steroids, which can return the indices to normal (Nozaki, Francisco, Fonseca, Miyadahira, & Zugaib, 2009). In the absence of ruptured membranes, post-dates or renal anomalies, participants were excluded if the AFI persisted below the 5th percentile on standard nomograms, as oligohydramnios can reflect reduced fetal kidney perfusion as a consequence of fetal vascular redistribution caused by placental insufficiency (Bamfo & Odibo, 2011).
Participants who were identified as smoking anytime in the pregnancy or using any illicit substances were excluded. Nicotine, heroin, cocaine and amphetamines are linked to IUGR (Society of Obstetricians and Gynecologists of Canada, 2011; Soto & Bahado-Singh, 2013). Women who smoke during pregnancy have approximately 3.5 the risk of IUGR compared to non-smokers and the increased risk is dose, duration and GA dependent (Suhag & Berghella, 2013; M. Williams, Malik, Francis, & Gardosi, 2011).

Participants with pre-gestational or gestational diabetes mellitus were excluded as the growth rate of fetuses of these mothers differs from that of the normal population (Lepercq et al., 2001; Mohammadbeigi et al., 2013; Wong, Lee-Tannock, Amaraddio, Chan, & McIntyre, 2006). Fetal complications associated with maternal diabetes include macrosomia, and maternal complications include hypertension and preeclampsia (Setji, Brown, & Feinglos, 2005).

There is a significant association between maternal coeliac disease and IUGR (Suciu et al., 2014). Inflammatory bowel diseases including ulcerative colitis and Crohn’s disease increase the risk of adverse birth outcomes such as stillbirth, growth restriction and preterm birth, especially, if exacerbations occur during pregnancy (Broms et al., 2014). As several participants who experienced bowel disorders were admitted to hospital or investigated for abdominal pain, this group of participants were excluded from the nAGA sample.

Preeclampsia is a risk factor for the development of IUGR and chronic hypertension increases the likelihood of developing both IUGR and preeclampsia (Srinivas et al., 2009). HELLP (haemolysis, elevated liver enzyme levels and low platelet levels) syndrome is a variant of preeclampsia involving blood and liver complications in pregnancy. In a subsequent pregnancy, women with a history of HELLP syndrome, at or
before 28 weeks gestation, are at increased risk for preterm birth, pregnancy induced hypertension and increased neonatal mortality (Haram, Svendsen, & Abildgaard, 2009). Hypertension or preeclampsia documented by the referring doctor or a history of HELLP syndrome excluded participants from the nAGA group.

Fetal cardiac abnormities and a single UA are well documented structural causes of IUGR (S. Lee & Walker, 2010). Participants who carried a fetus with these anomalies were excluded from the nAGA group. Maternal infections such as toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, varicella-zoster virus (shingles) and syphilis are associated with approximately 5 to 15% of IUGR (Longo, Borghesi, Tzialla, & Stronati, 2014). Participants were eliminated from the nAGA group if any of these infections were documented by their referring doctor.

Prematurity, low birthweight, admission to neonatal intensive care, delivery by caesarean and fetal death are more prevalent in women with chronic kidney disease compared to women without renal disease (Kendrick et al., 2015). Recent data suggested that for women with mild kidney disease, 65% of pregnancies experienced no complications, such as preeclampsia, IUGR or preterm delivery (Bili, Tsolakidis, Stangou, & Tarlatzis, 2013). However, the incidence of preterm delivery, SGA and decline of maternal renal function increased with a reduction in the estimated glomerular filtration rate (Feng, Minard, & Raghavan, 2015), and consequently participants with chronic renal disease were excluded from the nAGA group.

Opinions vary about the outcomes of pregnancies complicated by hepatitis B or C. Reddick, Jhaveri, Gandhi, James, and Swamy (2011) reported that there was no association between hepatitis B and/or hepatitis C and IUGR or pre-eclampsia. However, when possible confounders had been controlled, Dunkelberg, Berkley, Thiel,
and Leslie (2014) and Safir, Levy, Sikuler, and Sheiner (2010) found that there was a significant association between a positive hepatitis B or C status and a birthweight less than 2,500 g. During pregnancy, women with hepatitis C are not treated, whereas women with chronic hepatitis B may undergo antiviral treatment (Dunkelberg et al., 2014). Due to the possibility of treatment and the lack of consensus regarding the association of hepatitis with IUGR, participants with hepatitis B or C were excluded from the nAGA group.

Current guidelines (Government of South Australia, 2012; Royal College of Obstetricians and Gynaecologists, 2014a; The American Congress of Obstetricians and Gynecologists, 2008), national asthma handbook (National Asthma Council Australia, 2015) and several studies (Johnston & Said, 2012; Little, 2016; MacMullen, Shen, & Tymkow, 2010) identified a low risk of IUGR in mothers with mild or well controlled asthma. As asthma was not given as the clinical reason for any nAGA group participant to undergo an ultrasound examination, and no participant was hospitalised during their pregnancy for exacerbation of asthmatic symptoms, participants with mild asthma were retained in the sample.

Women with seizure disorders are not at increased risk of IUGR (McPherson, Harper, Odibo, Roehl, & Cahill, 2013). Intrahepatic cholestasis of pregnancy has been associated with an increased incidence of spontaneous preterm delivery, respiratory distress syndrome, meconium staining of the amniotic fluid and stillbirth, but not IUGR (Williamson & Geenes, 2014). Participants with either of these conditions were included in the nAGA group.

Hyperthyroidism and hypothyroidism can be associated with IUGR (Saki et al., 2014). Graves’ disease is associated with hyperthyroid function. Goitre can be caused
by either hyperthyroidism or hypothyroidism. Hyperthyroidism and hypothyroidism in pregnancy are only risk factors for IUGR if the conditions are severe or uncontrolled (Collins et al., 2013; Patil-Sisodia & Mestman, 2010). Participants with previous abnormal thyroid function tests were medically managed by their referring medical officer and presented with euthyroid function during pregnancy and therefore they were included in the nAGA sample.

Women with thalassaemia are predisposed to severe maternal anaemia, which is associated with IUGR (Royal College of Obstetricians and Gynaecologists, 2014b). Higher rates of IUGR were found among patients with moderate to severe thrombocytopenia and were attributed to preeclampsia and HELLP syndrome (Parnas et al., 2006). As none of the participants identified with thalassaemia, anaemia or thrombocytopenia suffered any complications of their disorders during their pregnancies, they were not eliminated. Publication bias controlled meta-analyses (Facco, You, & Grobman, 2009; Howley, Walker, & Rodger, 2005; Rodger et al., 2010) and a recent clinical study (Coriu et al., 2014) indicate no significant association between Factor V Leiden and IUGR. Participants with this thrombophilia would not be excluded from the nAGA group. The most significant fetal complication of red cell antibodies is fetal anaemia that may result in hydrops (Royal College of Obstetricians and Gynaecologists, 2014c; Williamson & Geenes, 2014). As no fetus of a participant with maternal antibodies exhibited hydrops or elevated Doppler indices associated with fetal anaemia, these participants were included in the nAGA sample.
3.10 Stratification of the Small for Gestational Age (SGA) Category

In this research the SGA category was defined as those fetuses born with a birthweight below the 10th percentile based on Australian percentiles for GA and gender. This broad category encompassed normally growing small fetuses as well as fetuses that failed to reach their growth potential and were growth restricted (Parra-Saavedra et al., 2014; Sovio et al., 2015). One of the research aims was to determine if the GA related reference ranges, developed from the nAGA data, were able to identify growth restricted fetuses. To achieve this aim, a practical, historically relevant and clinically applicable stratification of the SGA birthweight category into normally growing small fetuses and pathologically small fetuses was required.

In order to isolate IUGR fetuses from SGA fetuses and use criteria comparable with previous research, a review of definitions used in nine relevant publications that had analysed the diameter, velocity or blood flow in the UV of IUGR fetuses was undertaken. The criteria used to define IUGR in these publications are shown in Table 3.5. The most common definition was birthweight or AC less than a predetermined value and this was frequently supported by abnormal UA PI readings. Considering the aim was to identify the in utero presence of growth restriction, it was anticipated that EFW would be part of the definition, which was supported by the POP study (Sovio et al., 2015). The POP study advocated that EFW and AC below the 10th percentile were the best predictors of delivering a SGA neonate with a high morbidity risk (Sovio et al., 2015). Including the EFW was consistent with results published by Ott (2002), who found that either EFW or AC were good predictors of IUGR and that accuracy was improved with Doppler evaluation of the UA. The inclusion of EFW was also supported by Unterscheider et al. (2013a) who reported that an EFW less than the 3rd percentile
was associated with adverse outcomes \((p = 0.0131)\). von Beckerath et al. (2013) supported the inclusion of UA Doppler results as pathologically small fetuses can be discriminated from constitutionally SGA fetuses by Doppler examination of fetal vessels, namely an elevated UA PI, or absent reversed end diastolic flow, or decreased MCA PI.

Table 3.5

Parameters Defining IUGR

<table>
<thead>
<tr>
<th>Research Papers</th>
<th>Sample size</th>
<th>Gestational age (weeks)</th>
<th>Diameter, velocity or blood flow</th>
<th>Parameters used to define a intrauterine growth restriction (IUGR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiserud et al. (1994)</td>
<td>38 IUGR</td>
<td>17 to 39</td>
<td>Blood flow</td>
<td>Diameter &lt;2.5%</td>
</tr>
<tr>
<td>Ferrazzi et al. (2000)</td>
<td>37 IUGR</td>
<td>25 to 38</td>
<td>Diameter, velocity and blood flow</td>
<td>&lt;2 SD below mean</td>
</tr>
<tr>
<td>Rigano et al. (2001)</td>
<td>21 IUGR</td>
<td>23 to 36</td>
<td>Diameter, velocity and blood flow</td>
<td>&lt;2 SD below mean</td>
</tr>
<tr>
<td>Boito et al. (2002)</td>
<td>33 IUGR</td>
<td>20 to 36</td>
<td>Diameter, velocity and blood flow</td>
<td>&lt;5th percentile</td>
</tr>
<tr>
<td>Di Naro et al. (2002)</td>
<td>15 IUGR</td>
<td>32 to 40</td>
<td>Blood flow</td>
<td>&lt;5th percentile</td>
</tr>
<tr>
<td>Bellotti et al. (2004)</td>
<td>56 IUGR</td>
<td>20 to 38</td>
<td>Blood flow</td>
<td>&lt;10th percentile</td>
</tr>
<tr>
<td>Rigano et al. (2008)</td>
<td>26 IUGR</td>
<td>20 to 30</td>
<td>Diameter, velocity and blood flow</td>
<td>&lt;2 SD below mean</td>
</tr>
<tr>
<td>Liao et al. (2014)</td>
<td>18 MCDA twins</td>
<td>29.5 to 32.4</td>
<td>Diameter, velocity and blood flow</td>
<td>&lt;10th percentile on Brazilian charts</td>
</tr>
<tr>
<td>Parra-Saavedra et al. (2014)</td>
<td>95 IUGR</td>
<td>&gt;34</td>
<td>Blood flow</td>
<td>&lt;10th percentile</td>
</tr>
</tbody>
</table>

Note: IUGR = intrauterine growth restriction.
As there was no identifiable consistent definition of IUGR/FGR, several combinations of ultrasound measurements and pregnancy outcomes were analysed. Using the SGA birthweight category as a baseline, multiple combinations were developed using:

i. Birthweight percentiles (< 10th, 5th and 3rd percentiles).

ii. EFW percentiles (≤ 10th, 5th and 3rd percentiles).

iii. AC ≤ 5th percentile.

iv. UA S/D ratio and/or UA PI > 95th percentile.

v. AFI < 5th percentile.

vi. MCA PI < 5th percentile.

vii. Apgar scores at 1 and 5 min.

viii. Stillbirths.

These combinations are shown in Table 10.4. The combination of birthweight < 10th percentile, EFW ≤ 10th percentile, AC ≤ 5th percentile and UA S/D ratio > 95th percentile was chosen to define IUGR for further analysis in this research and is referred to as the IUGR/FGR group. This decision was based on the rationale:

i. AC is a widely accepted indicator of poor intrauterine growth and the inclusion of this parameter makes the definition clinically robust.

ii. The 10th percentile is an established cut-off for both birthweight and EFW classification of IUGR, whereas the 5th and 3rd percentiles isolate severely growth restricted fetuses.

iii. This definition of IUGR is in keeping with findings in relevant publications (Table 3.5), in which four out of nine research papers investigating UV characteristics defined IUGR as reduced AC and elevated UA indices.
iv. Most research has used an elevated UA PI as a diagnostic indicator of IUGR. Review of research data found there were statistically inadequate numbers of elevated UA PI values, as only Participant 66 presented with three elevated UA PI readings, and therefore UA S/D ratio was used as an indicator of placental function.

v. This definition defines a mild to moderate form of IUGR which is commonly encountered and monitored in regional areas.

3.11 Ultrasound Equipment, Examination and Sonographers

3.11.1 The ultrasound machines.

Toshiba Aplio XG Version 3, model SSA-790A (Toshiba Medical Systems Corporation, Otawara-shi, Tochigi-ken, Japan) ultrasound machines manufactured in 2008 and 2009 were used for all ultrasound examinations. All measurements were recorded using either a transabdominal curved PVT-375BT transducer with a central frequency of 3.5 MHz or a transabdominal curved PVT-674BT transducer with a central frequency of 6 MHz depending on maternal body habitus. All examinations were completed using obstetric presets (Appendix J) tailored for specific transducers with optimised B-mode and Doppler imaging parameters. All ultrasound machines underwent routine servicing and calibration to ensure their safety and accuracy.

3.11.2 The ultrasound examination.

An ultrasound examination may take up to an hour depending on GA and diagnostic intent of the examination. In this research, at each participant presentation,
the sonographer took three additional optimised B-mode images of the UCV for measurement of the diameter and three additional Doppler spectral traces for measurement of the UCV PV.

The research actions extended the examination time by approximately 3 min and there were no other changes to routine scanning practices. To minimise any possible detrimental effects of the ultrasound examinations, all care was taken to reduce overall scanning and dwell time, lower the acoustic power, and lower the frame rate (Moderiano et al., 2015). Imaging the UCV reduced direct fetal exposure and all forms of Doppler avoided the fetal eyes. The obstetric presets had an initial MI of 1.3 for the 3.5 MHz transducer and 1.0 for the 6 MHz transducer, which are both compliant with previously discussed guidelines. The TI was also compliant with the guidelines: the Toshiba Aplio XG only displayed the TI if the value exceeded 0.4.

In all obstetric ultrasound examinations the care of the mother and fetus is paramount. Data collection was suspended if maternal or fetal well-being was compromised, the participant’s referring doctor was notified and the participant was escorted to the Maternity Ward. The measurements of the UCV diameter and PV were not included in the formal imaging report nor did the measurements influence the participant’s obstetric management. Neither the participants nor NSW Health and Ageing incurred any additional costs for the completion of this research. All sonographers respected the participants’ rights for dignity, comfort and privacy.

3.11.3 Sonographers and reliability.

Three sonographers at OHS and two sonographers at BHS received ethics approval to participate in this research. Subsequently, in August 2011, one of the OHS
sonographers resigned. The remaining four sonographers were senior staff members with scanning experience ranging from 15 to 21 years.

The intersonographer and intrasonographer reproducibility was maximised by using a strict protocol. This protocol required that the measurements be undertaken using the Toshiba Aplio XG (Version 3) ultrasound machine, with standardised image parameters at both sites, and by averaging three UCV measurements. I reviewed all images to ensure adherence to the protocol.

The intersonographer and intrasonographer reliability was assessed by retrospectively selecting B-mode images of the UCV diameter and Doppler spectral images of the UCV PV from the stored images of ten randomly chosen participants who had unmeasured images stored. These images were copied and downloaded to the hard drive of a single ultrasound machine. Blinded to the results of their colleagues, the four remaining research sonographers measured the diameter or the PV on each of the 20 images and stored the images. Each sonographer repeated the measurement three times for each image, with the images randomly selected from the hard drive list of files so that the three measurements were not consecutively repeated. All measurements were retrieved from the recorded images and entered into a spreadsheet. The intersonographer and intrasonographer reliability was assessed by a linear mixed model and the intraclass correlation coefficient (ICC) calculated according to Gwet (2014) using R statistical software version 3.1.1 (R Core Team, 2014). The ICC value ranges from 0 to 1: with poor agreement 0 to 0.2, fair agreement 0.21 to 0.4, moderate agreement 0.41 to 0.61, good agreement 0.61 to 0.80, and 0.81 to 1.0 indicating almost perfect agreement (Costa-Santos, Bernardes, Ayres-de-Campos, Costa, & Costa, 2011; Fernández et al., 2008).
3.12 Ultrasound Measurements and Calculations

The ultrasound machines and images were used for making a range of measurements and calculations.

3.12.1 Gestational age and estimated delivery date.

A reliable recollection of the first day of the LMP of a regular 28 day cycle, a known embryo transfer date or ultrasound anthropometric parameters measured before 14 weeks gestation determined the EDD of a fetus for the purpose of this research. If none of these criteria were upheld the participant was excluded from the research.

If there was a discrepancy of more than five days between the EDD estimated by LMP and ultrasound EDD by CRL in the first trimester, the ultrasound EDD was assigned (American College of Obstetricians and Gynecologists Opinion 611, 2014; New South Wales Government, 2014) (Table 2.4). Ideally pregnancy dating was undertaken at approximately eight weeks GA. Once the EDD was established, this date was used for all calculations of GA, even if this date varied slightly from the EDD used by the clinical team providing obstetric care to the participant.

The GA at the time of the ultrasound examination was calculated by the ultrasound machine software upon the entry of the EDD. The ultrasound machines automatically calculated the additional day added by the leap year in 2012.

3.12.2 Fetal biometry, growth and estimated fetal weight.

The anthropometric parameters of BPD, HC, AC and femur length were measured according to Australasian Society for Ultrasound in Medicine (ASUM) guidelines and plotted on ASUM 2000 graphs (Australasian Society for Ultrasound in Medicine, 2001).
The ASUM guidelines and graphs were standardised at both hospitals and parameters were measured at intervals of no less than 14 days for the purpose of fetal growth. The fetal weight was estimated by Hadlock C regression formula (Hadlock, Harrist, Sharman, Deter, & Park, 1985):

$$
\log_{10} = 1.335 - (0.0034 \times \text{abdominal circumference} \times \text{femur length}) \\
+ (0.0316 \times \text{biparietal diameter}) + (0.1623 \times \text{femur length}) \\
+ (0.0457 \times \text{abdominal circumference}).
$$

3.12.3 Amniotic fluid index.

The AFI was the sum of the deepest, unobstructed, vertical amniotic fluid pool in each quadrant of the pregnant uterus. The sum was plotted on a nomogram (Moore & Cayle, 1990) with < 5th percentile classified as oligohydramnios, a value between the 5th and 95th percentiles classified as normal, and an AFI > 95th percentile classified as polyhydramnios. Prior to approximately 24 weeks GA, the quantity of amniotic fluid was subjectively assessed by the sonographer and only quantified by the AFI if suspected to be abnormal.

3.12.4 Umbilical cord artery Doppler.

Localisation of the umbilical cord was predominantly undertaken in B-mode. Colour, power or advanced dynamic flow (ADF™) Doppler was used to enhance cord localisation for participants with suboptimal body habitus or low amniotic fluid volume. A mid portion of a free loop of umbilical cord was identified and the sweep speed, velocity scale and gain were optimised for recording spectral traces of the UA. Ideally the portion of umbilical cord was aligned parallel to the beam axis to minimise the error in measuring the Doppler shift. A small gate size and locating the gate over the central portion of the artery reduced spectral broadening. The pulse repetition
frequency (PRF) was optimised to avoid aliasing. UA Doppler measurements were taken during fetal quiescence (apnoea and absence of movement) from a frozen spectral trace displaying at least three, consecutive, uniform arterial waveforms. The traces of the umbilical cord artery were quantified by calculating the S/D ratio and PI (Figure 3.1). The S/D ratio and PI are angle independent indices quantifying downstream impedance. The lowest UA S/D and PI values were recorded and documented as abnormal if the S/D or PI > 95th percentile for GA according to nomograms (Arduini & Rizzo, 1990; Hecker, Campbell, Doyle, Harrington, & Nicolaides, 1995), or when there was absent or reversed end diastolic flow. All indices were calculated when the fetal heart rate was between 120 and 180 beats per minute to eliminate errors in the indices induced by aberrant diastole velocities.

![UA spectral trace with indices formulae. Adapted from “Doppler in obstetrics,” by K. Nicolaides, G. Rizzo, K. Hecker, and R. Ximenes, 2002, Diploma in Fetal Medicine and ISUOG Education Series, p. 23.](image-url)
3.12.5 Umbilical cord vein diameter.

A curve 3.5 MHz or 6 MHz transducer was used to identify a mid-portion of intra-amniotic umbilical cord in real-time. Lateral resolution was optimised by locating the focal zone at the level of the UCV and narrowing the sector width, and axial resolution was optimised by using the highest possible scanning frequency. The UCV was magnified to occupy approximately 25% of the screen using the depth and zoom functions prior to freezing (write zoom) as this improved the spatial resolution (Hedrick, 2013). A horizontal segment of cord was identified with the vein walls perpendicular to the ultrasound beam and the scanning plane aligned to the central widest part of the vessel, as shown in Figure 3.2c. The dynamic range and overall gain were optimised to improve resolution of the vein wall. The diameter was measured with the inner border of the horizontal line of the calipers placed on the line that defined the inner vein walls (Figures 3.3 and 3.4) excluding the vessel wall from the measurement. This measurement technique was chosen as all participating sonographers were practised in this method as it was routinely employed in measuring the nuchal translucency (Nicolaides et al., 1999; Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2011). The diameter measurements were recorded in millimetres (mm) to one decimal point which was the default setting of the ultrasound machine.

If possible, the diameter measurement was repeated thrice on different segments. All images were saved and formed part of the participants’ medical examination stored in PACS. The images and measurements were retrieved from PACS and the measurements entered into a re-identifiable spreadsheet. The mean of the three diameter measurements was referred to as the average UCV diameter. The
average UCV diameter rounded to one decimal point was used for statistical analyses of the cord diameter, and the unrounded average value was used in $Q_{ucv}$ calculations to eliminate truncation errors.

Figure 3.2. Schematic diagrams illustrating correct and incorrect measurements of the widest UCV diameter. (a) the ultrasound plane is parallel to the long axis of the vessel, but on the edge of the vessel and under measures the diameter, (b) a tilted ultrasound plane will yield curved vessel walls instead of horizontal lines and will incorrectly measure the diameter and (c) the ultrasound plane is aligned to the widest, central portion of the vessel enabling the maximum diameter to be correctly measured. Adapted from “Integration of umbilical venous and arterial Doppler flow parameters for prediction of adverse perinatal outcome,” by H. Shripad, R. Zainab, A. Prashant, and R. Lavanya, 2015, *International Journal of Health & Allied Sciences, 4*(4), pp. 224-225.
Figure 3.3. Measurement technique for the UCV diameter based on nuchal translucency protocol. (a) correct technique with the cross hairs of the calliper defining the inner-to-inner vein diameter and (b) incorrect calliper placement. Adapted from “Protocols for measuring the nuchal translucency,” by Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2011. Retrieved from http://www.nuchaltrans.edu.au/certification/protocols-for-measuring-the-nuchal-translucency

Figure 3.4. B-mode ultrasound images of the UCV showing measurement of the lumen diameter. De-identified images used with permission of the individuals.

The mean of three UCV diameters was used for statistical analysis because:

i. Averaging three diameters of the UCV provided the best approximation of the true size of the vessel as the UCV diameter progressively decreased, in a portion nearer the fetus, from the placenta to the fetal ends of this portion of the cord (W. Li et al., 2006).
ii. $Q_{ucv}$ calculations involving small vessels improves by increasing the number of repeated diameter measurements (Kiserud & Rasmussen, 1998).

iii. International research supports the use of an average diameter value as published by Acharya et al. (2005), who used the average of five measurements, whereas Ferrazzi et al. (2000) and Rigano et al. (2008) used the average of three diameter measurements.

In this research, the intra-amniotic UCV instead of the intra-abdominal portion of the umbilical vein was examined because:

i. Intra-amniotic cord segments were easier to locate, especially later in a pregnancy when the fetus was larger.

ii. Sonographers already had an established protocol and were familiar with performing very similar Doppler measurements on the UA.

iii. The Doppler shift equation ($f_D = 2vf \cos 2vf \cos \theta/c$) computes the velocity of blood ($v$) from the average speed of sound through tissue ($c$), the frequency shift ($f_D$), the transmitted frequency ($f$), and the angle of insonation ($\theta$) relative to the long axis of the vessel (Hofer, 2001). The best estimation of blood velocity occurs when the beam alignment is 0°, or parallel to the long axis of the vessel, and when the cosine of the insonation angle approaches one. With an angle of insonation approaching 90°, the cosine of the angle approaches zero, the frequency shift approaches zero, and there is an increasing relative error in calculating blood velocity. Achieving an insonation angle that is 0°, or close to 0°, is easier using the intra-amniotic portion, as some segment of the cord will
be vertical, whereas, the orientation of the intra-abdominal UV is highly
dependent on fetal position.

iv. Direct insonation of the fetus was reduced in keeping with the ALARA
principle.

3.12.6 Umbilical cord vein peak velocity.

A vertical portion of free-floating UCV was identified in real-time B-mode.
Ideally the longest length of vertical cord was used as this allowed maximum
development of the parabolic flow profile (Najafzadeh et al., 2016). Colour, power or
ADF™ Doppler imaging was used to enhance cord localisation for participants with
suboptimal body habitus or low amniotic fluid volume and the image was optimised in
both modalities. The Doppler gate, with a default width of 3 mm, was placed in the
centre of the vessel, as the blood profile in the UCV was assumed to be symmetrically
parabolic (Kiserud et al., 1994) and laminar (Sutton et al., 1990) with the peak velocity
found in the centre of the vessel. Careful placement of the Doppler gate avoided
contamination of the waveform by adjacent umbilical arteries.

Ideally, the Doppler angle was 0° to improve the estimation of blood velocity; if
unachievable, an angle of less than 15° with angle correction was used in all cases
included in the data. The scale, PRF and baseline were set so that the venous
waveform occupied approximately three quarters of the available spectral window.
With B-mode and colour Doppler frozen, the spectral trace ran for at least four
seconds and the trace was optimised for sweep speed and gain. The umbilical vein
trace was visually inspected for the presence and extent of fluctuations in the flow
profile and it was only included in the research if there was a continuous, monophasic
flow pattern recorded during fetal quiescence.
The PV was visually selected on the frozen image of the spectral trace and manually measured in units of centimetre per second (cm/s) to one decimal point, which was the default limit on the ultrasound machine (Figure 3.5). This procedure was repeated thrice, on different segments of cord if possible. All images were saved and formed part of the participant’s PACS ultrasound examination. The images and measurements were retrieved from PACS and the measurements were entered into a re-identifiable spreadsheet. The mean of the three PV measurements was referred to as the average UCV PV. The average UCV PV rounded to one decimal point was used for statistical analyses and construction of the GA related reference ranges. The unrounded average PV value was used for $Q_{ucv}$ calculations to eliminate truncation errors.

*Figure 3.5. Ultrasound image measuring UCV PV. De-identified image used with permission of the individual.*
The segment of cord used for Doppler velocity measurements was different to the portion used to measure the diameter, as the ideal measurement in spectral Doppler and B-mode necessitate vertical and horizontal orientation of the cord, respectively.

The UCV PV measurements were repeated thrice because:

i. The average of three UCV peak velocities negated the different flow patterns that exist along the length of the UCV.

ii. The current and established method in ultrasound research is the use of the average of three PV measurements in calculating $Q_{uv}$ (Barbera et al., 1999; Di Naro et al., 2002; El Behery et al., 2011; Flo et al., 2009).

3.12.7 Calculation of umbilical cord vein blood flow.

To compute the $Q_{ucv}$ the UCV $V_{\text{mean}}$ and area needed to be calculated from the raw data. The UCV $V_{\text{mean}}$ was calculated using the unrounded UCV PV value and the following formula (Acharya et al., 2005; Flo et al., 2010; Najafzadeh & Dickinson, 2012; Rizzo et al., 2016), in which 0.5 was the spatial velocity profile coefficient applicable to a parabolic profile:

$$UCV V_{\text{mean}} (cm/s) = \text{peak velocity (cm/s)} \times 0.5.$$  

The calculation of the cross-sectional area assumed the UCV was circular in shape, used the unrounded average UCV diameter measurements and the following sequence of computations. Converting the average UCV diameter measurements recorded in millimeters to centimetres:

$$Diameter(cm) = \frac{Diameter(mm)}{10},$$

where the radius ($r$) was the diameter divided by two and expressed in centimetres:

$$Radius(cm) = \frac{Diameter(cm)}{2}.$$
Calculating the UCV area used the formula for the area of a circle and a 15-digit value of \( \pi \) automatically generated by Excel®:

\[
UCV\ area (cm^2) = \pi \times r^2.
\]

The following formula was used to calculate \( Q_{ucv} \) (Acharya et al., 2005; Kiserud, 2003a; Rigano et al., 2008):

\[
Q_{ucv}\ (ml/min) = UCV\ area \times UCV\ V_{mean} \times 60
\]

\[
Q_{ucv}\ (ml/min) = (\pi \times r^2) \times (UCV\ PV \times 0.5) \times 60.
\]

Multiplying by 60 converted \( Q_{ucv} \) to a value in minutes; as this value was an exact count, it did not alter the number of significant figures. To avoid truncation errors the intermediate results were unrounded. The final \( Q_{ucv} \) value, rounded to one decimal point, was used for statistical analyses and construction of the GA related reference ranges. The \( Q_{ucv} \) value was expressed in millilitres per minute (ml/min) where 1 cm\(^3\) equals 1 cc equals 1 ml.

3.12.8 Ratios and longitudinal trends.

The ratios of the UCV diameter:PV and the UCV PV:diameter were calculated from the rounded average values for each nAGA group attendance and GA related reference ranges were developed. \( Q_{ucv} \), derived from the diameter and PV, was not involved in this ratio assessment as the aim was to develop a simple parameter with the least possibility for measurement or calculation errors.

In order to assess longitudinal trends all UCV diameter, PV and \( Q_{ucv} \) data points for SGA participants with more than two scans, with more than three scans in the LGA category and with more than five scans in the AGA birthweight category were selected and plotted against GA. In order to simplify the trend assessment, all SGA category participants with two or more scans were retained, giving 38 participants. Nineteen
participants from both the nAGA and LGA categories, with two or more scans, were randomly selected to give a combined data set of comparable size to the SGA group. The first and last scan UCV variables values were selected for these 76 participants and plotted against GA.

Using the previous data from the first and last attendances of the SGA, nAGA and LGA participants and combining this with similar data from the IUGR/FGR group, the slope of the longitudinal data from these subgroups was calculated:

$$slope = \frac{measurement_{final} - measurement_{first}}{GA_{final} - GA_{first}}.$$  

### 3.13 Data Analysis

#### 3.13.1 Data management.

All data were re-identifiable when entered into spreadsheets as each participant was assigned a sequential number determined by the date and time that the first image was recorded during the participant’s first ultrasound examination. The first presentation was demarcated as “a”, the second “b”, and so on. The characteristics of the participant were entered at the time of the first ultrasound examination. Pregnancy outcomes were entered once available. When data collection was completed, all information was stored in a non-identifiable format for statistical analyses.

The electronic data were stored on a password-restricted computer. Consent Forms and digital backups of data were stored in a locked filing cabinet. Both the computer and filing cabinet were located in a lockable office within the Medical Imaging Department of OHS. All care and diligence was taken in the transportation and storage of information.
All data collected in this research will be retained for 20 years after the project is completed in accordance with the State Records Authority of New South Wales GDA 23 guidelines (New South Wales Government, 2005b). The ultrasound images acquired for this research project will remain as part of the participants’ medical imaging record in PACS and will be disposed of in accordance with the State Records Authority of New South Wales GDA 17 guidelines (New South Wales Government, 2009b). These guidelines consider the fetus as the patient, therefore the obstetric images must be retained until the patient reaches the age of 25 years, or for a minimum of seven years after the last attendance for a diagnostic procedure, whichever is longer. All paper records relating to the participants’ obstetric ultrasounds were digitised, stored and disposed of in accordance with the State Records Authority of New South Wales GA 36 guidelines relating to imaged records (New South Wales Government, 2009a).

At the end of the retention periods, electronically stored data will be deleted and paperwork will be shredded. PACS images were stored in a centralised, offsite, unlimited capacity, secure, long-term archive provided by NSW Health.

3.13.2 Statistical analysis.

All statistical analyses were undertaken using R statistical software version 3.1.1 (R Core Team, 2014) in collaboration with Sharon Nielsen, Director Quantitative Consulting Unit, Charles Sturt University. Continuous variables were reported as means and standard deviations. Categorical, ordinal and discrete variables were reported as numbers and/or percentages. Where appropriate p values or confidence intervals were provided. Descriptive data analyses used scatterplots, boxplots and histograms.

The Chi-squared Test for Independence was used to determine whether there was a significant association between two categorical variables, such as sex of the
neonate or smoking in relation to birthweight category. The requirements of the Chi-
squared Test were that the individual observations were independent of each other,
that the value of the cells in the contingency table was five or more in at least 80% of
the cells, and that no cell had a value less than one (Martin & Pierce, 1994). When
these requirements were not met, the Fisher’s Exact Test was applied.

Like the Chi-squared Test for Independence, the Fisher’s Exact Test was used to
determine whether there was a significant association between two categorical
variables. There was a requirement that the individual observations were independent
of each other, but as this test was an exact test, there are no requirements on the cell
sizes in the contingency table (Everitt, 1998). Fisher’s Exact Test was used to analyse
the association between birthweight category and mode of delivery, maternal
ethnicity, ART, gestational age at delivery, cord artery Doppler indices, and AFI.

Ordinal regression was used when the variable was ordinal (categorical variable
with an order implied) such as the Apgar scores or parity in relation to birthweight
category. When fitting an ordinal regression model to the data there was a
requirement that the Condition Hessian Value was less than 10,000. Numbers less than
10,000 indicate that the model was not ill defined.

Analysis of variance (ANOVA) was used to determine if there was a significant
association between the three dependent variables (UCV diameter, PV and $Q_{ucv}$), and
the two independent variables (birthweight category and GA). ANOVA methods were
also used in UCV longitudinal data analysis. Weighted least squares regression was
applied in UCV slope analysis to account for different variances across the four
subgroups.
Linear mixed models were used to analyse maternal age at conception, GA at delivery in relation to birthweight category, and to analyse UCV diameter, \( PV \), \( Q_{ucv} \) in relation to birthweight categories and GA. Statistical analysis methods have assumptions or requirements to ensure that the results are reliable (Kuzma & Bohnenblust, 2001). The model assumptions were that: the residuals were normally distributed; they had a constant variance; they were independent; and, the factor level variances were equal. The Shapiro-Wilk test of normality was used to determine if the residuals were normally distributed and residual plots were used to visually determine if model assumptions were met. A Brown-Forsythe Test was used to determine if the factor level variances were equal. The coefficient of determination \( (R^2) \) was used to describe the goodness of fit of some linear mixed models to the data points; an \( R^2 \) value approaching one indicated the regression line was a good fit to the data. The greater the \( R^2 \) value the more the variability in one variable (UCV parameter and GA) is accounted for in terms of the variability in the other variable (birthweight category) (Polgar & Thomas, 2008). Linear mixed regression models where graphed showing the 95% CIs. The 95% CI provided the range, between the upper and the lower limits of the confidence interval, that 95% of the time will contain the true or population mean (Polgar & Thomas, 2008).

Quantile regression using the Quantreg procedure in R software was used to model the GA related reference ranges for UCV diameter, \( PV \), \( Q_{ucv} \) and ratios. Polynomial linear, quadratic and cubic quantile regression models were applied with backward selection of power and the most parsimonious model was selected. For each percentile, coefficients standard errors, \( t \) values and \( p \) values were produced and tabulated.
The polynomial regression equation expressing percentiles \((Q_p)\) of the UCV variables \((y)\) (diameter, PV, \(Q_{ucv}\) and ratio) as a function of GA was written:

\[
Q_p(y) = b_{0p} + b_{1p}GA + b_{2p}GA^2 + b_{3p}GA^3 \ldots \ldots \ldots \ldots
\]

Where \(b_{0p}\) represents the intercept, \(b_{1p}\), \(b_{2p}\) and \(b_{3p}\) the regression coefficients, \(p\) the 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th percentiles and GA the gestational age in days.

Quantile regression has been used as an alternative to standard regression models for statistical modelling of ultrasound data in recent years as it has been shown “to offer a more flexible approach for the estimation of growth curves” (Rizzo et al., 2016, p. 706). Quantile regression has several features that overcome assumptions of standard regression analysis. Quantile regression is more robust to outliers, does not assume a normal distribution of the data, or that the residuals have a mean of zero and constant variance (homoscedasticity). Quantile regression considers the distribution of data rather than the mean of data thereby allowing all portions of the distribution to be assessed, such that for each percentile a different fitted function is produced in response to advancing GA (Rizzo et al., 2016; Sandlin et al., 2014). A percentile is a value below which certain percentage of observations will fall on the reference ranges. Percentiles are easier to comprehend, are more intuitive to apply in clinical practice, and indicate the expected prevalence. However, percentiles perform poorly at quantifying outliers, as extreme values are combined in the highest or lowest percentiles (Wang & Chen, 2012).

A significance level of less than 0.05 \((p < 0.05)\) was used for all analyses. A \(p\) value is a measure of the “strength of evidence” against the null hypothesis, where the null hypothesis assumes no relationship or clinical difference between the variables.
(Dorey, 2010, p. 2297). This $p$ value indicates a 5%, or “1 in 20 probability of observing an association as large or larger than that found in the study by chance alone, given that there is really no association” (Hennekens & Burning, 1987, p. 246). Conversely, chance alone cannot be excluded as a possible explanation of the findings when the $p$ value is greater than 0.05 ($p > 0.05$) (Hennekens & Burning, 1987).

### 3.13.3 Dissemination of results.

Women participating in the study will receive a copy of the final publication if this was requested on their Consent Forms. Referring medical officers will receive a copy of the final publication. Journal publications, oral presentations and posters are noted throughout the exegesis and portfolio.
SECTION IV RESULTS AND DISCUSSION: THE SAMPLES

Chapter 4 Whole Research Sample Results

4.1 Introduction

In this chapter, the sample size and participation rates for the whole research sample will be detailed. Maternal characteristic, pregnancies outcomes, UA Dopplers, and AFI variables of this sample will be described and analysed in relation to three birthweight categories. The raw data are contained in Appendix K1 and statistical analysis in Appendix L1. Continuous variables are reported as means ± SD. Categorical, ordinal and discrete variables are reported as numbers or percentages. Where appropriate p values are provided.

4.2 Whole Research Sample Size and Participation Rates

There were 661 clients who consented to participate in the research while attending for obstetric ultrasound examinations at the Medical Imaging Departments of Orange and Bathurst Health Services. The women attended these hospitals between 5 April 2011 and 22 March 2013 from the central west New South Wales towns of Orange, Bathurst, Mudgee, Wellington, Parkes, Forbes, Condobolin, Cowra, and surrounding localities (Figure 4.1).

Based upon inclusion criteria, 661 women were accepted into the research project, 36 participants were later excluded due to:

i. Unavailable pregnancy outcomes (20).

ii. Early termination due to lethality (1).
iii. Noncompliance of ultrasound measurements with research protocols (13),

and

iv. GA not established prior to 14 weeks gestation (2).

The final sample size of 625 participants is referred to as the whole research sample in the exegesis and portfolio.

Figure 4.1. Map of NSW Heath Districts showing the locality of the research sample. Adapted from “NSW allied health scholarships rural classification maps,” by Health Education and Training Institute. Retrieved from http://www.heti.nsw.gov.au/content/scholarship-rural-classification-maps/

BHS conducted ultrasound examinations on 29 participants and OHS 596 participants. Greater than half of the participants underwent more than one ultrasound examination (Table 4.1) with BHS completing 32 (2.3%) and OHS 1,342
(97.7%) of the total 1,374 ultrasound examinations. These examinations represent 34.2% and 3.6% of possible examinations at OHS and BHS, respectively, and gave an overall participation rate of 28.6% based on the number of similar ultrasound examinations \((n = 4,810)\) undertaken at these Health Services during the data collection period.

Table 4.1

<table>
<thead>
<tr>
<th>Number of ultrasound examinations per participant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>275</td>
<td>167</td>
<td>84</td>
<td>49</td>
<td>23</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

4.3 Birthweights and Birthweight Categories

Birthweights in the whole research sample ranged from 880 g to 5,010 g with a mean live birthweight of 3,297±530 g and a median of 3,300 g. The mean live born birthweight of males was 3,351±550 g which was 103 g heavier than their female counterparts at 3,248±507 g. Neonates of mothers who used ART, compared to those who did not, had a mean live birthweights of 3,117±647 g and 3,301±527 g, respectively. For Indigenous mothers the mean live birthweight was 3,144±537 g which was 160 g less than their non-indigenous equivalents (3,304±529 g). Using national birthweight percentiles, defined by GA and gender (Dobbins et al., 2012), the participants were divided into three categories based on the birthweight percentile band of their neonate: SGA (< 10th percentile), AGA (10th to 90th percentiles) and LGA (> 90th percentiles). The majority of neonates/participants where categorised as AGA (Table 4.2).
Table 4.2

*Number of Participants/Neonates in Each Birthweight Category (n = 625)*

<table>
<thead>
<tr>
<th>Birthweight categories</th>
<th>Small for gestational age (SGA)</th>
<th>Appropriate for gestational age (AGA)</th>
<th>Large for gestational age (LGA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants/neonates</td>
<td>62 (9.9%)</td>
<td>515 (82.4%)</td>
<td>48 (7.7%)</td>
</tr>
</tbody>
</table>

4.4 Whole Research Sample Maternal Characteristics in Relation to Birthweight Categories

4.4.1 Ethnicity.

In the sample, 4% of mothers identified themselves as being Indigenous. The remainder of the sample identified as white (90.6%), black (0.8%), East Asian (0.8%), South Asian (1.6%), Southeast Asian (0.4%) and mixed race (1.8%) (Table 4.3). The Fisher’s Exact Test ($p = 0.569$) showed birthweight category and maternal ethnicity were not dependent.

Table 4.3

*Ethnicity of Participants According to Birthweight Category (n = 625)*

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous</td>
<td>25</td>
<td>3</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Black</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>East Asian</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>South Asian</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>White</td>
<td>566</td>
<td>56</td>
<td>464</td>
<td>46</td>
</tr>
<tr>
<td>Mixed</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
<td>515</td>
<td>48</td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.
4.4.2 Maternal age at conception.

Maternal age at conception ranged from 15 to 42 years, with a mean of 27.5±5.6 years and a median of 28 years. The maternal ages were recorded as continuous variables and grouped for demographic summary (Table 4.4). Figure 4.2 shows the maternal ages were symmetrically distributed. ANOVA determined no dependent relationship existed between maternal age at conception and birthweight category ($p > 0.05$).

Eighty percent of Indigenous mothers had their babies before the age of 30 compared to 63% of non-Indigenous mothers (Table 4.4). Indigenous mothers had a younger mean age of conception of 25.2±2.8 years compared to 27.6±5.6 years for non-Indigenous mothers. Linear mixed modelling found no significant relation between maternal age and ethnicity ($p = 0.055$).

Table 4.4

<table>
<thead>
<tr>
<th>Maternal age at conception (years)</th>
<th>Birthweight categories</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole sample</td>
<td>SGA</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>54</td>
<td>9</td>
</tr>
<tr>
<td>20 - 24</td>
<td>144</td>
<td>15</td>
</tr>
<tr>
<td>25 - 29</td>
<td>202</td>
<td>18</td>
</tr>
<tr>
<td>30 - 34</td>
<td>155</td>
<td>14</td>
</tr>
<tr>
<td>35 - 39</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
</tr>
</tbody>
</table>

*Note: SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.*
4.4.3 Methods of conception.

In the sample, 4.3% of participants conceived as a result of ART. The most commonly used ARTs were clomiphene citrate (37%) and IVF (48%) as shown in Table 4.5. Clomiphene citrate was used most frequently by 25 - 29 year olds and IVF by 30 - 34 year olds. IVF (42.8%) and clomiphene citrate (50%) were used most frequently for conception of the first child, and after two children no ART was used by the participants to conceive. The birthweight category of the newborn and the use of ART were not dependent based on the Fisher’s Exact Test ($p = 0.731$). However, the same analysis demonstrated a significant relationship ($p = 0.006$) between maternal age at conception and the use of ART, as the mean age of women who used ART was 30.1±4.9 years compared to 27.5±5.6 years for participants who did not use ART.
Table 4.5

Conception Methods According to Birthweight Category (n = 625)

<table>
<thead>
<tr>
<th>Method of conception</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>598</td>
<td>60</td>
<td>493</td>
<td>45</td>
</tr>
<tr>
<td>Clomiphene citrate</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracytoplasmic sperm injection</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro fertilisation</td>
<td>13</td>
<td>2</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Letrozole</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
<td>515</td>
<td>48</td>
</tr>
</tbody>
</table>

Note. SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

4.4.4 Parity.

Parity ranged from zero to eight children (Table 4.6). Nearly three quarters of participants were expecting their first or second child and the mean age of nulliparous mothers was 25.2±5.6 years. Using an ordinal logistic regression model, birthweight category was dependent on parity (p = 0.01) with 50% of SGA participants being first time mothers. Table 4.7 shows that the heaviest neonates in the SGA, AGA and LGA categories tended to be born to multigravida mothers; whereas the lightest babies in each category were all born to mothers with different parities and were never born to first time mothers.
Table 4.6

Parity According to Birthweight Category (n = 625)

<table>
<thead>
<tr>
<th>Parity</th>
<th>Whole sample</th>
<th>Birthweight categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGA</td>
</tr>
<tr>
<td>0</td>
<td>227</td>
<td>31</td>
</tr>
<tr>
<td>1</td>
<td>212</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>102</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

Table 4.7

Mean Birthweight by Birthweight Category and Parity

<table>
<thead>
<tr>
<th>Parity</th>
<th>Birthweight (g) in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA</td>
</tr>
<tr>
<td>0</td>
<td>2,589</td>
</tr>
<tr>
<td>1</td>
<td>2,679</td>
</tr>
<tr>
<td>2</td>
<td>2,557</td>
</tr>
<tr>
<td>3</td>
<td>2,713</td>
</tr>
<tr>
<td>4</td>
<td>2,320‡</td>
</tr>
<tr>
<td>5</td>
<td>2,910†</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>2,720</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age. NA = not applicable.  
†Lightest.  
‡Heaviest.

4.4.5 Maternal diseases.

In all birthweight categories at least three quarters of mothers reported having no major health problems. Chronic disorders (mild asthma, chronic fatigue, renal disease, rheumatoid arthritis, personality disorders, vitamin deficiency, hypotension, osteoporosis, epilepsy, allergies) were the most frequently stated medical conditions
of the mothers, followed by diabetes and thyroid disorders as seen in Table 4.8. The large number of cells with zero or low numbers meant that it was not possible to carry out statistical analyses on this data and therefore any possible relationship between maternal disorders and birthweight categories could not be determined.

Table 4.8

<table>
<thead>
<tr>
<th>Maternal disorders</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No maternal diseases</td>
<td>497</td>
<td>51</td>
<td>410</td>
<td>36</td>
</tr>
<tr>
<td>Diabetes</td>
<td>24</td>
<td>3</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Bowel disorders</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Blood disorders</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Gynaecological problems</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17</td>
<td>2</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid disorders</td>
<td>23</td>
<td>1</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Other complications of pregnancy</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Chronic disorders</td>
<td>33</td>
<td>3</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Viral diseases</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
<td>515</td>
<td>48</td>
</tr>
</tbody>
</table>

Note. SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

4.4.6 Smoking status during pregnancy.

Overall, 24.6% of participants reported smoking at some time during their pregnancy (Table 4.9). Three quarters of smokers were under the age of 30 years, with the mean maternal age at conception for mothers who smoked being 25.7±5.9 years compared to 28.1±5.5 years for non-smokers. More Indigenous mothers (36%) reported smoking at some time during their pregnancy compared to non-Indigenous mothers (24.2%). Birthweight category and maternal smoking were dependent
according to the Chi-squared Test for Independence ($p = 0.02$), as a greater number of SGA neonates had a mother who reported smoking during the pregnancy.

Table 4.9

Smoking Status of Participants According to Birthweight Category ($n = 625$)

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>154</td>
<td>24</td>
<td>121</td>
<td>9</td>
</tr>
<tr>
<td>No</td>
<td>471</td>
<td>38</td>
<td>394</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
<td>515</td>
<td>48</td>
</tr>
</tbody>
</table>

Note. SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

4.5 Whole Research Sample Pregnancy Outcomes in Relation to Birthweight Categories

4.5.1 Perinatal and infant deaths.

There were 625 singleton births including three stillbirths attributed to antepartum haemorrhages, giving a fetal death rate of 0.48% (4.8 per 1000 births). All stillbirths were in the AGA birthweight category. There was one infant death, from the AGA birthweight category, at six months of age due to a cardiac anomaly.

4.5.2 Gestational age at delivery.

The mean GA at birth was 273.9±11.6 days (39 weeks and 1 day). The earliest birth was 181 days (25 weeks and 6 days) and the latest was 294 days (42 weeks). The 39th week of pregnancy (Table 4.10) was the commonest delivery period with 29% of births occurring during this week.
Table 4.10

GA at Delivery (n = 625)

<table>
<thead>
<tr>
<th>Gestational age at delivery (weeks)</th>
<th>25</th>
<th>30</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
<th>41</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neonates</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>11</td>
<td>22</td>
<td>51</td>
<td>144</td>
<td>181</td>
<td>135</td>
<td>64</td>
<td>1</td>
</tr>
</tbody>
</table>

Note. Only the weeks during which births occurred are included in the table.

The GA at delivery was divided into three standard categories (Hilder et al., 2014) (Table 4.11). The majority (92%) of babies were born at term, 7.8% were born preterm, and 0.2% were born post term. ANOVA (p = 0.356) demonstrated that birthweight category and GA at delivery were not dependent.

Table 4.11

Number of Neonates in Three Delivery Categories According to Birthweight Category (n = 625)

<table>
<thead>
<tr>
<th>Gestational age at delivery</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm (&lt; 37 weeks)</td>
<td>49</td>
<td>5</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Term</td>
<td>575</td>
<td>57</td>
<td>472</td>
<td>46</td>
</tr>
<tr>
<td>Post term (≥ 42 weeks)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>625</td>
<td>62</td>
<td>515</td>
<td>48</td>
</tr>
</tbody>
</table>

Note. SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

4.5.3 Sex of the newborn.

There were 329 (52.6%) females born and 296 (47.4%) males. In the SGA and AGA birthweight categories there were more girls than boy neonates, with the reverse ratio seen in the LGA birthweight group (Table 4.12). The Chi-squared Test for Independence (p = 0.594) did not demonstrate a relationship between the newborns’ birthweight category and sex.
4.5.4 Mode of delivery.

The majority of births were normal vaginal deliveries (60.6%) (Table 4.13). The 30 - 34 year old group had the highest percentage of caesarean deliveries (33.8%), and the 25 - 29 year old group achieved more vaginal deliveries (35.1%). About one third of caesareans were performed on first time mothers. The newborns’ birthweight category and mode of delivery were not dependent according to the Fisher’s Exact Test ($p = 0.709$).

4.5.5 Apgar scores at one and five minutes after birth.

The three stillborn babies had Apgar scores of zero at both one and five minutes. The Apgar score was not recorded for four newborns at one minute and for three newborns at five minutes after birth (Table 4.14). The neonates’ Apgar scores at
one and five minutes after birth, classified by birthweight category, are shown in Table 4.15. The Apgar score at one and five minutes was recorded as an ordinal variable, and an ordinal logistic regression analysis model was used to assess the relationship between birthweight category and Apgar scores. This type of analysis required that the Condition Hessian Value was less than 10,000 which was true for both scores. The neonates’ birthweight categories were not dependent at the one minute Apgar score ($p > 0.05$) or the five minute Apgar score ($p > 0.05$).

Table 4.14

**Number of Neonates in Apgar Score Groups at One and Five Minutes after Birth ($n = 625$)**

<table>
<thead>
<tr>
<th>Apgar scores at 1 min</th>
<th>0 to 3</th>
<th>4 to 6</th>
<th>7 to 10</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neonates</td>
<td>12</td>
<td>39</td>
<td>570</td>
<td>4</td>
</tr>
</tbody>
</table>

| Apgar scores at 5 min | 4     | 10    | 608    | 3               |

Table 4.15

**Number of Neonates in Apgar Score Groups at One and Five Minutes after Birth According to Birthweight Category ($n = 625$)**

<table>
<thead>
<tr>
<th>1 min Apgar scores</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 3</td>
<td>12</td>
<td>1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>4 to 6</td>
<td>39</td>
<td>9</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>7 to 10</td>
<td>570</td>
<td>51</td>
<td>477</td>
<td>42</td>
</tr>
<tr>
<td>Not available</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5 min Apgar scores</th>
<th>4</th>
<th>0</th>
<th>4</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 3</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>4 to 6</td>
<td>608</td>
<td>60</td>
<td>501</td>
<td>47</td>
</tr>
<tr>
<td>Not available</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Note.** SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.
4.6 Whole Research Sample Ultrasound Indices in Relation to Birthweight Categories

4.6.1 Umbilical cord artery Dopplers.

A normal UA S/D ratio was recorded in 942 out of 1,374 ultrasound examinations and 46 examinations recorded an S/D ratio above the 95th percentile (Table 4.16). The ratio was not measured in 386 examinations as the GA was less than the lower limit (28 weeks) on the relevant nomogram (Hecker et al., 1995) or the measurement of ratio was inappropriate under the clinical context of the examination. The birthweight category and the UA S/D ratio were dependent ($p < 0.001$) by the Fisher’s Exact Test.

A normal PI was recorded during 1,053 out of 1,374 ultrasound examinations (Table 4.16). The index was not measured in 318 examinations due to a GA less than the lower limit (20 weeks) on the relevant nomogram (Arduini & Rizzo, 1990) or the index measurement was inappropriate under the clinical context of the examination. Only one SGA fetus (Participant 64/ Visits 66a - c) recorded an elevated UA PI on three separate ultrasound examinations. The newborns’ birthweight category and UA PI were dependent ($p = 0.003$) by the Fisher’s Exact Test.
Table 4.16

Number of Ultrasound Examinations According to UA S/D ratio (n = 988), PI (n = 1,056) and Birthweight Category

<table>
<thead>
<tr>
<th>Birthweight categories</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical artery S/D ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>942</td>
<td>119</td>
<td>742</td>
<td>81</td>
</tr>
<tr>
<td>&gt; 95th percentile</td>
<td>46</td>
<td>24</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>988</td>
<td>143</td>
<td>764</td>
<td>81</td>
</tr>
<tr>
<td>Umbilical artery PI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1,053</td>
<td>145</td>
<td>821</td>
<td>87</td>
</tr>
<tr>
<td>&gt; 95th percentile</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,056</td>
<td>148</td>
<td>821</td>
<td>87</td>
</tr>
</tbody>
</table>

Note: S/D = systolic/diastolic, PI = pulsatility index. SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.

4.6.2 Amniotic fluid index.

Greater than 95% of all examinations recorded a normal AFI and an AFI less than the 5th percentile was commonest in the SGA birthweight category (Table 4.17).

The newborns’ birthweight category and AFI were dependent ($p < 0.001$) by the Fisher’s Exact Test.

Table 4.17

Number of Ultrasound Examinations According to AFI and Birthweight Category (n = 1,374)

<table>
<thead>
<tr>
<th>Amniotic fluid index</th>
<th>Whole sample</th>
<th>SGA</th>
<th>AGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3rd percentile</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 5th percentile</td>
<td>35</td>
<td>13</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 95th percentile</td>
<td>15</td>
<td>0</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
<td>1,306</td>
<td>147</td>
<td>1,043</td>
<td>115</td>
</tr>
<tr>
<td>Total</td>
<td>1,374</td>
<td>169</td>
<td>1,086</td>
<td>118</td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age. AGA = appropriate for gestational age. LGA = large for gestational age.
4.7 Conclusion

Birthweight was used to divide the whole research sample into three categories and statistical analysis was undertaken to define the relationships between these categories, maternal characteristics and pregnancy outcomes. A dependent relationship was identified between parity, smoking, UA Doppler indices, AFI and birthweight categories. The remaining characteristics and outcomes did not have dependent relationships with the birthweight categories.
Chapter 5 Whole Research Sample Discussion

5.1 Introduction

In this chapter the sample size, participation rates, bias and the use of birthweight as a method of categorising the participants will be discussed. Participant and neonate features of the whole sample will be compared to broader populations. The relationships between maternal characteristics, pregnancy outcomes, ultrasound indices and birthweight categories of the whole research sample will be discussed and compared.

5.2 Whole Research Sample

5.2.1 Sample size.

To detect the difference between AGA and SGA fetuses for PV and Q\textsubscript{uv}, at the 5% significance level and with a dropout rate of 10%, 176 participants were required. The final sample size of 625 participants was approximately four times the recommended sample size which halved the sample error to 0.04 and increased the sample’s relevance.

5.2.2 Participation rate.

OHS was the major recruitment site and achieved a 34.2% participation rate, which was lower than the mean participation rate of 55.6±19.7% reported in a review of 141 academic papers (Baruch, 1999). The low participation rate of 3.6% at BHS was a consequence of Bathurst being a satellite research site and I was not present to encourage recruitment. An overall participation rate of 28.6% of all eligible ultrasound examinations undertaken at Orange and Bathurst Health Services was comparable to
an American study in which the participation response rates, in medical research, varied with the type of recruitment procedure and was as low as 27% when written advance permission was required (K. Nelson et al., 2002).

5.2.3 Bias.

There was no perceivable difference between participants and non-participants, and no unintended bias was introduced by the non-participants. There was no intentional selection bias as no client in the population was favoured in, or excluded from, the selection process. However, selection bias cannot be wholly excluded as there was systematic favouritism by clients who self-selected to attend the public hospitals for their obstetric ultrasounds. As Medicare bulk billing was available at the public health services, there was a higher attendance of lower socio-economic clients as there were no out-of-pocket expenses for ultrasound examinations. OHS operates antenatal clinics for diabetes and maternal obesity which may have skewed the sample towards mothers with these disorders. Orange and Bathurst Health Services both provide monitoring of fetuses with anomalies or growth discrepancies and as such the whole research sample may not be wholly typical of a low risk obstetric population. Neither hospital provided tertiary obstetric care and clients requiring this level of care would not have been available as potential participants.

The lower and upper 10% of birthweights were defined as SGA and LGA, respectively. Both the SGA (9.9%) and LGA (7.7%) categories contained approximately 10% of the participants indicating that the whole research sample was not biased towards any particular birthweight category.
5.3 Birthweight Categories

Participants and neonates were retrospectively classified as SGA, AGA or LGA based on birthweight as there was an obvious disparity between EFW and birthweight.

The difference between these two weights stems from two broad areas:

i. The ultrasound measurements: The EFW formula is dependent on the accuracy of the measurements which it incorporates (Nesbitt-Hawes, Tetstall, Gee, & Welsh, 2014) and errors occur through poor calliper placement and noncompliance with standardised measurement protocols. These measurement errors may be intensified by sonographer inexperience, awkward fetal position, reduced amniotic fluid, suboptimal image quality and equipment faults (Edwards, 2001; Nahum, 2014).

ii. The EFW formula: In this research, EFW was calculated using the Hadlock C formula (Hadlock et al., 1985). This formula was derived from a cross-sectional study of 109, mainly white, middle-class, American women who are not representative of the current Australian population.

In a study comparing ultrasound EFW to actual birthweight the Hadlock C formula had an overall error of 14.7%, but this was as high as 37.8% for birthweights less than 1,000 g and as low as 9.9% for birthweight between 2,000 - 2,999 g (Anderson, Jolley, & Wells, 2007). A comparison of the six most commonly used ultrasound EFW formulae against 1999 Australian birthweights demonstrated that after 37 weeks gestation, ultrasound EFW significantly overestimated the birthweight (Campbell Westerway, 2012). ASUM recognises this imprecision in EFW formulae and does not recommend any specific formula for the calculation of fetal weight, since
none have achieved sufficient accuracy to be endorsed (Australasian Society for Ultrasound in Medicine, 2001).

Given these irregularities, it was appropriate to use Australian, sex stratified, actual birthweights to classify fetal growth in this research. Other researchers have also used local birthweights as a method of defining fetal growth (Boito et al., 2002; Rigano et al., 2001). It was acknowledged that all fetuses classified below the 10th percentile by birthweight were not growth restricted and some fetuses born within the normal weight range may have failed to reach their potential weight and be growth restricted. The aim of this study was to develop reference ranges for characteristics of the UCV using the best classification system available, which arguably is not the ultrasound derived EFW.

5.4 Whole Research Sample Characteristics Summary

A comparison of the maternal characteristics and pregnancy outcomes of the whole research sample to Local Health District (LHD), state and national data was published in the Australasian Journal of Ultrasound in Medicine, 19(3), pp. 118-122 in 2016 (Appendix M). In summary, the whole research sample was typical of the Australian population with similar proportions of Indigenous mothers, ART usage, parity, delivery by caesarean section, and the numbers of female and male neonates. Mothers in the whole research sample were younger, especially non-Indigenous mothers, more mothers smoked at some point in their pregnancy, and neonates were lighter than comparable Australian populations.
5.5 Whole Research Sample Maternal Characteristics in Relation to Birthweight Categories

Using the whole research sample and birthweight categories, there was no statistically dependent relation between birthweight categories and ethnicity, maternal age at conception or ART usage. However, parity and smoking status during pregnancy were shown to have a statistically dependent relationship with birthweight categories.

Maternal ethnicity and birthweight category and were not dependent \( (p = 0.569) \). National data from 2011, showed 12.6% of Indigenous mothers gave birth to a live born weighing less than 2,500 g, compared to 6% of non-Indigenous mothers (Z. Li et al., 2013). The research data did not reflect this trend, as 4% of Indigenous neonates weighed less than 2,500 g, which was similar to 5.5% of non-Indigenous neonates.

In the whole sample, 4% of mothers identified themselves as being Indigenous which was similar to the NSW data (3.4%) and identical to national data (4.0%) (Hilder et al., 2014). Western NSW LHD reported 13.8% of mothers as Indigenous in 2010 (Centre for Epidemiology and Evidence, 2012). The difference between the LHD and the whole research sample may reflect the more easterly geographical location of the research area compared to the whole LHD (Figure 4.1). The majority of the whole research sample identified themselves as white (90.6%). Comparison to a broader population was limited as maternal country of birth is the national reported maternal feature. However, in the 2011 national census, ancestry data was collected based on identification with cultural groups. Census data was difficult to interpret due to double counting and the inclusion of whole Australian population, nevertheless,
approximately 0.7% of respondents identified as being black, 4.6% as East Asian, 2.6% as South Asian and 2.5% as Southeast Asian (Australian Government, 2011). An ethnicity or ancestry defined as “white” was commonest in both the whole research sample and the Australian census report.

No relationship between maternal age at conception and birthweight category \((p > 0.05)\) was identified, with the 25 - 29 year age range being the most frequent range across all birthweight categories. This finding was at odds with a large 2006 meta-analysis that concluded a maternal age of 40 years or older was an independent risk factor for IUGR with an odds ratio of 3.2 (Odibo, Nelson, Stamilio, Sehdev, & Macones, 2006). SGA rates were also higher in 35 years or older women compared with women aged 20 - 24 years, in a large Canadian study (Joseph et al., 2005). Kramer (1987) proposed that age alone was not a risk factor in mothers older than 34 years, but that age amplified the impact of other risk factors. This last statement was reflected in the research sample as there were 70 participants aged 34 years and older and only 6 (8.6%) had an SGA neonate.

National data from 2013 showed Indigenous women had their babies at younger ages (median 24.9 years) compared with non-Indigenous women (median 30.8 years) (Australian Bureau of Statistics, 2014). In the whole research sample the difference between the two groups was not as extreme as the national data (median age at conception of 24 years versus 28 years, respectively). The overall trend of Indigenous women being younger mothers was reflected in the research data, with 52% of Indigenous mothers having their babies before the age of 24 years compared to 30.8% in the non-Indigenous mothers, and 80% before 30 years compared to 63.3% non-Indigenous.
The birthweight category of the newborn and ART was not dependent \((p = 0.731)\), as a low percentage of women used ART in all birthweight categories. In Australia in 2011, the mean birthweight was 3,331 g for live born ART singletons which was 67 g lower than the mean birthweight of all live born singletons (Macaldowie, Wang, Chughtai, & Chambers, 2014). Using only IVF and ICSI data, the mean weight of ART live born neonates was 3,117±647 g, which was 180 g lighter than the whole research sample mean and consistent with the national trend. There was a significant \((p = 0.006)\) relation between maternal age at conception and ART usage, as a greater proportion of older participants used ART. In 2011, the average age of Australian women at the time of delivery was 30.0 years, which was five years younger than women who used IVF, GIFT or ICSI (mean age 34.9 years) (Macaldowie et al., 2014). A similar trend was seen in the whole research sample, as participants who conceived using IVF and ICSI data had a mean age of 30.0±3.1 years compared to 27.4±5.6 years for participants using all other methods of conception.

A dependent relationship between birthweight category and parity \((p = 0.01)\) was demonstrated and is consistent with a recent meta-analysis of 41 studies showing nulliparity significantly increased the risk of low birthweight and SGA births (Shah, 2010). Shah’s (2010) finding was reflected in the whole research sample, as 50% of all SGA neonates were born to nulliparous participants. Kramer (1987) showed that a parity of five or greater was not associated with low birthweight and this was reflected in the sample, as there were only 19 participants with a parity of five or greater, of which only two had SGA neonates. Generally, the greater the parity, the heavier the birthweight (Varvarigou, Asimakopoulou, & Beratis, 2008), which was reflected in the research data, as the heaviest neonates in the AGA and LGA birthweight categories
were born to mothers with the highest parity, whereas the heaviest SGA category neonate was born to the mother with the second highest parity.

As could be expected from published research, birthweight category and maternal smoking were dependent variables ($p = 0.02$). Overall, 24.6% of participants reported smoking at some time during their pregnancy. A larger percentage (38.7%) of mothers who gave birth to an SGA baby reported smoking during the pregnancy compared to smoking mothers who gave birth to AGA babies (23.5%) or LGA babies (18.8%). Cigarette smoking is the most significant maternal environmental factor for IUGR and there is a linear correlation between the number of cigarettes smoked per day and the degree of growth restriction (Wollmann, 1998). Recent research confirmed that tobacco smoke inhibits fetal growth and proposed that tobacco smoke causes vasoconstriction of the umbilical arteries (Milnerowicz-Nabzdyk & Bizoń, 2014).

Nationally in 2012, approximately 48% of Indigenous mothers and 11% of non-Indigenous mothers reported smoking during pregnancy (Hilder et al., 2014). Compared to national data, a lower percentage of Indigenous mothers (36%) in the whole research sample reported smoking at some time during their pregnancy, and a greater proportion of non-Indigenous mothers (24.2%) reported smoking. Three quarters of smokers were under the age of 30 years, and higher levels of smoking have been associated with youth unemployment (Blank & Burau, 2013). This may explain the higher rates of smoking identified in the sample, as there was 13.5% to 14.1% youth unemployment within the local area, compared to 11.8% for NSW (Brotherhood of St Laurence, 2014). Smoking during pregnancy is linked to socio-economic factors including smoking behaviour by close family and peer groups, status as a single mother, unemployment of the mother or a partner, low levels of education and
occupational skills, poor living standards, and larger families (Gillies, Madeley, & Power, 1989; Zolnierczuk-Kieliszek, Chemperek, & Koza, 2004).

5.6 Whole Research Sample Pregnancy Outcomes in Relation to Birthweight Categories

In relation to birthweight categories there was no statistically established dependent relation with GA at delivery, sex of the newborn, mode of delivery or Apgar scores in the whole research sample. In the whole research sample, birthweight category and GA at delivery were not dependent ($p = 0.758$), nor was the newborns’ birthweight category and sex ($p = 0.594$). This was the expected result as the birthweight percentiles employed in this research (Dobbins et al., 2012) were stratified according to GA and gender. The newborns’ birthweight category and method of delivery were not dependent variables ($p = 0.709$), as the percentage of neonates delivered by either caesarean, instrumental vaginal or normal vaginal delivery was similar in all three birthweight categories for the research sample.

In the research sample, the neonates’ birthweight categories were not dependent on the one ($p > 0.05$) or the five minute Apgar score ($p > 0.05$). In developed countries, approximately 1% of newborns have an Apgar score less than seven at five minutes, which if prolonged, is associated with an increased risk of death and neurological incapacity (Ehrenstein, 2009). Excluding the three stillbirths and unavailable results, 1.8% of all neonates had an Apgar score of less than seven at five minutes consistent with Ehrenstein’s (2009) findings. By birthweight category 1.6% of SGA, 1.8% of AGA and 2.1% of LGA category neonates had an Apgar score of less than seven at five minutes, which is not consistent with a review of 17 articles, undertaken
by Ehrenstein (2009), who concluded that extremes of neonatal size more frequently received low Apgar scores. The association with low birthweight and low Apgar scores was evident in earlier research undertaken in Taiwan from 1982 to 1987, which found that infants with low and very low birthweights had low Apgar scores (Mori, Shiraishi, Negishi, & Fujimura, 2008). Australian birth statistics also shows that 9% of low birthweight babies (< 2,500 g) had an Apgar score of less than seven, whereas only 1% of babies weighing 2,500 g or more had similar scores (Australian Institute of Health and Welfare, 2015).

The three neonatal deaths within the sample had an AGA birthweight classification and all deaths were attributed to antepartum haemorrhages. In a recent Scottish study reviewing 784,576 term singleton births between 1992 and 2008, the authors found that 33% of antepartum stillbirths and 17% of term delivery related deaths occurred in neonates with a birthweight outside the 20th to 97th percentile range (Moraitis, Wood, Fleming, & Smith, 2014). This association with extreme birthweight was also acknowledged by Simchen et al. (2000) who found IUGR preterm infants had a higher mortality compared to AGA preterm infants and proposed that the long held belief that IUGR fetuses are more mature at delivery, due to prolonged intrauterine stress, was incorrect.

In the whole research sample the mean male live birthweight was 103 g heavier than the female equivalent. A Canadian study (van Vliet, Liu, & Kramer, 2009) suggested that the difference between male and female birthweights had decreased between 1981 and 2003 and proposed that environmental exposures to endocrine disrupters may be responsible. Such a reduction in the difference between male and female birthweights was not evident in a NSW study of term singleton deliveries.
between 1990 and 2007, although the mean birthweight increased by 23 g for boys and 25 g for girls during this 16 year period, the gap between the sexes remained at approximately 120 g (Hadfield et al., 2009). This continued difference between the birthweight of the sexes is further supported by Australian data (Table 5.1), which showed that singleton live born males were approximately 120 g heavier than their female counterparts, between 1998 and 2007 (Dobbins et al., 2012). This trend has continued with the gap between the sexes being 119 g in 2011 and 115 g in 2012 based on all live births during this period (Hilder et al., 2014; Z. Li et al., 2013).

Table 5.1

Mean Birthweight for Infants by Sex in Australia between 1998 and 2012

|------|------|------|------|------|------|------|------|------|------|------|------|------|


Females typically have a higher incidence of low birthweight compared to male babies (Hilder et al., 2014; Mondal, 1998). This was reflected in the research data, as 34 female neonates and 28 male neonates were classified as SGA. A review of 66 articles concluded that there is a relative risk of 1.19 for females neonates having a low birthweight compared to males (Kramer, 1987).
5.7 Whole Research Sample Ultrasound Indices in Relation to Birthweight Categories

In the whole research sample there was a significant relation between UA indices, AFI and birthweight categories. Doppler assessment of the UA, in an uncomplicated pregnancy, showed that the impedance to flow within the arteries declines with advancing GA (Nicolaides et al., 1999). The newborns’ birthweight category and the UA S/D ratio \( p < 0.001 \) and PI \( p = 0.003 \) were dependent. The fetuses classified as SGA had a higher percentage (16.8%) of elevated UA S/D ratio compared to other birthweight categories, and no LGA birthweight babies had an elevated ratio. No AGA or LGA birthweight neonates had an elevated UA PI. One SGA fetus had an elevated UA PI on three separate occasions.

The neonates’ birthweight category and AFI were found to be dependent \( p < 0.001 \) with a larger percentage of SGA (13%) neonates having an AFI below the 5th percentile compared to the AGA and LGA neonates. In uteroplacental insufficiency IUGR there is redistribution of the cardiac output away from the fetal abdomen and limbs to the vital organs, decreasing perfusion of the fetal kidneys and lungs and consequently reducing amniotic fluid production (Manning, 2009), as reflected in the research findings.

5.8 Conclusion

The whole sample of 625 participants was in excess of the size recommended for research purposes and was recruited without intentional bias. The whole research sample was relatively typical of the Australian population except that mothers were younger, more mothers smoked, and neonates were lighter than comparable
Australian populations. In relation to birthweight categories there was no statistically established dependent relation with ethnicity, maternal age at conception, ART, GA at delivery, sex of the neonate, mode of delivery or Apgar scores in the whole research data. There was a statistically dependent relationship between birthweight categories and parity, smoking status during pregnancy, umbilical cord indices and AFI.
Chapter 6 “normal” Appropriate for Gestational Age (nAGA) Results

6.1 Introduction

The nAGA group was a subgroup of the AGA birthweight category participants who had no significant risk factors for abnormal fetal growth. nAGA participants delivered a live, term neonate, whose conception was unassisted, and normal AFI and UA Doppler measurements were recorded throughout the pregnancy. This subgroup of 321 participants represented “normal” pregnancies and was used to construct the GA related reference ranges. Sample features, maternal characteristics and pregnancy outcomes of this subgroup will be detailed in this chapter. The raw data are contained in Appendix K2 and statistical analysis in Appendix L2. Continuous variables are reported as means ± SD. Categorical, ordinal and discrete variables are reported as numbers and/or percentages.

6.2 nAGA Group Sample Size and Participation Rates

The elimination of confounders for abnormal intrauterine fetal growth from the AGA birthweight category resulted in a nAGA group sample of 321 participants. Fourteen (4.4%) participants in the nAGA group underwent their examinations at BHS and the remaining 307 (95.6%) were scanned at OHS. Approximately half (50.5%) of participants underwent more than one ultrasound examination as shown in Table 6.1, producing a total of 603 ultrasound examinations, of which 15 examinations were undertaken at BHS. The processes of establishing the birthweight categories and the nAGA group are summarised in Figure 6.1.
Table 6.1

*Number of Ultrasound Examinations Undertaken on nAGA Group Participants (n = 603)*

<table>
<thead>
<tr>
<th>Number of ultrasound examinations per patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>159</td>
<td>93</td>
<td>35</td>
<td>25</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 6.1. Flowchart showing construction of research samples.
6.3 nAGA Group Maternal Characteristics

In the nAGA cohort, the majority of participants were white with less than 3% of participants in any of the other ethnicity categories (Table 6.2). Maternal age at conception ranged from 15 to 42 years with a mean age of 27.8±5.4 years. The median age of the group was 28.5 years and the maternal ages were normally distributed. All Indigenous mothers had their babies before the age of 30 compared to 61.9% of non-Indigenous mothers (Table 6.3). Indigenous mothers had a younger mean age of conception of 23.3±2.8 years compared to 27.9±5.4 years for non-Indigenous mothers.

Table 6.2

*Ethnicity of the nAGA Group (n = 321)*

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous</td>
<td>9</td>
</tr>
<tr>
<td>Black</td>
<td>5</td>
</tr>
<tr>
<td>East Asian</td>
<td>4</td>
</tr>
<tr>
<td>South Asian</td>
<td>7</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>290</td>
</tr>
<tr>
<td>Mixed</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 6.3

*Number of nAGA Participants per Age Group and Indigenous Status (n = 321)*

<table>
<thead>
<tr>
<th>Maternal age at conception (years)</th>
<th>nAGA</th>
<th>Non-Indigenous</th>
<th>Indigenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>17</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>20 - 24</td>
<td>76</td>
<td>71</td>
<td>5</td>
</tr>
<tr>
<td>25 - 29</td>
<td>109</td>
<td>106</td>
<td>3</td>
</tr>
<tr>
<td>30 - 34</td>
<td>80</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>35 - 39</td>
<td>35</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>321</td>
<td>321</td>
<td>9</td>
</tr>
</tbody>
</table>

*Note.* nAGA = “normal” appropriate for gestational age.
All conceptions in the nAGA group were spontaneous, as IVF and ICSI conceptions were eliminated. Hormonal therapy was used by 10 participants to improve successful ovulation and implantation. Eight participants used clomiphene citrate, one DHEA and one letrozole. Parity in the nAGA group ranged from zero to eight children (Table 6.4). Three quarters (74.5%) of participants were expecting their first or second child.

Table 6.4

<table>
<thead>
<tr>
<th>Parity</th>
<th>Number of participants</th>
<th>Mean maternal age±SD (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>116</td>
<td>26.0±5.5</td>
</tr>
<tr>
<td>1</td>
<td>123</td>
<td>27.6±4.6</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>29.4±5.0</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>32.9±5.4</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>30.0±2.8</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>31.5±6.4</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>37.5±3.5</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>30</td>
</tr>
</tbody>
</table>

*Note. NA = not applicable.*

6.4 nAGA Group Pregnancy Outcomes

The live birthweight of the nAGA group ranged from 2,630 g to 4,320 g with a mean of 3,398±317 g, and a median of 3,390 g. The mean live birthweight of males was 3,458±321 g and females 3,348±305 g, both being approximately 100 g heavier than the whole sample mean live birthweights. The mean live birthweight of neonates of nAGA indigenous mothers was 3,196±358 g and 3,403±314 g for babies of non-
Indigenous mothers, being 50 g and 100 g heavier than their whole sample equivalents, respectively.

Retaining only term deliveries restricted the nAGA group GA at delivery range to be between 259 days to 293 days. The majority of the nAGA group babies (35.2%) were born between 39 and 40 weeks GA as shown in Table 6.5 with the mean GA at delivery being 276±7.5 days (39 weeks and 3 days).

Table 6.5

The Number of Deliveries between 37 and 42 Weeks in the nAGA Group (n = 321)

<table>
<thead>
<tr>
<th>Gestational age at delivery (weeks)</th>
<th>37 - 38</th>
<th>38 - 39</th>
<th>39 - 40</th>
<th>40 - 41</th>
<th>41 - 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of births</td>
<td>31</td>
<td>68</td>
<td>113</td>
<td>80</td>
<td>30</td>
</tr>
</tbody>
</table>

There were 146 male neonates born and 175 female neonates in the nAGA group. There were 321 deliveries in the nAGA group: 63.9% were normal vaginal deliveries, 9% were instrumental vaginal deliveries, and 27.1% of deliveries were by caesarean. The mean age of participants undergoing a caesarean section was 29.3±5.5 years, instrumental vaginal delivery 25.1±5.4 years, and normal vaginal delivery 27.5±5.2 years. More first time mothers underwent a caesarean (31.9%) or instrument delivery (17.2%).

The Apgar score was not recorded for one newborn at one minute. All newborns at five minutes after birth had an Apgar score recorded as shown in Table 6.6. There were 19 newborns with an Apgar score of less than seven at one minute after delivery and only three newborns with a score of less than seven at five minutes.
Table 6.6

Apgar Scores at One and Five Minutes after Birth for the nAGA Group (n = 321)

<table>
<thead>
<tr>
<th>1 min Apgar scores</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 3</td>
<td>3</td>
</tr>
<tr>
<td>4 to 6</td>
<td>16</td>
</tr>
<tr>
<td>7 to 10</td>
<td>301</td>
</tr>
<tr>
<td>Not available</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5 min Apgar scores</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 3</td>
<td>0</td>
</tr>
<tr>
<td>4 to 6</td>
<td>3</td>
</tr>
<tr>
<td>7 to 10</td>
<td>318</td>
</tr>
<tr>
<td>Not available</td>
<td>0</td>
</tr>
</tbody>
</table>

6.5 Conclusion

The nAGA group was a subgroup of AGA birthweight participants numbering 321. This subgroup incorporated only those participants that met strict inclusion criteria and as such some maternal characteristics and pregnancy outcomes reflected the selection criteria. Mothers had no confounders for fetal growth, a majority of mothers identified as white, Indigenous mothers were younger than non-Indigenous mothers, and three quarters of mother were expecting their first or second child. Pregnancy outcomes defined by the selection criteria, included delivery of a live neonate with a GA between 37 completed weeks and 42 weeks, and birthweight between the 10th and 90th percentiles by Australian standards. In the nAGA group there were more male neonates delivered, male neonates and babies born to non-Indigenous mothers were heavier, and a majority of babies were delivered vaginally.
Chapter 7 “normal” Appropriate for Gestational Age (nAGA) Discussion

7.1 Introduction

The sample, bias, maternal characteristics and pregnancy outcomes of the nAGA group will be discussed in this chapter and compared to the Australian population. The portion of analysis will determine if this subgroup, used for the creation of the reference ranges, was typical of broader populations.

7.2 nAGA Group Sample

7.2.1 Sample size.

After removal of confounders for abnormal fetal growth (Table 3.4), the nAGA group consisted of 321 participants, which is twice the recommended sample size.

7.2.2 Bias.

Data on which reference ranges are constructed should relate to “normal fetuses”; individuals with maternal or fetal conditions known to affect fetal growth should be excluded from any reference group (Altman & Chitty, 1994). Ideally, for a normal sample, data should be collected on women not requiring ultrasound examinations, as there is bias in using data collected from women referred for an ultrasound due to the clinical reason for the investigation (Altman & Chitty, 1994). This form of selection bias was minimised in this research design, as the participants in the nAGA group were refined from the whole research sample after data collection was completed and therefore no participant was recruited, or not recruited, based on their potential to be part of the nAGA group. Additionally, many initial referrals were made as a component of routine antenatal care, rather than due to pathological reasons.
Altman and Chitty (1994) proposed that serial measurements be used for the development of fetal growth reference percentiles, whereas cross-sectional data is preferred for fetal size reference percentiles. Current statistical analyses are able to accommodate for variances within, and between participants, negating the sole use of either longitudinal or cross-sectional data collection (Littell et al., 2000). Altman and Chitty (1994) also indicated that if only cross-sectional data was used, the number of observations per fetus would vary and sampling bias would result since fetuses who are delivered early will be underrepresented. This form of sampling bias was minimalised in the research design by including only term deliveries.

7.3 nAGA Group Maternal Characteristics

The maternal characteristic of ART usage, diseases and smoking are not discussed as these variables were eliminated by the exclusion criteria. Participants in the nAGA group had similar ethnicity as the 2012 Australian population, but tended to be younger and have a higher parity.

In the nAGA group, 2.8% of mothers identified themselves as Indigenous which was not significantly different to the 2012 Australian ($p = 0.341$) or NSW ($p = 0.663$) proportions (Hilder et al., 2014). The remaining nAGA participants identified as being white (90.3%), black (1.6%), East Asian (1.2%), South Asian (2.2%), and mixed ethnicity (1.9%); there were no Southeast Asian participants. Comparison of the nAGA ethnicity diversity with a wider group was difficult due to the various methods of ethnical classification and data collection. However, “white” ethnicity or ancestry was commonest in both the nAGA group and the 2011 Australian census report (Australian Government, 2011).
The maternal age at conception of the nAGA participants ranged from 15 to 42 years, which was narrower than the 15 to 56 years reported for Australia in 2012 (Hilder et al., 2014). The nAGA group mean maternal age of 27.8±5.4 was significantly less ($p = 0.001$) than the 2012 mean Australian age of 30.1 years (Hilder et al., 2014). Teenage (< 20 years) mothers made up 3.6% of the national population in 2012, which was not significantly different ($p = 0.139$) to the 5.3% recorded in the nAGA group; however, the national data would have included ages less than 15 years. Nationally 4.3% of mothers were aged 40 and over and only 1.3% of the nAGA group fell into this age range.

The nAGA Indigenous mothers had a mean age of conception of 23.3±2.8 years, which was similar ($p = 0.083$) to the 2012 national mean of 25.2 years old at delivery. The nAGA non-Indigenous mothers had a mean age of conception of 27.9±5.4 years, which was significantly less ($p < 0.001$) than the 2012 national average of 30.3 years old at delivery (Hilder et al., 2014). There is an exaggerated difference in maternal ages between the nAGA group and the government data. The former was calculated from the maternal age at conception and the latter from maternal age at the birth of the baby, which could make the nAGA group mothers 38 weeks younger than government data for a term delivery.

Compared to 2012 NSW ($p = 0.027$) and national ($p = 0.005$) data, there were significantly fewer mothers having their first baby in the nAGA group. Proportionally more mothers in the nAGA group had given birth previously compared to 2012 NSW ($p = 0.005$) and national ($p = 0.004$) data (Table 7.1).
Table 7.1

Percentage of Women Who Gave Birth by Parity in the nAGA Group, NSW and Australia

<table>
<thead>
<tr>
<th>Parity</th>
<th>nAGA group</th>
<th>Percent of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.2%</td>
<td>44.0%</td>
</tr>
<tr>
<td>1</td>
<td>38.3%</td>
<td>33.2%</td>
</tr>
<tr>
<td>2</td>
<td>15.9%</td>
<td>14.3%</td>
</tr>
<tr>
<td>3</td>
<td>5.9%</td>
<td>5.1%</td>
</tr>
<tr>
<td>4 or more</td>
<td>3.7%</td>
<td>3.3%</td>
</tr>
<tr>
<td>Not stated</td>
<td>0%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>


7.4 nAGA Group Pregnancy Outcomes

Due to the inclusion criteria, all nAGA group neonates were singleton, live born, neonates delivered between 37 weeks and 41 weeks and 6 days GA. The nAGA group had similar birthweights, modes of delivery rates and Apgar scores compared to state and national data, but the remaining outcomes were significantly different.

Table 7.2 provides evidence for the following comparisons. The mean live birthweight of the nAGA group was very similar to the 2012 national mean ($p = 0.085$) (Hilder et al., 2014). The nAGA group male ($p = 0.183$) and female ($p = 0.081$) mean live birthweights were very similar to 2012 national data (Hilder et al., 2014). The nAGA group Indigenous birthweights were within the range of weights identified by states, with the highest mean Indigenous birthweight recorded in Victoria (3,298 g) and the lowest in Western Australian and the Northern Territory (3,128 g) (Hilder et al., 2014). There was no significant difference between the nAGA birthweights and national birthweights of Indigenous ($p = 0.9$) and non-Indigenous ($p = 0.089$) neonates (Hilder et al., 2014).
Table 7.2

Mean Live Birthweights of the nAGA Group Compared to 2021 Australian Data

<table>
<thead>
<tr>
<th></th>
<th>Mean live birthweight (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nAGA</td>
<td>Australia 2012 (Hilder et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Whole sample</td>
<td>3,398±317</td>
<td>3,367</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3,458±321</td>
<td>3,422</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3,348±305</td>
<td>3,307</td>
<td></td>
</tr>
<tr>
<td>Indigenous mothers</td>
<td>3,196±358</td>
<td>3,211</td>
<td></td>
</tr>
<tr>
<td>Non-Indigenous mothers</td>
<td>3,403±314</td>
<td>3,373</td>
<td></td>
</tr>
</tbody>
</table>

Note: nAGA = “normal” appropriate for gestational age.

The nAGA group had a sex ratio of 83.4 male births per 100 female births. Compared to 2012 national sex ratio of 106.4 males births per 100 female births ($p = 0.033$) and NSW sex ratio of 107.4 males births per 100 female births ($p = 0.027$), there were significantly less males born in the nAGA group.

The mode of delivery of the nAGA group was compared to state and national data in Table 7.3. The caesarean rate in the nAGA group was less than the Australian rate ($p = 0.049$), but similar to the NSW caesarean rate ($p = 0.137$). The nAGA group had a similar rate of all vaginal deliveries compared to 2012 state ($p = 0.137$) and national data ($p = 0.053$) (Hilder et al., 2014).

Table 7.3

Birth Method and Locality

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Caesarean</td>
<td>27.1%</td>
<td>31.1%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Instrumental vaginal</td>
<td>9%</td>
<td>11.4%</td>
<td>12.4%</td>
</tr>
<tr>
<td>Normal vaginal</td>
<td>63.9%</td>
<td>57.5%</td>
<td>55.3%</td>
</tr>
</tbody>
</table>

There were only three (0.9%) newborns with an Apgar score of less than seven at five minutes in the nAGA group, which was not significantly different ($p = 0.398$) to the 2012 national rate of 1.7%. In the same year, Apgar scores between 0 and 3 were recorded at five minutes for 0.3% of live born babies nationally, compared to 0% in the nAGA group and respective rates of 1.4% and 0.9% for scores 4 to 6 at five minutes (Hilder et al., 2014).

### 7.5 Conclusion

Overall the nAGA group was similar to the Australian population in respect to the proportion of Indigenous mothers, vaginal deliveries and low Apgar scores at five minutes. There was no significant difference in the mean birthweight between the nAGA group and 2012 Australian data. nAGA group mothers were significantly younger than the 2012 average Australian mother, with non-Indigenous mothers dominating this trend. Significantly fewer mothers were expecting their first child and less male babies were born compared to 2012 national data. The major characteristics of the whole research sample and the nAGA group are summarised in Table 7.4.
Table 7.4

Comparison of Major Maternal Characteristic and Pregnancy Outcomes between the Whole Research Sample and the nAGA Group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Whole research sample</th>
<th></th>
<th>nAGA group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD or %</td>
<td></td>
<td>mean±SD or %</td>
<td></td>
</tr>
<tr>
<td>Participants numbers</td>
<td>625</td>
<td></td>
<td>321</td>
<td></td>
</tr>
<tr>
<td>Number of examinations</td>
<td>1,374</td>
<td></td>
<td>603</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>27.5±5.6</td>
<td></td>
<td>27.8±5.4</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>36.3%</td>
<td></td>
<td>36.2%</td>
<td></td>
</tr>
<tr>
<td>&gt; 1</td>
<td>63.7%</td>
<td></td>
<td>63.8%</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indigenous</td>
<td>4%</td>
<td></td>
<td>2.8%</td>
<td></td>
</tr>
<tr>
<td>Non-Indigenous</td>
<td>96%</td>
<td></td>
<td>97.2%</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>24.6%</td>
<td></td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>Fetal death rate</td>
<td>0.48%</td>
<td></td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>273.9±11.6</td>
<td></td>
<td>276.2±7.5</td>
<td></td>
</tr>
<tr>
<td>(days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3,297±530</td>
<td></td>
<td>3,398±317</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean</td>
<td>31.7%</td>
<td></td>
<td>27.1%</td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>68.3%</td>
<td></td>
<td>72.9%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>47.4%</td>
<td></td>
<td>45.5%</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>52.6%</td>
<td></td>
<td>54.5%</td>
<td></td>
</tr>
<tr>
<td>Apgar score &lt; 7 at 5 min</td>
<td>1.8%</td>
<td></td>
<td>0.9%</td>
<td></td>
</tr>
</tbody>
</table>

Note: nAGA = “normal” appropriate for gestational age.
SECTION V RESULTS AND DISCUSSION: RELIABILITY

Chapter 8 Reliability Results

8.1. Introduction

This chapter will report the intersonographer and intrasonographer reliability for measurements of the UCV diameter and PV.

8.2. Umbilical Cord Vein Diameter and Peak Velocity Reliability Results

The data of three repeated UCV diameters and PV measurements made by four sonographers on images from 10 randomly selected participants are contained in Appendix K3 and related statistical analysis in Appendix L3. Boxplots displaying this data are shown in Figures 8.1 and 8.2. The GA of the selected participants ranged from 23 weeks 6 days to 39 weeks 5 days.

The intersonographer and intrasonographer reliability for measurements of the UCV diameter and PV were assessed by a linear mixed model including participants and sonographers, and the ICC calculated according to Gwet (2014) using R statistical package (R Core Team, 2014). All model assumptions were met, with the exception of UCV PV residuals which were not derived from a normally distributed population as indicated by the Shapiro-Wilk test ($p = 0.011$); however, ANOVA techniques are robust against such departures and this was not considered to be important for this analysis. The ICC for UCV diameter and PV measurements are shown in Table 8.1.
Figure 8.1. Boxplots of repeated UCV diameter measurements recorded by four sonographers on 10 random participants’ images. (a) sonographers and (b) participants.

Figure 8.2. Boxplots of UCV PV measurements recorded by four sonographers on 10 random participants’ images. (a) sonographers and (b) participants.
Table 8.1

*Intersonographer and Intrasonographer ICC Results*

<table>
<thead>
<tr>
<th></th>
<th>Intersonographer</th>
<th>Intrasonographer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICC</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>UCV diameter</strong></td>
<td>0.98</td>
<td>[0.41, 0.99]</td>
</tr>
<tr>
<td><strong>UCV peak velocity</strong></td>
<td>0.98</td>
<td>[0.43, 0.99]</td>
</tr>
</tbody>
</table>

Note. UCV = umbilical cord vein. CI = confidence interval.

ICC values can range from 0 to 1: with poor agreement 0 to 0.2, fair agreement 0.21 to 0.4, moderate agreement 0.41 to 0.60, good agreement 0.61 to 0.80 and 0.81 to 1.0 indicating almost perfect agreement (Costa-Santos et al., 2011; Fernández et al., 2008). Therefore, the intersonographer and intrasonographer ICCs for measurements of the UCV diameter and PV demonstrate almost perfect agreement.

8.3. Conclusion

The intersonographer and intrasonographer reliabilities were assessed using a linear mixed model and the ICC calculated for measurements of the UCV diameter and PV. The intersonographer and intrasonographer ICC demonstrated excellent agreement within and between sonographers.
Chapter 9 Reliability Discussion

9.1 Introduction

In this chapter, the intersonographer and intrasonographer ICC results will be discussed and compared with previous publications. Reliability assessment is necessary to determine if the raw data collected by the research sonographers had little variability and provided the best possible data to construct the GA related reference ranges. Reliability, reproducibility and precision are terms used to describe the similarity of measurements repeated by the same sonographer and by different sonographers at various sites, on different machines and at different times (Barbieri, Cecatti, Souza, Marussi, & Costa, 2008).

9.2 Umbilical Cord Vein Diameter and Peak Velocity Reliability Discussion

Intersonographer reliability refers to the systematic variation between sonographers undertaking a measurement. The greater the agreement between sonographers means the reliability is higher. Intrasonographer reliability refers to the deviation between repeated measurements by a sonographer measuring a particular object and the more uniform the measurements, the higher the reliability.

The ICC is an assessment of the uniformity of measurements made by individuals measuring the same item (Shrout & Fleiss, 1979). The ICC compares the variance of different measurements with the total variance of all measurements. In this analysis, variance was derived from ANOVA using a linear mixed model. ICC value ranges from 0 to 1, with a value greater than 0.81 indicating almost perfect agreement (Costa-Santos et al., 2011; Fernández et al., 2008). The ICC is considered superior to
both Pearson and Spearman analyses, as the ICC takes into account the differences between observers (Shrout & Fleiss, 1979) and the variance of all measurements (Figueras et al., 2008).

The sonographer boxplots for measurement of the UCV diameter and PV in Figures 8.1 and 8.2 showed no outliers; the data sets were relatively symmetrical for each sonographer and the interquartile range length was similar, which indicated little variation in the repeated measurements. As expected, the participant boxplots showed that the UCV diameters and PV were different for each participant, as the participants were randomly selected, independent and had different gestational ages.

In this research, the UCV diameter and PV intersonographer and intrasonographer ICC values were greater than 0.81, which illustrates there was consistency of measurements by each sonographer and between sonographers. These results were compared with five published works in Table 9.1, and the diameter ICC results were similar to three previously published papers and superior to those reported by Fernandez et al. (2008) and Figueras et al. (2008). An excellent intersonographer ICC result for UCV diameter measurements was reported by Barbieri et al. (2008): the ICC was 0.91 for interobserver variability between two observers in a cross-sectional study of 221 women. Nyberg and colleagues (2010) reported an excellent interobserver ICC of 0.93 for one diameter measurement made by each of two observers on 22 participants and an excellent interobserver ICC was reported by Najafzadeh et al. (2016) for the variability between two observers repeating the diameter measurement three times on 18 participants.
Table 9.1

Comparison of Intersonographer and Intrasonographer ICC Measurements

<table>
<thead>
<tr>
<th>UCV measurement</th>
<th>Intraclass correlation coefficient (ICC)</th>
<th>95% CI</th>
<th>Intraclass correlation coefficient (ICC)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intersonographer ICC</td>
<td></td>
<td>Intrasonographer ICC</td>
<td></td>
</tr>
<tr>
<td>Research diameter</td>
<td>0.98</td>
<td>[0.41, 0.99]</td>
<td>0.99</td>
<td>[0.36, 0.99]</td>
</tr>
<tr>
<td>Diameter (Fernández et al., 2008)</td>
<td>0.65</td>
<td>[0.48, 0.78]</td>
<td>0.70</td>
<td>[0.55, 0.81]</td>
</tr>
<tr>
<td>Diameter (Figueras et al., 2008)</td>
<td>0.65</td>
<td>[0.48, 0.78]</td>
<td>0.70</td>
<td>[0.55, 0.81]</td>
</tr>
<tr>
<td>Diameter (Barbieri et al., 2008)</td>
<td>0.91</td>
<td>-</td>
<td>0.94</td>
<td>-</td>
</tr>
<tr>
<td>Diameter (Nyberg et al., 2010)</td>
<td>0.93</td>
<td>[0.88, 0.96]</td>
<td>0.94</td>
<td>[0.90, 0.97]</td>
</tr>
<tr>
<td>Diameter (Najafzadeh et al., 2016)</td>
<td>0.94</td>
<td>[0.85, 0.98]</td>
<td>0.953</td>
<td>[0.90, 0.98]</td>
</tr>
<tr>
<td></td>
<td>Research PV</td>
<td>0.98</td>
<td>[0.43, 0.99]</td>
<td>0.99</td>
</tr>
<tr>
<td>T_{amqav} (Fernández et al., 2008)</td>
<td>0.46</td>
<td>[0.23, 0.64]</td>
<td>0.59</td>
<td>[0.4, 0.74]</td>
</tr>
<tr>
<td>T_{amqav} (Figueras et al., 2008)</td>
<td>0.46</td>
<td>[0.23, 0.64]</td>
<td>0.59</td>
<td>[0.4, 0.74]</td>
</tr>
</tbody>
</table>

Note. CI = confidence interval. UCV = umbilical cord vein. PV = peak velocity. T_{amqav} = time-averaged maximum velocity.

In this research, the ICC results for the UCV diameter and PV were identical. Other researchers recorded lower ICC values for velocity measurements: Fernández et al. (2008) and Figueras et al. (2008) compared measurements of the $T_{amqav}$ between two observers on 63 singleton pregnancies and reported the interobserver ICC as 0.46 and the intraobserver ICC as 0.59. A possible cause of these lower velocity ICC values is that the diameter is measured from a B-mode image with crisp borders, whereas the velocity is a fluctuating spectral trace with either the sonographer or the automatic software determining which fluctuation to measure, making the PV measurement
more susceptible to variation and subjective choice. Scherjon, Kok, Oosting, and Zondervan (1993) reported the UA PI interobserver ICC as 0.39 and Nakai and Oya (2002) reported the UA resistive index interobserver ICC for as 0.49. These indices are the accepted benchmarks in Doppler assessment of the UA and consequently, the results calculated in this research demonstrate exceptional reliability and reproducibility compared to current clinical benchmarks.

9.3 Conclusion

In this study, intersonographer and intrasonographer reliability were excellent, with similar or superior values to other published results and higher than ultrasound benchmark indices currently used in clinical practice. These high reliability results demonstrate that by following a strict protocol, several sonographers can produce similar measurements. The reliable measurement of the UCV diameter and PV ensured that the data used to construct reference ranges in this research were reproducible at Orange and Bathurst Health Services.
SECTION VI RESULTS AND DISCUSSION: UMBILICAL CORD VEIN

Chapter 10 Umbilical Cord Vein Diameter Results

10.1 Introduction

In this chapter, the range and measures of central tendency of the average UCV diameter will be reported and scatterplots constructed to display the relationship between average UCV diameter and GA, for the whole research sample and the nAGA group. Statistical modelling will determine if a significant difference exists between the average UCV diameters of three different birthweight categories, plus construct a reference range from the nAGA group data. Using stratified SGA category data, analysis will be undertaken to assess the performance of the reference range in identifying growth restricted fetuses. Continuous variables are reported as means ± SD and discrete variables as numbers or percentages. Where appropriate p values are provided.

10.2 The Umbilical Cord Vein Diameter Data and Data Checking

The diameter of the UCV was measured three times and the mean calculated to produce a UCV diameter measurement for each participant at each attendance, which is referred to as the average UCV diameter. In the whole research sample (n = 1,374) (Appendix K1), the average UCV diameter was recorded during 1,359 (98.9%) examinations. In the remaining 15 examinations, the diameter was not recorded by the research sonographer. There were 168 average UCV diameter measurements in the SGA birthweight category, 1,075 in the AGA category and 116 in the LGA category.
The narrowest average UCV diameter in the whole research sample (n = 1,359) was 2.3 mm at 127 days (18 weeks 1 day) (Participant 100/Visit 103a) from an AGA category fetus. The widest average UCV diameter was 10.8 mm at 263 days (37 weeks 4 days) (Participant 172/Visit 175f) from a LGA category fetus. The whole research sample average UCV diameter median was 7.0 mm and the mean 6.4 mm.

In the nAGA group (n = 603) (Appendix K2), the average UCV diameter was recorded in 598 (99.2%) examinations and not recorded in five examinations. The narrowest average UCV diameter was 2.5 mm at both 115 (16 weeks 3 days) (Participant 146/Visit 149a) and 127 days (18 weeks 1 day) (Participant 49/Visit 51). The widest average UCV diameter was 10.2 mm at 268 days (38 weeks 2 days) (Participant 451/Visit 462c). The nAGA group average UCV diameter median was 7.0 mm and the mean 6.3 mm.

Scatterplots (Figure 10.1) of the average UCV diameter plotted against GA show a general trend of a wider diameter with advancing GA in both the whole research sample and the nAGA group. There was increasing variability of the average UCV diameter measurements with advancing GA in both samples, with the variability more apparent after 200 days GA. Both the whole research sample and the nAGA group data were divided into second (< 26 weeks GA) and third trimesters (≥ 26 weeks GA), and boxplots (Figure 10.2) confirm that during the third trimester there was greater variability in the average UCV diameter measurements, as indicated by wider interquartile ranges (Q₃ - Q₁). The third trimester data sets were symmetrical compared to the second trimester sets, whose median was not central in the interquartile range box.
Figure 10.1. Scatterplots of average UCV diameters with increasing GA. (a) whole research sample ($n = 1,359$) and (b) nAGA group ($n = 598$).

Figure 10.2. Boxplots of average UCV diameters during the second and third trimesters. (a) whole research sample ($n = 1,359$) and (b) nAGA group ($n = 598$).

Figure 10.3. Scatterplots of ln(average UCV diameter) against advancing GA. (a) whole research sample ($n = 1,359$) and (b) nAGA group ($n = 598$).
The data were more variable with advancing GA and a natural logarithm (ln) transformation of the average UCV diameter values was performed to address this heterogeneity. This is a standard transformation used when data sets exhibit increased variance and the linear mixed model assumption of constant variance of residuals are not met (Osborne, 2002; Troy, 2006). Scatterplots of the transformed data (Figure 10.3) show less variance of the ln(average UCV diameter) with advancing GA, compared to raw data (Figure 10.1).

### 10.3 Modelling the Three Birthweight Categories

This portion of data analysis will determine if a significant difference exists between average UCV diameters of the SGA, AGA and LGA birthweight categories (Appendix L4). The ln(average UCV diameter) data were colour coded for the three categories and plotted in Figure 10.4. This scatterplot displays a difference between the SGA and LGA categories, but some overlap with the AGA category is evident.

![Colour coded scatterplot](image)

*Figure 10.4. Colour coded scatterplot of the whole research sample ln(average UCV diameter) at different GA (n = 1,359).*
Visual inspection of Figures 10.3 and 10.4 shows that the relation between ln(average UCV diameter) and GA is potentially curvilinear. Using the transformed data, a linear mixed model was fitted using ASReml-R computer program (Butler, Cullis, Gilmour, & Gogel, 2007) to model the relationship between ln(average UCV diameter) and GA. The linear mixed model expressed as a quadratic polynomial regression can be symbolically written as:

\[ \ln(\text{AV diam}) \sim \text{birthweight category} + \text{gestational age} + (\text{gestational age})^2 \]

\[ + \text{birthweight category: gestational age} \]

\[ + \text{birthweight category: (gestational age)}^2. \]

ANOVA (Table 10.1) shows a significant curvilinear relation between ln(average UCV diameter) and GA \((p < 0.001)\); a significant difference between the birthweight categories \((p < 0.001)\), but with similar shaped curves for all three birthweight categories \((p = 0.069)\). The model had a coefficient of determination close to one \((R^2 = 0.977)\) indicating the regression lines were a good fit to the data. The mean squared error \((0.079)\) of the model approached zero, also indicating the regression lines were a close fit to the data, and the variance and bias were small.

<table>
<thead>
<tr>
<th>Source</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>15,540.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GA^2</td>
<td>1,502.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category</td>
<td>40.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category: GA</td>
<td>9.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category: GA^2</td>
<td>268.00</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Note. GA = gestational age.
The quadratic polynomial regression equations for the three birthweight categories are written below. As the term birthweight category: GA\(^2\) was not significant, it was dropped from the model. The dependent variable is the \(\ln(\text{average } \text{UCV diameter})\) and GA in days is the explanatory variable.

**SGA**

\[
\ln(\text{average } \text{UCV diameter}) = -1.54897809 + 0.02786511 \times GA - 0.00005546 \times GA^2
\]

**AGA**

\[
\ln(\text{average } \text{UCV diameter}) = -1.63826365 + 0.02863189 \times GA - 0.00005546 \times GA^2
\]

**LGA**

\[
\ln(\text{average } \text{UCV diameter}) = -1.62676926 + 0.0288991 \times GA - 0.00005546 \times GA^2.
\]

The model assumptions that the residuals were normally distributed, had constant variance and were independent were assessed. The residuals were not derived from a population that was normally distributed as defined by Shapiro-Wilk test of normality \((p < 0.001)\). In Figure 10.5, the Q-Q plot and the histogram display an approximate normality of the residuals. The residual and sequence plots allow visual assessment of the distance of each observation from the fitted line and a horizontal band pattern suggest that the variance of the residuals was constant. The residuals were assumed to be independent as the data was based on different participants and the same participants at different times.
Figure 10.5. Residual plots derived from the linear mixed model.

The quadratic polynomial regression equations for each of the three birthweight categories are plotted in Figure 10.6 with 95% CI displayed. Confidence intervals were calculated for each curve using the standard error of prediction and set at 95%. In medical research the 95% CI is most often cited (Attia, 2005) and the CI is a statement about the true mean population and not the spread of data, with the 95% CI indicating there is a 95% chance that the true population mean lies within these limits. In Figure 10.6 the shape of the curves are similar, but with overlap of the CIs until 181 days (25 weeks and 6 days), after which there is a distinction between the upper CI of the SGA group and the lower CI of the AGA group and the same distinction between the AGA and LGA groups.
In Figure 10.6, the width of the CI interval on the SGA and LGA birthweight category curves is wider than the AGA birthweight category as there were fewer observations in the former categories. The denominator of the standard error is the square root of the number of observations; therefore, more observations in the AGA birthweight category made the denominator larger, the standard error smaller, and consequently the width of the CI narrower.

The CIs of the three birthweight curves were separate after 25 weeks and 6 days GA and to ensure this cutoff was robust the graph was redrawn with the LGA and AGA groups combined (Figure 10.7) (Appendix L5). This graph shows an overlap of the CIs of the two birthweight categories until 170 days (24 weeks 2 days) GA was achieved, at which point there was a definite distinction between the upper CI of the
SGA and the lower CI of the combined group. The most advanced GA of 25 weeks and 6 days was chosen as the cut-off to define the separation between the birthweight curves, as above this GA there was no overlap.

![Graph showing predicted ln(average UCV diameter) for SGA and combined AGA and LGA birthweight groups.](image)

*Figure 10.7.* Predicted ln(average UCV diameter) for the SGA and combined AGA and LGA birthweight groups. Dashed lines indicate 95% CI. Vertical line indicates GA where separation of the CIs occurs.

In summary, linear mixed modelling of the birthweight categories showed a curvilinear relationship between average UCV diameter and advancing GA. The quadratic polynomial regression equations demonstrated a significant difference among the three birthweight categories ($p < 0.001$). The regression curves had a similar shape and a separation of the curves was not evident until after 25 weeks and 6 days GA.
10.4 Developing a Reference Range (Nomogram) using Quantile Regression and the nAGA Group

The development of a GA related reference range, from the nAGA group data, will be reported in this portion of analysis. Visual inspection of Figures 10.1b and 10.3b and previous analysis of the whole research sample indicated the relation between UCV diameter and GA was potentially curvilinear.

Initially, the nAGA average UCV diameters were transformed using natural logarithms and linear mixed modelling was used to model the correlation within participants using the ASReml-R computer program (Butler et al., 2007). The model assumptions that the residuals were normally distributed, had constant variance, were independent, and that the factor level variances were equal, were assessed. The residuals were not derived from a normally distributed population as assessed by the Shapiro-Wilk test of normality ($p = 0.006$). The Q-Q plot and the histogram also showed the assumptions of normality were not met. The residual plot showed the variance of the residuals was constant. The residuals were assumed independent. The $\ln$(average UCV diameters) were back-transformed and the 95% CI described the data, but did not encapsulate the variability of the data (Appendix L4).

The linear mixed model provided a reliable estimate of the data, but hypothesis testing was unreliable as the data were not derived from a normally distributed population, thereby failing to comply with one of the linear mixed model assumptions. Most importantly, back-transformation of this model failed to accommodate the variability in the data and therefore the CI was not estimated correctly. Linear regression modelling was applied to the UCV PV and $Q_{uv}$ nAGA data and back-transformation showed that the poor estimation of the 95% CI was even more
pronounced for these UCV features. To overcome these shortcomings an alternative analysis by quantile regression (Appendix L6) was undertaken and is the only modelling reported in the UCV PV and $Q_{ucv}$ results.

As previously indicated the relationship between average UCV diameter and GA was potentially curvilinear, therefore a polynomial quantile regression model was applied with backward selection of power and a quadratic (order 2) quantile regression model was selected for analysis. The model can be symbolically written as:

$$Umbilical\ cord\ vein\ diameter \sim \text{mean} + GA + GA^2.$$  

The regression equation can be written:

$$UCV\ diameter = b_0 + b_1 GA + b_2 GA^2.$$  

Where $b_0$ = intercept  
$P$ = percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97th  
$GA$ = gestational age in days.

Quantile regression is a non-parametric model and has no expectations about the underlying distribution of data and therefore no testing for assumptions of normality was required. The regression coefficients, standard errors of the coefficients, and $p$ values for all percentiles are shown in Table 10.2. The $p$ values indicate the significance of each order of the polynomial in the quadratic quantile regression, given that $p < 0.05$ was considered statistically significant. The standard error of the coefficients relates to quantile regression analysis and provides an estimate of the range of the errors for coefficient estimation. The predicted values from the model for each percentile, at weekly GA intervals, are shown in Table 10.3. The quadratic quantile regression percentiles are plotted in Figure 10.8 with the nAGA data superimposed.
### Table 10.2

**UCV Diameter Coefficients**

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Parameter</th>
<th>Coefficient Value</th>
<th>Standard Error of the Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>$b_0$</td>
<td>-8.527519424</td>
<td>1.5138425</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
<td>0.11231372</td>
<td>0.0171089</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_2$</td>
<td>-0.000213769</td>
<td>0.0000447</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>5th</td>
<td>$b_0$</td>
<td>-8.757804046</td>
<td>1.3993055</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
<td>0.115346739</td>
<td>0.0158825</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_2$</td>
<td>-0.000219541</td>
<td>0.0000420</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>10th</td>
<td>$b_0$</td>
<td>-8.765219078</td>
<td>0.9897232</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
<td>0.11585926</td>
<td>0.0108758</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_2$</td>
<td>-0.000214001</td>
<td>0.0000277</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>25th</td>
<td>$b_0$</td>
<td>-10.3431763</td>
<td>0.7401659</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
<td>0.133454751</td>
<td>0.0083730</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_2$</td>
<td>-0.000250931</td>
<td>0.0000220</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>50th</td>
<td>$b_0$</td>
<td>-10.84895487</td>
<td>0.9280011</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
<td>0.140566158</td>
<td>0.0106144</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>$b_2$</td>
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<td>0.0000284</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>75th</td>
<td>$b_0$</td>
<td>-10.54703323</td>
<td>0.7361624</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
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<td>0.0080516</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_2$</td>
<td>-0.000251588</td>
<td>0.0000204</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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Table 10.3

*Predicted UCV Diameter Values*

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<th>GA (days)</th>
<th>GA (weeks)</th>
<th>3rd</th>
<th>5th</th>
<th>10th</th>
<th>25th</th>
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Note. GA = gestational age.
All values rounded to one decimal place.
Figure 10.8. Nomogram of the predicted UCV diameter with advancing GA derived from quadratic quantile regression. nAGA data plotted in black ($n = 598$). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

10.5 Identification of IUGR/FGR Group using the Reference Range

Having determined that a significant difference existed between the average UCV diameters in each birthweight category, further analysis was undertaken using the nomogram and SGA birthweight category data. The SGA birthweight category was stratified using multiple combinations of birthweight percentiles, EFW percentiles, AC, UA Doppler indices, AFI, MCA PI, Apgar scores and stillbirths (Appendix K4). The data from several of these combinations were plotted on the nomogram (Appendix L7) and the percentage of data points falling below specified percentiles are tabulated in Table 10.4. In Table 10.4, data points were considered as at risk of IUGR if the average UCV diameter plotted below a specified percentile and normal if the data point plotted
Table 10.4

**Combinations of Birthweight, EFW, AC, UA Doppler Indices, AFI, MCA PI, Apgar Scores and Stillbirths for the SGA Birthweight Group and the Percentage of Data Points Falling Below Specified Percentiles**

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<th>Percent below percentile</th>
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</tr>
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<td>19.8</td>
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</tr>
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<tr>
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</tr>
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<tr>
<td>BW&lt;10th, EFW≤10th, AC≤5th and UA S/D&gt;95th (IUGR/FGR group)</td>
<td>28.3</td>
</tr>
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<td>BW&lt;10th, EFW≤10th, AC≤5th, UA S/D&gt;95th and AFI≤5th‡</td>
<td></td>
</tr>
<tr>
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<td>BW&lt;5th, EFW≤5th, AC≤5th and UA S/D&gt;95th</td>
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</tr>
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<td>Apgars≤7 at 1 min including stillbirths‡</td>
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</tr>
<tr>
<td>Apgars≤7 at 1 min excluding stillbirths‡</td>
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<tr>
<td>Stillbirths‡</td>
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<td>Apgars≤7 at 5 min including stillbirths‡</td>
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<td>Apgars≤7 at 5 min excluding stillbirths‡</td>
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<td>Apgar&lt;4 at 5 min including stillbirths‡</td>
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<tr>
<td>Apgar&lt;4 at 1 min excluding stillbirths‡</td>
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</table>

*Note. SGA = small for gestational age. BW = birthweight. EFW = estimated fetal weight. AC = abdominal circumference. S/D = systolic/diastolic ratio. AFI = amniotic fluid index. PI = pulsatility index. MCA = middle cerebral artery. ‡Similar combination assessed with poor identification of IUGR/FGR. †Three or fewer participants.*
above the same specified percentile. Not all combinations were analysed as there were three or fewer participants in some categories, thus providing an insufficient number of data points for statistical analysis. In other cases, more severe IUGR combinations had been analysed and the results were inconclusive, inferring that less severe combinations would not provide any more useful information and therefore analysis was not undertaken.

Three combinations had similar detection rates and two combinations had equal highest rates of 60.9% of attendances using the 10th percentile as a cutoff. The two combinations with the highest detection rate at the 10th percentile contained the same seven participants providing 46 data points, and one of these two combinations was the previously defined the IUGR/FGR group (Subsection 3.10). The IUGR/FGR group average UCV diameters are plotted on the nomogram in Figure 10.9.

![Figure 10.9](image_url)

*Figure 10.9*. Nomogram of the UCV diameter with advancing GA with the IUGR/FGR group data points plotted in red (n = 46). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
10.6 Conclusion

The narrowest UCV diameter recorded in the whole research sample was 2.3 mm and the widest was 10.8 mm. The narrowest UCV diameter recorded in the nAGA group was 2.5 mm and the widest was 10.2 mm. The narrowest UCV 50th percentile predicted diameter was 1.6 mm and the widest was 7.9 mm during the 37th to 40th weeks. A curvilinear relation between average UCV diameter and GA was established. Linear mixed regression was used to model the three birthweight categories. A significant difference between the ln(average UCV diameters) in each birthweight categories was identified. The regression curves had a similar shape and a separation of the CI curves was not evident until after 25 weeks and 6 days GA.

Initially a linear mixed regression was used to model the nAGA group data, but all model assumptions were not upheld. Consequently, quadratic quantile regression was used to model the nAGA data and develop a reference range of the UCV diameter with advancing GA. The coefficients for the regression formula and the calculated values for each percentile were presented. Multiple combinations of birthweight percentiles, EFW percentiles, AC, UA Doppler indices, AFI, MCA PI, Apgar scores and stillbirths were established from the SGA birthweight category and analyses against the nomogram. Using the reference range, 60.9% of the IUGR/FGR group data plotted below 10th percentile.
Chapter 11 Umbilical Cord Vein Diameter Discussion

11.1 Introduction

In this chapter, the raw data and predicted values of the UCV diameter will be compared to published results and explanations offered for variations in values. Errors in measuring the diameter and variations in techniques will be discussed in the context of the methods used in this research. The characteristics of the reference range developed in this research will be compared to other published trends and its performance in identifying poor intrauterine growth will be compared to other research results. The limitations, strengths and potential applications of the UCV diameter research will also be discussed.

11.2 Comparison of Umbilical Cord Vein Diameter Values

Previously, after completing a review of relevant literature, I concluded that UV diameter increased with GA from approximately 2.5 mm at 16 weeks to 8 mm at term. This range is consistent with the research results (Table 11.1).

Table 11.1

<table>
<thead>
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<tr>
<td>Umbilical cord diameter (mm)</td>
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<tr>
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</tr>
<tr>
<td>Narrowest</td>
</tr>
<tr>
<td>Widest</td>
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<tr>
<td>Whole research sample</td>
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<tr>
<td>nAGA group</td>
</tr>
<tr>
<td>Predicted 50th percentile</td>
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</table>
The UV diameter measurements and methods published in research spanning 26 years are summarised in Table 11.2. In the following discussion, I explore the similarities and differences of these publications with the research results and propose possible reasons for variations. In Table 11.2, UV diameters acquired from either longitudinal or transverse planes were relatively similar. Weissman et al. (1994) determined that longitudinal and transverse measurement of the umbilical cord and vessels were very similar and they preferred the longitudinal plane, as it was easier to obtain measurements.

In Table 11.2, the intra-abdominal measurements were narrower than intra-amniotic ones and both were narrower than measurements at the abdominal wall. The intra-abdominal UV diameter was expected to be the smallest, as it is within the confines of the fetal abdominal cavity and liver. It was unexpected that the measurement at the abdominal wall was wider than the UCV measurement, as there is a significant reduction in the UCV diameter as it approaches the fetus (W. Li et al., 2006) and it has been shown that the UV diameter at the abdominal wall was smaller than in the cord, but similar to the intra-abdominal measurement (Skulstad, Kiserud, & Rasmussen, 2004). Barbieri et al. (2012) were the only authors to include the vein wall in the UV diameter and as expected their diameters were larger than all inner to inner measurements after 20 weeks GA.

Table 11.2 shows greater variability of UV diameter values with advancing GA. At 22 weeks GA, when data from all eight publications were available, the range was 3.5 mm to 5.4 mm with a difference of 1.9 mm. At 39 weeks, the oldest GA when all results are available, the range was 6.6 mm to 9.0 mm a difference of 2.4 mm. This
increase in the range of diameters at comparable GA reflects the increased variance displayed in raw data from this research.

Predicted maximum and term diameter measurements reflect the model chosen to describe the data. Linear modelling resulted in a continual rise and a maximum diameter at the oldest GA, whereas curvilinear modelling may predict a higher or lower end value depending on the regression formula. The 50th percentile UCV diameter calculated in this research recorded a maximum diameter between 37 and 40 weeks GA followed by a decline. Only the research values followed this trend in Table 11.2, however, this trend has been reported in other researchers (Chen et al., 1986; Togni et al., 2007) and will be discussed in more detail later in this chapter.

In summary, there was a similarity in the diameter values in Table 11.2, as they ranged from approximately 4 mm at 20 weeks to less than 10 mm at term. The diameter values were similar despite different methods of acquiring the diameter measurements, sample sizes, the year of the research, and whether the values were obtained from raw data or from regression formulae. In conclusion, the UCV diameter values from this research are in accordance with previously published diameter values. The major differences in results arise from measurement techniques, location of diameter measurement and the choice of models used to describe the data.
Table 11.2

**Published and Research UV Diameters**

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<th>Published research articles</th>
<th>Participants</th>
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<th>Measurement method</th>
<th>Scan plan for measurement Model</th>
<th>Acquisition of measurement</th>
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Note: All measurements rounded to one decimal place for comparison
11.3 Errors and Method Variations in Measuring the Umbilical Vein Diameter

There are several possible errors and variations in UV diameter measurement techniques including averaging diameter measurements, UV measurement sites, measurement planes and fetal behavioural states. Measurement accuracy has improved with enhanced computer measurement tools and new technologies such as tissue harmonics.

11.3.1 Averaging measurements.

The research protocol used the mean of three UCV diameter measurements to improve accuracy of the diameter value used in subsequent calculations, as a small error in the diameter measurement results in a large error in the area calculation and $Q_{uv}$ (Link et al., 2007; Rizzo et al., 2016). Kiserud and Rasmussen (1998) repeated measurements of the intra-abdominal UV between two and 20 times on 163 participants and found that by increasing the repeated measurements from one to five, the error in the measurement was halved (Figure 11.1). Kiserud, Saito, Ozaki, Rasmussen, and Hanson (1999) used silicone rods implanted into sheep to validate their hypothesis that UV diameters were highly reproducible when averaging repeated measurements; these researchers concluded that variations in measurements was halved when three to five measurements were averaged, but further improvement was difficult to achieve. Using Kiserud et al.’s (1999) research, Figueras et al. (2008) concluded that repeating the UV diameter between three and five times greatly improved clinical accuracy. Both Figueras et al.’s (2008) and Kiserud et al.’s (1999) findings support the research method of using the mean of three UCV diameter measurements and they highlight the trade-off between precision requirements and the need to conduct a clinical examination within a limited time frame.
Figure 11.1. Precision improvement by increasing the number of UV diameter repeated measurements. The 95% CI is shown. Adapted from “How repeat measurements affect the mean diameter of the umbilical vein and the ductus venosus,” by T. Kiserud and S. Rasmussen, 1998, *Ultrasound in Obstetrics and Gynecology, 11*(6), p. 422.

### 11.3.2 Measurement sites.

The UV diameter can be measured intra-abdominally or as a free floating loop within the amniotic fluid and the advantages and disadvantages of these methods were discussed in Chapter 2. The intra-amniotic portion of cord was used in this research, as all of the research sonographers were familiar with imaging this portion of cord while recording UA Doppler indices. In addition, this portion was easy to locate, Doppler angle correction was simple to apply, and direct insonation of the fetus was reduced. Although W. Li et al. (2006) documented a progressive decrease in the width of the UCV, in practice no significant difference was found between cross-sectional...
areas measured at three points along the UCV (Boito et al., 2002), thus supporting the research method of averaging three diameter measurements from different portions of the UCV.

Figueras et al. (2008) compared the cross-sectional areas of the UV recorded by other researchers at 30 weeks gestation. The mean intra-abdominal area was 22.6 mm$^2$; the mean intra-amniotic area was 32.2 mm$^2$ using the diameter and assuming a circular vein for calculations, and was 40 mm$^2$ using direct area measurements. Figueras et al. (2008) proposed that standard measurement techniques ought to be adopted because techniques were not interchangeable. Thus, Figueras et al.’s (2008) work provides a rationale for the need to use a standardised measurement method, as employed in this research.

11.3.3 Measurement planes.

Both the longitudinal and transverse measurement planes have limitations with incorrect orientations causing erroneous diameter measurements. In the transverse plane the orientation of ultrasound beam and the UV may be oblique, causing a false cross-section and incorrect measurement of the diameter (Lees et al., 1999). Also, the accuracy of the UV diameter in the transverse plane may be reduced if a horizontal, as opposed to a vertical measurement, is recorded, as lateral resolution is poorer than axial resolution in ultrasound imaging. Measurement in the longitudinal plane also encounters errors in orientation. The ultrasound beam must be perpendicular to the mid portion of the vessel in order to measure the most accurate central diameter as illustrated in Figure 3.2.
11.3.4 Vein shape.

Irrespective of the measurement plane there is an assumption that the diameter is measured in a circular shaped vessel (Flo et al., 2010; Hoskins, 2011; Raio et al., 1999a). This assumption may be invalid as the UCV can be distorted by extrinsic pressure (Najafzadeh & Dickinson, 2012) and the UCV is not circular as it enters the abdomen (Skulstad et al., 2004). The availability of trace, spline and autotrace tools allows the circumference of odd shaped vessels to be outlined, but incurs errors of poor calliper placement. Tracing the area of the UCV has been reported in UV research undertaken by Boito et al. (2002), Lees et al. (1999) and Togni et al. (2007).

11.3.5 Structural variations.

Excluding the slight reduction in diameter as the cord enters the fetal abdomen; there is an assumption that the UV has a relatively uniform diameter. However, areas of segmental thinning of the cord have been reported (Raio et al., 1999a) and hypercoiling may compress the UCV causing folding and narrowing (Louis, 2011). Portions of the umbilical cord may be enlarged due to structural anomalies such as tumours, urachal cysts, omphalomesenteric cysts, knots (Bosselmann & Mielke, 2015; Di Naro, Ghezzi, Raio, Franchi, & D'Addario, 2001) and intra- and extra-abdominal UV varices (Yagel et al., 2010). A large umbilical cord has been reported in cases of gestational diabetes (Weissman & Jakobi, 1997); however, more recent research has not confirmed that finding (Saha, Farhat, & Karmaker, 2014). The inclusion of grossly abnormal diameters will distort normative assessment.

11.3.6 Calliper placement.

Incorrect calliper placement introduces measurement errors (Lees et al., 1999). The use of a strict protocol and a familiar technique, as was employed in this research,
helps reduce calliper placement errors. An automated measurement function designed for nuchal translucency measurement was used for UV diameter measurements by Rizzo et al. (2016) with the intention of reducing human error in calliper placement. The use of the nuchal translucency measurement guidelines by Rizzo and colleagues (2016) corroborates the choice of this technique in this research.

**11.3.7 Fetal behavioural states.**

Boito et al. (2004) showed that the traced UCV cross-sectional area significantly increased \((p = 0.02)\) in size between fetal quiescence and a behavioural state when the fetus exhibited gross body and eye movements. These researchers proposed that fetal activity raised cardiac inflow increasing the mean venous pressure in the UCV, which in turn increased the vessel size due to an extremely compliant wall (Boito et al., 2004). Thus, Boito et al.’s (2004) research reinforces the study protocol of undertaking ultrasound UCV measurements during fetal quiescence.

**11.3.8 Tissue harmonics.**

Tissue harmonics imaging utilises the reflected harmonic echoes produced by the non-linear propagation of ultrasound through soft tissues and are produced at multiples of the fundamental frequency. The advantages of harmonic imaging are decreased side lobe artefacts, reduced reverberation and near-field artefacts, increased axial and lateral resolution, increased signal to nose ratio and increased penetration without loss of detail providing improved imaging in obese patients (Paladini, 2009; Shapiro et al., 1998; van Neera et al., 2011).

Tissue harmonics imaging has not been investigated in umbilical vein imaging (Najafzadeh & Dickinson, 2012). Extensive research into harmonics imaging versus conventional imaging technology was undertaken in the measurement of the nuchal
translucency in which the use of harmonic underestimated the width of translucency (Pasquini et al., 2010). However, RANZCOG (2011) guidelines recommend that the use of harmonic be reviewed in respect to the nuchal image quality. The use of harmonics, as in this research, should be reported in the methods of research papers to allow reproduction of imaging parameters (Appendix J).

11.4 Comparison of the Nomogram with Published Reference Ranges

The predicted UCV diameters in the research project showed an increase with advancing GA until 37 weeks, then a four week plateau, followed by a slight decrease after 40 weeks GA; this progression was described by the quadratic quantile regression equation: $UCV \text{ diameter} = b_0 + b_1 GA + b_2 GA^2$. Using the coefficients provided in Table 10.2, the reference ranges are plotted in Figures 11.2 and 11.3, with the latter showing the nAGA data points. The final reference ranges graphs omitted the 3rd and 97th percentile lines because:

i. Both linear and cubic regression equations were used to describe the $Q_{ucv}$ producing reference ranges with percentile lines that were overlapping and visually confusing. Omission of the 3rd and 97th percentile linear lines on the $Q_{ucv}$ reference ranges improved the graph’s clarity. In the interest of continuity, all graphs were made similar.

ii. No other recent publication had included nomograms with the 3rd and 97th percentiles (Barbieri et al., 2012; Flo et al., 2010; Rizzo et al., 2016).

iii. The percentage of data points plotting below the 3rd percentile in Table 10.4 were always less than a third of all the data points and therefore was not clinically useful.
Figure 11.2. Nomogram of UCV diameter with advancing GA derived from quadratic quantile regression. Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

Figure 11.3. Nomogram of UCV diameter with advancing GA derived from quadratic quantile regression with the nAGA data points plotted in black (n = 598). Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
In the following section I will discuss the varied opinions about the relationship between UV diameter and GA, with researchers describing a linear increase of UV diameter with advancing GA, or a curvilinear trend with an early peak followed by either a plateau or a decline in diameter towards term.

11.4.1 Linear relation with gestational age.

A positive linear trend has been used to describe the relationship between UCV diameter and advancing GA including early work by Sutton et al. (1990), who described a linear relationship between the UCV diameter and advancing GA from 19 to 40 weeks, with the equation: $UCV\ diameter = 0.027 + 0.021GA$. Barbera et al. (1999) described a linear relationship between the UCV diameter and GA with the formula: $UCV\ diameter = 0.231585GA - 0.550006$. Lees et al. (1999), in a cross-sectional study of 129 singleton pregnancies between 23 and 33 weeks GA, used an ellipse trace measurement of the internal cross-sectional area of the UCV and demonstrated a linear relation between area and GA. A cross-sectional study from Thailand also described a linear relation between UCV diameter and advancing GA (Somprasit et al., 2010).

11.4.2 Curvilinear relation with gestational age.

Other researchers found UV diameter increased rapidly until the mid-third trimester and then the rate of increase slowed or declined. Early research by Chen et al. (1986) showed a curvilinear relationship with intra-abdominal UV diameter and advancing GA until 38 weeks GA, then a three week plateau and then a decline. Weissman et al. (1994) constructed reference ranges from 14 to 42 weeks GA and described the trend by the formula: $UCV\ diameter = -6.9 + 0.72GA - 8.8 \times$
This trend had bimodal peaks, but has been interpreted as a steady increase up to 32 weeks GA and then stabilising (Togni et al., 2007).

Acharya et al. (2005) showed a curvilinear relation between GA and intra-abdominal UV diameter, with a steady linear increase of 0.2 to 0.3 mm per week in diameter up until the 34th week. From this point to term, the weekly increase in diameter was 0.1 mm until a maximum at 41 weeks. Togni et al. (2007) studied 312 healthy Brazilian women with EFW between the 10th and 90th percentile based on the Hadlock formula, with normal UA Dopplers and AFI. Togni et al. (2007) showed that the cross-sectional area of the UCV increased up to the 34th week, then remained relatively stable until the 38th week and then decreased after the 39th week. Although Togni and colleagues traced the vein and automatically calculated the area, their study showed a similar trend to the research findings. Flo et al. (2010) found a curvilinear relationship between UCV diameter and increasing GA. While Barbieri et al. (2012) showed a rapid increase in UCV diameter from 12 to 27 weeks, followed by a slowed increase in diameter to a maximum at 40 weeks GA as described by the formula:

\[ \log_{10}(UCV) = -1.717 + 0.21GA - 0.0056GA^2 + 0.00005GA^3 \]

The whole umbilical cord diameter close to the placental insertion site, collected from 973 placenta specimens, was shown to have a non-linear relation with GA which peaked during the 37th week and then declined until 41 weeks (Pinar & Iyigun, 2012). The pathological specimens were 30 to 40% smaller than ultrasound measurements, which may be explained by post-delivery cord exsanguination. The nomograms developed from postpartum, entire umbilical cord specimens support the general curvilinear relation between diameter and GA and a decline close to term.
A large recent study (Rizzo et al., 2016) using quantile regression modelled a curvilinear trend between increasing UV diameter with increasing GA, unfortunately, the published coefficients were incorrect and therefore the formula cannot be included. Rizzo et al.’s (2016) research showed a 0.2 to 0.3 mm increase per week up until approximately 32 weeks GA and then only 0.1 mm per week until term. This trend of rapid increase of the UV diameter earlier in the pregnancy with a slowing of the growth towards term was reflected in an Australian study that found a 107% increase in UCV diameter between results 18 and 26 weeks GA and only a 31% increase between 26 and 34 weeks (Najafzadeh et al., 2016).

In my opinion, the consensus of published data is that the widest UV diameter occurs during the third trimester, somewhere between 32 and 42 weeks GA. The majority of researchers found that the rate of UV diameter increase slowed in the third trimester and a few found a decline after a peak. In this research, the diameter reached a peak value at 37 weeks GA and remained stable until 40 weeks GA, before declining. A decline after a third trimester peak was also described by Chen et al. (1986), who measured the intra-abdominal UV, Togni et al. (2007), who used the cross-sectional area of the UCV, and Raio et al. (1999a), who described the whole umbilical cord. A third trimester peak and then decline was also supported by assessment of clinical specimens by Pinar and Iyigun (2012). The decline after 40 weeks GA documented in this research may be the result of a small number of observations, as only six average UCV diameters were recorded during the 40 to 42 week period.

Variations in the diameter of the UV in published reference ranges may be a reflection of improvement of imaging machinery, variations in where and how the measurements were taken, and perhaps most significantly, the interpretation of the
raw data. Early research proposed linear relations between UV diameter and GA, whereas more recent publications have exclusively proposed curvilinear relations and used quantile regression, which has been reflected in the regression formulae chosen to describe the research data.

### 11.5 Identifying IUGR/FGR Group using the Reference Range

The IUGR/FGR group average UCV diameters are plotted on the nomogram in Figure 11.4 to display the detection rates from Table 10.4. Assuming that growth compromised fetuses have a smaller UCV diameter and using the 10th percentile line as an arbitrary cut-off, the nomogram correctly classified 60.9% of attendances in which the fetus was identified as IUGR; however, this cut-off also erroneously classified 39.1% of these attendances within normal range.

Linear mixed modelling of the three birthweight categories determined that after 25 weeks and 6 days GA the diameters of the three birthweight categories could be reliably separated. The GA at the time of scanning the 46 IUGR/FGR data points ranged from 139 (19 weeks and 6 days) to 278 days (39 weeks and 5 days). Removal of the single data point recorded prior to 25 weeks and 6 days GA from the IUGR/FGR group, left 28 of 45 (62.2%) data points below the 10th percentile. After 26 weeks GA no IUGR/FGR group data plotted above the 50th percentile, giving 100% detection rate using this percentile as a cut-off. A larger IUGR/FGR group sample with more data points around 26 weeks GA would have provided more robust testing of the proposed cut-off.
Figure 11.4. Nomogram of UCV diameter with advancing GA with the IUGR/FGR group data points plotted in red (n = 46). Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

Other published results have showed a wide range of percentages for the detected IUGR fetuses on reference ranges. Ferrazzi et al. (2000) plotted the UCV diameters of 37 IUGR fetuses against a linear nomogram provided by Barbera et al. (1999) and concluded that UCV diameter was reduced in IUGR fetuses, but not significantly. Ferrazzi et al.’s (2000) graph was difficult to interpret due to the large symbol size, but at least 15 of the 37 (40.5%) IUGR fetuses plotted below the 10th percentile. Using the UCV cross-sectional area Boito et al. (2002) found that 28 of 33 (84%) IUGR fetuses plotted below the 5th percentile on their reference range.

\[^{31}\text{AC < 2 SD below mean and birthweight < 10th %}\]
\[^{32}\text{AC and birthweight < 5th %}\]
The mean UCV diameter of the nAGA group was 6.3 mm (mean GA = 207 days) and the mean IUGR/FGR group diameter was also 6.3 mm (mean GA = 235 days), but at an older mean GA. Given that the diameter increases with advancing GA, the trend of reduced diameter in the IUGR/FGR group is in keeping with previous research by Rigano et al. (2001), who reported the UCV diameter in 21 IUGR fetuses was 5.09 mm and 6.42 mm in 36 age matched control fetuses. A later research group led by Rigano et al. (2008) found that the mean UCV diameter in 22 control fetuses was 5.1 mm, compared to 4.4 mm in 14 IUGR live births and 3.2 mm in 12 IUGR stillbirths. In addition, a Thailand study of 70 IUGR and 70 AGA age matched fetuses found the IUGR UCV diameter was less than comparable AGA diameters (Somprasit et al., 2010).

Although used in several studies, direct comparison of the mean of the UV diameter between normal and IUGR fetuses fails to consider the natural increase in diameter with advancing GA, which is accentuated if the groups are not age matched.

In summary, the IUGR detection rate in this research was comparable to earlier research based on the UV diameter. The research project detection rate of 62.2% also compared favourably with other methods of IUGR identification with similar rates of IUGR detection quoted for third trimester ultrasound biometry (67 %) (Hammad et al., 2016) fetal weight (65.8%), AC (62.2%) and UA S/D (66.7%) (Ott, 2005).

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33 AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
34 AC < 2 SD below mean, UA PI > 95th % and abnormal uterine artery
11.6 Limitations and Advantages of the Umbilical Cord Vein Diameter Results

The weaknesses of the UCV diameter measurement, development of the reference ranges, and identification of IUGR/FGR in this research can be summarised as follows:

i. There was sparsity of data between 23 to 28 weeks and after 39 weeks GA. The reference range would have been more robust if the modelling did not rely on interpolation between available data. This sparsity of data is a reflection of referral patterns for obstetric ultrasounds in regional hospitals. Morphological assessments are undertaken between 18 and 22 weeks gestation and most growth scans are not requested until the third trimester, leaving a gap in late second trimester, when participants were not referred. The sparsity of data after 39 weeks GA again reflects referral patterns, as uncomplicated pregnancies seldom require further imaging at this GA.

ii. In the IUGR/FGR group there were seven participants, providing 46 data points. Again the sparsity of data between 23 and 28 weeks gestation prohibited extensive testing of the 25 week 6 day GA defined cut-off for separation of the three birthweight groups.

iii. The characteristics defining the IUGR/SGA group were in keeping with other IUGR definitions; however, UA S/D was used instead of the more specific UA PI. The use of an elevated UA PI would have isolated severe IUGR, whereas my definition was of moderate IUGR. The differences between the severities of IUGR definitions may explain the comparative lower identification rates in this research.
iv. Participants were classified on the basis of the birthweight, gender and GA at delivery into SGA, AGA or LGA using reference ranges developed by Dobbins et al. (2012). These charts used completed whole weeks of pregnancy to define the newborn, which introduced errors as a fetus born at 36 weeks and 1 day was classified as the same age at birth as a fetus born at 36 weeks and 6 days.

Overcoming the first two limitations could involve extending data collection in order to recruit more participants and hopefully even the spread of data across all gestational ages. Alternatively, and as suggested by Silverwood and Cole (2007), healthy women could be recruited specifically for developing reference ranges and thereby not rely on data collected from women referred for medical reasons; however, this alternative method has ethical, safety and funding issues.

The strengths of the UCV diameter measurement, development of the reference ranges and identification of IUGR in this research can be summarised as follows:

i. The nAGA group contained 321 participants resulting in 598 data points, which was comparable to sample sizes to other publications and exceeded the recommended sample size.

ii. The inclusion criteria for the nAGA group were extremely strict. This rigid selection process ensured the data used for the development of the reference ranges was as “normal” as possible, given the participants were referred for obstetric ultrasounds.

iii. A high ICC calculated in this research demonstrated that measurement of the UCV diameter was very reliable and this reproducibility should be
exportable to everyday clinical practice, as the documented measurement method was easy to follow.

iv. The UCV diameter measurement requires only B-mode imaging, which is readily available in vast range of health facilities including regional and remote centres.

v. The UCV diameter reference range is the only range developed from Australian data.

vi. The UCV diameters from both the raw data and the 50th percentile predicted from the quantile regression formula were in keeping with previous published data spanning 26 years.

vii. The reference range was expressed in percentiles, which are easy to understand, and described the percentage of observations that fell below a predetermined value, e.g. 10th percentile.

viii. The UCV diameter reference range was developed using quantile regression, which is robust against outlines and variance within the data.

ix. The curvilinear reference range is similar to recently published results with clinical specimens supporting the slight decrease in diameter around term.

tax. The modelling of the three birthweight categories identified a significant difference between the birthweight groups after 26 weeks GA, which supports opinion that the UCV diameter is reduced in cases of SGA.

xi. The reference range was able to identify 60.9% of IUGR/FGR group attendances using only the 10th percentile and this rate increased to 62.2% when the cut-off of 25 weeks and 6 days GA was applied. This detection
rate compared favourably with other current ultrasound methods used for
the identification of IUGR.

11.7 Potential Application of the Umbilical Cord Vein Diameter Results

The potential of UCV diameter measurements and application of the
nomogram lies in its easy acquisition and application. The UCV diameter measurement
requires only B-mode imaging which is available on all ultrasound machines
throughout metropolitan, regional and remote Australia. The diameter is a simple,
straight forward, easily reproducible measurement that can be undertaken by
individuals of all skill levels. It is the combination of these two features that promote
the UCV diameter as a very useful diagnostic tool to individuals isolated from major
referral centres.

The detection rate was too low to permit the UCV diameter to be used as a
stand-alone parameter for the diagnosis of IUGR; however, application of the
nomogram may provide supporting evidence that could be a useful addition to the
assemblage of ultrasound parameters currently employed for the diagnosis and
monitoring of fetal growth restriction.

The UCV diameter may assist in separating normally growing small fetuses from
pathological small fetuses after 26 weeks GA. Normally growing small fetuses are
expected to have a UCV diameter which falls between the 10th and 90th percentiles.
Whereas, it would be expected that pathologically small fetuses will have UCV
diameters below the 10th percentile and never above the 50th percentile; however, a
larger sample size or a clinical trial would be needed to substantiate this expectation.
11.8 Conclusion

This research presents the first UCV diameter reference range developed from an Australian based “normal” obstetrics population using quantile regression. This research demonstrated a curvilinear increase in the UCV diameter with increasing GA up until 37 weeks, followed by a plateau lasting 4 weeks, and then a slight decline to 42 weeks GA; this trend is in keeping with other published works. Using linear mixed modelling, a significant difference was demonstrated between the UCV diameters recorded by the SGA, AGA and LGA birthweight categories and the CIs of these categories did not overlap after 25 weeks and 6 days GA. The reference range correctly identified 62.2% of IUGR/FGR group attendances after 25 weeks 6 days GA using the 10th percentile as a cut-off, suggesting the UCV diameter is reduced in fetal growth restriction and that this easy to measure feature may have a role to play in the diagnosis and monitoring of poor fetal growth.
Chapter 12 Umbilical Cord Vein Peak Velocity Results

12.1 Introduction

In this chapter, the range and measures of central tendency of the average UCV PV will be reported and scatterplots constructed to display the relationship between average UCV PV and GA, for the whole research sample and for the nAGA group. Statistical modelling will determine if a significant difference exists between the average UCV PV recorded for fetuses in the three birthweight categories. Further modelling will develop a reference range from the nAGA group data. Analysis will determine the nomogram’s ability to identify growth restricted fetuses. Continuous variables are reported as means ± SD and discrete variables are reported as numbers or percentages. Where appropriate p values are provided.

12.2 The Umbilical Cord Vein Peak Velocity Data and Data Checking

The PV of the blood coursing in the UCV was measured three times for each participant and these values were averaged to produce a UCV PV measurement for each participant at each attendance, which is referred to as the average UCV PV. In the whole research sample (n = 1,374) (Appendix K1) average PV measurements were acquired in 1,360 or 98.9% of examinations undertaken. There was no omitted data in either the SGA or LGA birthweight categories, giving 169 and 118 average UCV PV measurements, respectively. In the AGA birthweight category, three average PV measurements were eliminated due to phasic flow, one was eliminated due to a Doppler angle greater than 15°, and no measurements were recorded during 10
attendances, giving 1,073 average UCV PV measurements available for analysis in this category.

The slowest average UCV PV recorded in the whole research sample was 8.1 cm/s at both 138 days (19 weeks and 5 days) (Participant 608/Visit 13) and 161 days (23 weeks) (Participant 57/Visit 59b). The whole research sample average UCV PV measurements were plotted against GA (Figure 12.1) to visually determine the strength and type of relation between the two variables. There was an outlier with a velocity of 52.1 cm/s (Participant 58/Visit 60d). Review of the ultrasound images for Visit 60d showed that the Doppler technique met protocol requirements and the raw data values were all similar; however, this participant had six ultrasound examinations as part of this research, with average UCV PV measurements recorded as 14.0, 13.2, 17.1, 52.1, 19.6 and 23.0 cm/s. This indicated that the value of 52.1 cm/s was an outlier even within the data set collected on Participant 58, and it was therefore eliminated from further analysis. A revised scatterplot without the outlier and with appropriate scale changes was constructed (Figure 12.2a). The fastest velocity recorded during the research was revised down to 29.2 cm/s (Participant 41/Visit 43) at 247 days (35 weeks 2 days). The whole research sample average UCV PV median was 16.5 cm/s and the mean 16.9 cm/s.

In the nAGA group (n = 603) (Appendix K2) the average UCV PV was recorded in 593 (98.3%) examinations and a scatterplot of these measurements against GA constructed (Figure 12.2b). The average UCV PV was not recorded in seven examinations; two were eliminated due to phasic flow and one due to a Doppler angle greater than 15°. The slowest average UCV PV was 8.1 cm/s at 138 days (19 weeks 5 days) (Participant 608/Visit 13) and the fastest was 29.2 cm/s at 247 days (35 weeks 2
days) (Participant 41/Visit 43), these values were identical to the whole research sample. The nAGA group UCV PV median was 16.0 cm/s and the mean 16.6 cm/s.

![Figure 12.1](image1.png)

*Figure 12.1. Scatterplot of average UCV PV against increasing GA with outlier (n = 1,360).*

![Figure 12.2](image2.png)

*Figure 12.2. Scatterplots of average UCV PV with advancing GA. (a) whole research sample with the outlier removed (n = 1,359) and (b) nAGA group (n = 593).*
The scatterplots of both the whole research sample and the nAGA group (Figure 12.2) show greater variability with advancing GA. This was confirmed by boxplots dividing the data into second (< 26 weeks) and third trimesters (≥ 26 weeks) (Figure 10.3). These show greater variability in the average UCV PV measurements during the third trimester, indicated by wider interquartile ranges (Q₃ - Q₁). The data sets from both trimesters were symmetrical with the median central in the interquartile range box.

![Boxplots of UCV PV in the second and third trimesters.](image)

**Figure 12.3.** Boxplots of UCV PV in the second and third trimesters. (a) whole research sample (n = 1,359) and (b) nAGA group (n = 593).

As the data were more variable with advancing GA, a natural logarithm transformation of the average PV values was performed to address this heterogeneity. Scatterplots were constructed (Figure 12.4) and the ln(average UCV PV) data still showed increased variability with advancing GA, but less than the original data.
Figure 12.4. Scatterplot of ln(average UCV PV) with advancing GA. (a) whole research sample \((n = 1,359)\) and (b) nAGA group \((n = 593)\).

12.3 Modelling the Three Birthweight Categories

This portion of data analysis will explore the differences between the average UCV PV measurements of the three birthweight categories (Appendix L4). The previously transformed data were colour coded according to birthweight category and they were visually assessed. The difference between the three birthweight categories was not obvious as there was overlap of all categories as seen in Figure 12.5.
Visual inspection of Figures 12.4 and 12.5 shows the relation between ln(average UCV PV) and GA was potentially curvilinear. A linear mixed model was fitted to the transformed average UCV PV data using the ASReml-R computer program (Butler et al., 2007) to model the relationship between ln(average UCV PV) and GA. The linear mixed model expressed as a quadratic polynomial regression can be symbolically written as:

$$lnPV \sim mean + birthweight\ category + gestational\ age + (gestational\ age)^2$$

$$+ birthweight\ category: gestational\ age$$

$$+ birthweight\ category: (gestational\ age)^2$$

The ANOVA (Table 12.1) shows a significant curvilinear relationship between GA and ln(average UCV PV) depending on the birthweight category ($p < 0.001$), but with different shaped curves for all three birthweight categories ($p = 0.002$).
Table 12.1

ANOVA Table

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<tr>
<th></th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>779.8</td>
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<tr>
<td>GA²</td>
<td>0.69</td>
<td>0.407</td>
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<tr>
<td>Birthweight category</td>
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<tr>
<td>Birthweight category: GA</td>
<td>4.31</td>
<td>0.014</td>
</tr>
<tr>
<td>Birthweight category: GA²</td>
<td>6.50</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Note. GA = gestational age.

The model had a coefficient of determination close to one ($R^2 = 0.981$) indicating that the regression lines were a good fit to the data points. The mean squared error (0.15) of the model approached zero, also indicating the regression lines were a close fit to the data and the variance and bias were small. The quadratic polynomial regression equations for the three birthweight categories follow and the dependent variable is the natural logarithm of the average UCV PV and GA is expressed in days:

**SGA**

$$\ln(\text{average UCV PV}) = 1.11832725 + 0.1384195 \times GA - 0.00002786 \times GA^2$$

**AGA**

$$\ln(\text{average UCV PV}) = 2.33537671 + 0.00169619 \times GA - 0.00000244 \times GA^2$$

**LGA**

$$\ln(\text{average UCV PV}) = 2.09753037 + 0.00377939 \times GA - 0.00000066 \times GA^2$$

Assessment of the model assumptions was undertaken. These assumptions were that: the residuals were normally distributed, they had constant variance, and they were independent. The Shapiro-Wilk test of normality ($p = 0.008$) concluded that the residuals were not derived from a normally distributed population. In Figure 12.6,
the Q-Q plot and the histogram display an approximate normality of the residuals. The residual and sequence plots suggest that the variance of the residuals was constant. The residuals were assumed to be independent as the data were based on different participants and the same participants at different times.

Figure 12.6. Residual plots from the linear mixed model.

The quadratic polynomial regression equations for the three birthweight categories are plotted in Figure 12.7 and show three different shaped curves with marked overlap of the 95% CI of all three curves. The widths of the CI in the SGA and LGA birthweight categories were wider than the AGA birthweight category as there were fewer observations in the former categories. A wider CI reduces the chance of not including the population mean; however, a very wide CI will not provide a practically useful range of likely population means (Kuzma & Bohnenblust, 2001). The
LGA curve was not included in the following analysis due to the extensive overlapping of the large CI width. In Figure 12.7, there is divergence of the SGA and AGA birthweight category CIs prior to 141 days (20 weeks 1 day) and after 245 days (35 weeks 0 days) gestation. To confirm this observation, the LGA and AGA birthweight categories were combined and quadratic polynomial regression equations were developed and plotted in Figure 12.8 (Appendix L5). This graph shows no distinction between the upper 95% CI of the SGA and the lower 95% CI of the combined group between 144 days (20 weeks and 4 days) and 246 days (35 weeks and 1 day) GA. A lower cut-off of 20 weeks and 1 day and an upper cut-off of 35 weeks and 1 day were chosen to define the boundaries of the birthweight curve CI overlap; outside this GA range there was no overlap on either graph.

![Graph](image)

*Figure 12.7. Predicted ln(average UCV PV) from the regression formulae of the three birthweight categories. Dashed lines indicate 95% CI. Vertical lines indicate GA where separation of the CIs occurs.*
In summary, linear mixed modelling of the three birthweight categories showed a curvilinear relationship between ln(average UCV PV) and advancing GA. The quadratic polynomial regression equations demonstrated a significant difference between the three birthweight categories ($p < 0.001$). The regression curves had different shapes and CI separation was only evident prior to 20 weeks and 4 days and after 35 weeks and 1 day GA.

### 12.4 Developing a Reference Range (Nomogram) using Quantile Regression and the nAGA Group

In this portion of PV data analysis the development of a GA related reference range from nAGA data will be reported (Appendix L6). Visual inspection of Figures
12.2b and 12.4b and previous modelling of the AGA birthweight category shows the relation between UCV PV and GA is potentially linear. A polynomial quantile regression model was applied with backward selection of power and a linear (order 1) quantile regression model was selected for analysis. The model can be symbolically written as:

\[ Umbilical \ cord \ vein \ PV \sim mean + GA. \]

The regression equation can be written:

\[ UCV \ PV = b_{0p} + b_{1p} \cdot GA. \]

Where \( b_0 \) = intercept

\( p \) = percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97th

\( GA \) = gestational age in days.

Quantile regression is a non-parametric model and has no expectations about the underlying distribution of data and therefore no testing for assumptions of normality was required. The regression coefficients, standard errors of the coefficients, and \( p \) values for all percentiles are shown in Table 12.2. The \( p \) values indicate the significance of each order of the polynomial in the linear quantile regression given that \( p < 0.05 \) was considered statistically significant. The standard error of the coefficients relates to quantile regression analysis and provides an estimate of the range of the errors for coefficient estimation. The predicted values from the model for each percentile at weekly GA intervals are shown in Table 12.3. The percentile curves obtained from quantile regression are plotted in Figure 12.9 with the nAGA group data superimposed.
Table 12.2

*UCV PV Coefficients*

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Parameter</th>
<th>Coefficient Value</th>
<th>Standard Error of the Coefficient</th>
<th>p value</th>
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<tbody>
<tr>
<td>3rd</td>
<td>$b_0$</td>
<td>7.552415867</td>
<td>0.693438620</td>
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<tr>
<td></td>
<td>$b_1$</td>
<td>0.019188159</td>
<td>0.003409297</td>
<td>&lt; 0.001</td>
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<td>5th</td>
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<td>0.736247797</td>
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<td>0.067817884</td>
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Table 12.3

*Predicted UCV PV Values*

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<th>GA (days)</th>
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<td>25.5</td>
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</table>

Note: GA = gestational age.
All values rounded to one decimal place.
**Figure 12.9.** Nomogram of UCV PV with advancing GA derived from linear quantile regression with the nAGA data points plotted in black ($n = 593$). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

### 12.5 Identification of IUGR/FGR Group using the Reference Range

Having determined a significant difference existed between the average UCV PV birthweight categories, further analysis was undertaken using the nomogram and stratified SGA birthweight category data. The data from 15 of these combinations was plotted on the nomogram (Appendix L7) and visual inspection of these graphs found the data points were spread across all percentile lines and in five combinations the data points plotted above the 95th percentile. Table 12.4 shows the percentage of data points falling below specified percentiles, for birthweights less than the 10th percentile and the IUGR/FGR group. In this table, data points were described as normal...
if the average UCV PV plotted above a specified percentile and at risk of IUGR if the
data point plotted below the same specified percentile.

Table 12.4

*Birthweight < 10th and the IUGR/FGR Group and the Percentage of Data Points Falling Below Specified Percentiles*

<table>
<thead>
<tr>
<th>Stratified SGA Birthweight Group</th>
<th>Percent below percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd</td>
</tr>
<tr>
<td>Birthweight &lt; 10th</td>
<td>8.3</td>
</tr>
<tr>
<td>IUGR/FGR group</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Note. SGA = small for gestational age.

The IUGR/FGR group average UCV PV, consisting of seven participants providing 47 data points, are plotted on the nomogram in Figure 12.10.

*Figure 12.10. Nomogram of UCV PV with advancing GA derived from linear quantile regression with the IUGR/FGR group average UCV PV data plotted in red (n = 47). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).*
12.6 Conclusion

The slowest average UCV PV recorded in the whole research sample and the nAGA group was 8.1 cm/s and the fastest was 29.2 cm/s, after the elimination of an outlier. The slowest UCV 50th percentile predicted PV was 12.6 cm/s and the fastest was 19.8 cm/s.

There was increased variability in the UCV PV measurements with advancing GA and a natural logarithm transformation was undertaken to accommodate this data heterogeneity. Linear mixed modelling developed regression formulae for the three birthweight categories. A significant difference between the birthweight categories was identified and modelling showed that the curves were different in shape, with considerable overlap of the CIs between 20 weeks and 4 days and 35 weeks and 1 day GA.

Linear quantile regression was used to model the nAGA data and develop a reference range of the UCV PV with advancing GA. The coefficients for the regression formula and the calculated values for each percentile were presented. Using the IUGR/FGR group, the percentage of average UCV PV measurements plotting below the 3rd, 5th, 10th and 50th percentiles on the reference range were determined. With 27.7% of the IUGR/FGR group attendances plotting below 10th percentile on the reference range.
Chapter 13 Umbilical Cord Vein Peak Velocity Discussion

13.1 Introduction

In this chapter, the UCV PV raw data and predicted values from the quantile regression analysis of the nAGA group will be compared to published results and explanations offered for variations in values. Errors in measuring the UV velocity and variations in techniques will be discussed in the context of the methods used in this research. The reference range developed in this research and its ability to identifying IUGR/FGR group data will be compared to published information. The limitations, strengths and potential applications of the UCV PV research will also be discussed.

13.2 Comparison of Umbilical Cord Vein Peak Velocity Values

Over the last 30 years, published UCV PV measurements have been relatively constant. Reported values ranged from approximately 8 cm/s at 20 weeks to 20 cm/s at 40 weeks GA (Subsection 2.4.4.2). These values are comparable to the research values (Table 13.1).

Table 13.1

*Research UCV PV Values*

<table>
<thead>
<tr>
<th></th>
<th>Umbilical cord peak velocity (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slowest</td>
</tr>
<tr>
<td>Whole research sample</td>
<td>8.1 (19 weeks and 5 days, and 23 weeks)</td>
</tr>
<tr>
<td>nAGA group</td>
<td>8.1 (19 weeks and 5 days, and 23 weeks)</td>
</tr>
<tr>
<td>Predicted 50th percentile</td>
<td>12.6 (16 weeks, and 23 weeks)</td>
</tr>
</tbody>
</table>

Note: nAGA = "normal" appropriate for gestational age.
The velocity values from four publications are compared to the predicted 50th percentile PV values in Table 13.2, and comparing data across gestational ages there are striking similarities. Boito et al. (2002) published a regression formula: \( T_{\text{amaxv}} = 0.000216 \times GA^3 + 0.011526 \times GA^2 + 46.271224 \), which unfortunately did not compute realistic velocities and therefore was omitted from the comparison.

In Table 13.2, the slowest velocities were recorded at the earliest gestational ages. At 22 weeks GA, when data for all studies was available, the PV varied from 11.7 cm/s to 19.4 cm/s. In four out of five cases, the fastest velocities were recorded at the oldest GA. At 39 weeks GA, when all data was available, the velocity ranged from 17.8 cm/s to 22.9 cm/s, excluding the work by Acharya et al. (2005), who recorded a peak value of 24.7 cm/s during the 32nd and 33rd weeks of gestation. These different trends were due to modelling: the four studies with the fastest peak velocities at the oldest GA used linear modelling, whereas Acharya et al. (2005) chose a curvilinear model which declined towards term. Velocities published by Acharya et al. (2005) using the intra-abdominal UV were faster than all intra-amniotic velocities until 41 weeks GA and were faster than recent intra-abdominal velocities provided by Rizzo et al. (2016).

In Table 13.2, research undertaken in 2010 had the least number of participants and the slowest velocities across all gestational ages. The oldest study undertaken in 1999 documented the fastest velocities. Rizzo et al. (2016) and the current research were the most recent, had the largest number of participants and had velocities towards the lower end of the comparative range; perhaps indicating that sample size was not an influencing factor, but advancing technology and statistical analysis may account for some of the variation in published UV velocities.
Table 13.2

Published and Research UV PV

<table>
<thead>
<tr>
<th>Author</th>
<th>Participants</th>
<th>Data points</th>
<th>Velocity</th>
<th>Sample gate</th>
<th>Doppler angle</th>
<th>UV site</th>
<th>Model</th>
<th>Acquisition</th>
<th>Gestational age (weeks)</th>
<th>Umbilical vein PV (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbera et al. (1999)</td>
<td>n = 70</td>
<td>n = 70</td>
<td>$T_{\text{maxv PV}}$</td>
<td>Covered lumen</td>
<td>$&lt;20^\circ$</td>
<td>Intra-amniotic</td>
<td>Linear</td>
<td>Mean from formula</td>
<td>16</td>
<td>14.5</td>
</tr>
<tr>
<td>Archarya et al. (2005)</td>
<td>n = 130</td>
<td>n = 511</td>
<td>$T_{\text{maxv PV}}$</td>
<td>10-12mm gate</td>
<td>$&lt;15^\circ$</td>
<td>Intra-abdominal</td>
<td>Curvilinear</td>
<td>50th percentile from published charts</td>
<td>17</td>
<td>14.8</td>
</tr>
<tr>
<td>Flo et al. (2010)</td>
<td>n = 53</td>
<td>n = 232</td>
<td>$T_{\text{maxv PV}}$</td>
<td>Not specified</td>
<td>$&lt;15^\circ$</td>
<td>Intra-amniotic</td>
<td>Linear</td>
<td>50th percentile from published charts</td>
<td>18</td>
<td>15.1</td>
</tr>
<tr>
<td>Rizzo et al. (2016)</td>
<td>n = 852</td>
<td>n = 852</td>
<td>$T_{\text{maxv PV}}$</td>
<td>Not specified</td>
<td>$\leq 20^\circ$</td>
<td>Intra-abdominal</td>
<td>Linear</td>
<td>50th percentile from published charts</td>
<td>19</td>
<td>15.4</td>
</tr>
<tr>
<td>Spurway’s research</td>
<td>n = 321</td>
<td>n = 593</td>
<td>PV</td>
<td>3mm gate</td>
<td>$\leq 15^\circ$</td>
<td>Intra-amniotic</td>
<td>Linear</td>
<td>50th percentile from formula</td>
<td>20</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Note. $T_{\text{maxv}}$ = time averaged maximum velocity peak velocity. PV = peak velocity. Mean velocities converted to peak velocities for ease of comparison. All measurements rounded to one decimal place for ease of comparison.
All research in Table 13.2 used Doppler angles less than 20° and aimed at having a 0° angle of insonation, thereby eliminating angle of insonation as a cause of variation. The studies recording the $T_{amaxv}$ had marginally lower velocity values compared to the two studies recording PV. $T_{amaxv}$ is a software dependent automatic function that averages PV over time and the sample gate must cover the lumen; whereas, PV records the subjective chosen highest velocity from a centrally located sample gate and these different techniques may account for the variation in the tabled values.

In summary, there were no glaring differences based on the year the research was undertaken, the sample size, the method of recording the velocity or the angle of insonation between the range of velocities in the raw data or the predicted velocities in this research project and four previously published works.

13.3 Errors and Method Variations in Measuring the Umbilical Vein Velocity

There are several errors and variations in methods that are associated with measuring the UV velocity and some of these may account for the disparity between values documented by researchers. These broadly include measurement site and methods, variations in Doppler sampling and fluctuations in the spectral waveform.

13.3.1 Measurement sites.

Several researchers promote the intra-abdominal over the intra-amniotic UV for Doppler sampling, as the vein is separated from its accompanying arteries thereby eliminating any transmitted pulsations (Raio et al., 2003; Vasques et al., 2004); however, a good Doppler angle is sometimes difficult to achieve. Researchers have avoided the cord insertion sites as the UCV velocity near the placental cord insertion
has a flat flow profile (Pennati et al., 2004), may be obscured by fetal parts or is not identifiable due to oligohydramnios. The cord insertion into the fetal abdomen has also been avoided as the UCV velocity at this point is elevated due to umbilical ring narrowing (Skulstad et al., 2004). Choosing the free-floating portion of the UCV in this research avoided these insertion site issues.

13.3.2 Measurement methods.

The peak or maximum velocity can be measured manually as in this research or by an automated trace ($T_{\text{maxv}}$). Both methods have advantages and limitations, as discussed previously in Chapter 2. Both methods require conscientious and diligent appraisal by the sonographer so that the most accurate peak velocity is recorded, so that the angle of insonation is close to 0°, and so the sample gate contains the maximum velocity. The manual measurement of the PV is simple to perform and it is less technique and software dependent. It has been applied in this research as it is a method that is easily employed by all levels of users and machines models.

The research protocol averaged three UCV PV measurements to improve accuracy of the value used in subsequent calculations. This method of improving accuracy is recommended (Najafzadeh & Dickinson, 2012) and utilised in other similar ultrasound research (Barbera et al., 1999; Di Naro et al., 2002; El Behery et al., 2011; Flo et al., 2009; Flo et al., 2010).

13.3.3 Doppler sampling.

The UV velocity may be measured with a small sample gate placed entirely within the vein or by a large gate covering the entire vein. In this research, a 3 mm Doppler gate was placed in the middle of a vertical portion of UCV (Figure 3.5) with the lateral dimension of the sample gate limited by the Doppler beam dimensions
UMBILICAL CORD VEIN ULTRASOUND

(Figueras et al., 2008). The research protocol assumed that by identifying a vertical portion of umbilical cord, the parabolic flow profile would be developed and the maximum velocity would occur in the centre of the UV, as laminar flow is fastest in the centre and slowest close to the vein wall. The protocol also assumed that by using a small sample gate that only venous velocities would be sampled; however, arterial velocities may have been included at early gestational ages as the width of the sample gate may have exceeded the UCV width. If UCV $V_{\text{mean}}$ is measured then the width of the gate needs to cover the vessel so that mean velocity is not overestimated by exclusion of slower flow along the wall (Ferrazzi et al., 2000), but it also needs to exclude the neighbouring umbilical arteries’ flow, which may distort the Doppler waveform.

The peak velocity is overestimated by an amount that is relative to the insonation angle, with an overestimation of up to 30% as insonation angles approach 90° (Figueras et al., 2008; Hoskins, 2011; S. Li et al., 1993). There is also an inherent error in the visual alignment of the Doppler angle with the UCV (Figueras et al., 2008; Yamamoto et al., 2006). Both these errors were minimised by using a 0° insonation angle and a vertical portion of cord in this research.

13.3.4 Fluctuations in the spectral waveform.

The normal spectral trace of the UCV vein during the second and third trimesters is continuous with no variations (Najafzadeh & Dickinson, 2012). Spectral traces that demonstrate a repetitious single or double pulsatile waveform due to altered fetal heart or DV states should not be used for UV PV readings. The research protocol of measuring the PV during fetal quiescence was essential to eliminate fluctuations caused by fetal movement, breathing (Vasques et al., 2004), and
hiccupping (Zheng et al., 1998). Many researchers specified this state; however, achieving ideal traces sometimes proved difficult.

13.4 Comparison of the Nomogram with Published Reference Ranges

Quantile regression showed a linear relation between UCV PV and advancing GA. The linear quantile regression formula describing the percentiles is written:

\[ UCV \ PV = b_0 + b_1 p \ GA. \]

Where \( b_0 \) = intercept

\( p \) = percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97th

\( GA \) = gestational age in days.

Using the coefficients provided in Table 12.2, the reference range with the 3rd and 97th percentiles excluded are plotted in Figures 13.1 and 13.2, the latter showing the nAGA data points.

Figure 13.1. Nomogram of UCV PV with advancing GA derived from linear quantile regression. Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
In the following section I will discuss the three broad opinions that were identified in published research regarding the relationship between UV velocity and GA. Some authors assumed a constant velocity throughout pregnancy, others demonstrated an increase in PV with advancing GA in either a linear or curvilinear trend, and another described a mid-third trimester peak followed by a decline towards term.

### 13.4.1 Constant velocity with gestational age.

Several scholars have documented a constant UV velocity throughout pregnancy (Hofer, 2001; Link et al., 2007). Early research reported that the intra-abdominal UV velocity “did not change significantly during the last 16 weeks of pregnancy” (Chen et al., 1986, p. 320). Sutton and colleagues stated that the “umbilical
vein peak velocity varied little with age” (1990, p. 384); however, their range of values (7 to 23 cm/s) has been reproduced in later studies and was never described as having little variation with age. These early interpretations of the range of the PV may reflect a difference in knowledge and appreciation of ultrasound UV velocimetry.

13.4.2 Linear relation with gestational age.

The UV velocity has been reported by a majority of researchers to increase in a linear fashion with advancing GA, as found in this research. Lees et al. (1999) showed that between 23 and 33 weeks GA, the UV time-averaged velocity significantly increased in a positive linear fashion. Barbera et al. (1999) used linear regression to model the relationship between UCV $V_{\text{mean}}$ and GA and derived the formula:

$$UCV\ V_{\text{mean}} = 0.001513GA + 0.048182.$$ A similar positive linear relation was proposed by Ferrazzi et al. (2000) from a sample of 70 normal fetuses. Flo et al. (2010) described a linear association between $T_{\text{amaxv}}$ and GA with a continually increasing $T_{\text{amaxv}}$ with advancing GA. A very recent study using quantile regression found “a linear model better predicted the changes of the UV TAMXV$^{35}$ with advancing gestation” (Rizzo et al., 2016, p. 702). The majority of published works over the last 17 years have modelled the relation between the UV PV and GA as a positive linear trend, similar to that identified in this research.

13.4.3 Curvilinear relation with gestational age.

In recent years the relation between UV velocity and GA has also been described by curvilinear models. Some researchers have described a continued increase in velocity with advancing GA and others have shown an increase and then a declination nearer to term. Kiserud et al. (2000) described $V_{\text{mean}}$ in the intra-abdominal

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$^{35}$ Time-averaged maximum velocity
UV with the formula: 

\[ V_{\text{mean}} = 2.40 - 54.63 \, GA^{-1} + 17.27 \, GA^{-0.5} \]

This formula described a curve that steadily increased up to approximately 37 weeks GA and then very slightly slowed towards term. Boito et al. (2002) described their data as linear from 26 to 32 weeks GA with a slight upward deviation either side of this range. Acharya et al. (2005) calculated percentile ranges for the intra-abdominal UV PV and modelled a curvilinear relation, with the UV PV increasing rapidly from 19 to 25 weeks, slowing to a peak at 32 to 33 weeks GA, and then declining slowly until term. Even with curvilinear modelling there appears to be general trend of increasing UV velocity with advancing GA, with the exception of Acharya et al. (2005).

### 13.5 Identifying IUGR/FGR Group using the Reference Range

The IUGR/FGR group average UCV peak velocities are plotted on the nomogram in Figure 13.3 and visually display the data from Table 12.4. Using the 10th percentile as an arbitrary cut-off and assuming IUGR fetuses have reduced PV, the nomogram detected 27.7% of attendances where the fetus was identified as IUGR and erroneously classified 72.3% of fetuses as normal according to the definition used in this research.

Linear mixed modelling of the three birthweight categories demonstrated three different shaped, overlapping curves. There was separation of the CIs of the SGA and AGA birthweight categories prior to 20 weeks and 1 day and after 35 weeks 1 day GA, inferring that UCV PV would provide poor differentiation of these two categories between these gestational ages. The sample size of the SGA birthweight category \((n = 62)\) was small compared to the AGA category \((n = 515)\) and this was reflected by a wider CI. However, the mean curve for the SGA and AGA categories (Figure 12.7) were
superimposed between 182 days (26 weeks 0 days) and 222 days (31 weeks and 5 days) which would indicate that no matter how narrow the CI, these two categories could not be distinguished during this period.

Figure 13.3. Nomogram of UCV PV with advancing GA derived from the linear quantile regression formula with the IUGR/FGR group plotted in red \((n = 47)\). Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

The GA at the time of scanning the IUGR/FGR group participants ranged from 139 (19 weeks and 6 days) to 278 days (39 weeks and 5 days). Eliminating the 28 data points from the IUGR/FGR group that were collected between 20 and 35 weeks GA left 19 values for evaluation. Of the remaining 19 data points only three plotted below the 10th percentile and these were all at greater than 35 weeks, giving an IUGR/FGR group detection rate of 15%. Again, the spread of the IUGR/FGR group data points and the severity of IUGR fetuses inhibited extensive testing of the cut-offs.

The consensus of research is that the PV is reduced in IUGR fetuses compared to appropriately grown fetuses; however, published results showed a wide range of
detection rates. Jouppila and Kirkinen (1984) found that 8 of 11 (73%) IUGR\textsuperscript{36} fetuses had a last attendance UV $V_{\text{mean}}$ below the 10th percentile on their normal reference ranges, but four of these fetuses had an undetectable velocity at their last attendance, which they presumed to be low. If the IUGR detection rate is calculated on an attendance basis and the four attendances with no flow are eliminated, then 6 of 12 attendances were below the 10th percentile therefore correctly identifying 50% of IUGR fetuses. Ferrazzi et al. (2000) indicated the UCV $V_{\text{mean}}$ was significantly ($p < 0.001$) reduced in a sample of 37 IUGR\textsuperscript{37} fetuses compared to normally growing fetuses. In addition, 34 of the 37 IUGR fetuses plotted wholly below the 10th percentile, thereby correctly identifying 92% of IUGR fetuses; however, the physical size of the data points on the nomogram made interpretation difficult. Boito et al. (2002) found the $T_{\text{amaxv}}$ was below the 5th percentile in 66% of IUGR\textsuperscript{38} fetuses when compared to their nomogram.

All the above cited studies quote a higher rate of IUGR identification compared to this research. Reduced biometry and birthweight defined IUGR in these publications and both methods of measuring velocity (PV and $V_{\text{mean}}$) were used, thereby eliminating these possible reasons for variations in the results. The research ICCs were noticeably higher than those recorded by other researchers (Fernández et al., 2008; Figueras et al., 2008) and hence the nomograms developed in this research were based on more reliable data, which may explain the poor detection rate, as the values were not as variable.

The average UCV PV of the nAGA group was 16.6 cm/s (mean GA = 207 days) and the average of the IUGR/FGR group was 16.3 cm/s (mean GA = 235 days). The non-age matched mean values are relatively similar, which is in conflict with other

\textsuperscript{36} Birthweight < 10th %, reduced fetal growth, Apgar < 7 at 1 and 5 minutes, pathological CTG
\textsuperscript{37} AC < 2 SD below mean and birthweight < 10th %
\textsuperscript{38} AC and birthweight < 5th %
research. Rigano et al. (2001) concluded from a longitudinal study of 21 IUGR fetuses, between 23 and 36 weeks GA, that the UCV \( V_{\text{mean}} \) was significantly \( (p < 0.001) \) higher (0.9 cm/s) in the age matched control group than in the IUGR group (0.5 cm/s). In unpublished research, a comparison of the average UCV PV between an IUGR group (17.2±3.9 cm/s) and a non-age matched AGA group (13.1±3.0 cm/s), identified a statistically significant \( (p < 0.001) \) association between IUGR and reduced UCV PV (Spurway, 2006). Tchirikov et al. (2009) found the \( V_{\text{mean}} \) in a control group (15.95 cm/min) of fetuses with a good pregnancy outcomes was significantly higher \( (p = 0.0033) \) than in non-age matched, compromised fetuses (12.58 cm/min). Although used in several studies, direct comparison of the mean UV PV between control and IUGR fetuses fails to consider the natural increase in PV with advancing GA. If the control groups were constructed from fetuses that were older than the IUGR group fetuses, this alone would produce a difference in the means of the two groups and misrepresent the data.

In summary, modelling of the three birthweight categories has shown that the three categories overlapped between approximately 20 and 35 weeks GA, even though each birthweight category quadratic equation was significantly different. The overlap demonstrated by modelling the birthweight categories is perhaps one of the most significant outcomes of this research. Figures 12.7 and 12.8 show the gestational ages of separation of the categories, were too early and too late in pregnancy, to be clinically helpful in the diagnosis and monitoring of IUGR. Previously, Yagel et al. (2010, p. 106) stated “the considerable overlap with normal ranges precludes the usefulness

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39 AC < 2 SD below mean, UA PI > 2 SD above mean and abnormal uterine artery S/D ratio
40 EFW < 10th % or AC < 5th %
41 Master of Health Science thesis
42 Poor outcome based on the cord blood pH, Apgar score, birthweight percentile, length of pregnancy, requirement of respiratory support and transfer of the newborn to a specialist ward
of the UV flow velocity as a measure of IUGR”, which supports the research findings. This finding may explain why UCV PV has never become firmly established within the obstetric ultrasound community and it also provides a possible explanation for the low detection rate found in analysis of the IUGR/FGR group.

13.6 Limitations and Advantages of the Umbilical Cord Vein Velocity Results

In this research there were several weaknesses in the UCV PV measurement and identification of IUGR. These weaknesses are in addition to the sparsity of data in the late second and third trimesters, the IUGR/FGR sample size defining moderate growth restriction, and whole weeks used to define birthweight categories as previously discussed. These weaknesses can be summarised as follows:

i. A high ICC result demonstrated that measurement of the UCV PV was very reliable in this research; however, this reproducibility may not be exportable to everyday clinical practice, as the measurement of the UCV PV was extremely user dependent. Incorrect placement of the sample gate, poor angle of insonation and incorrect selection of the maximum velocity can make the reproducibility of the measurement difficult.

ii. The recording of a non-fluctuating UCV PV spectral trace was difficult to capture as it was dependent on the absence of fetal movement, breathing action, hiccupping, and a myriad of intrauterine conditions.

iii. The Doppler sample gate width may have exceeded the UCV width in early pregnancy thereby distorting the spectral trace by including adjacent UA velocities.

iv. When the 10th percentile was used as a cut-off, the reference range only correctly identified 27.7% of IUGR/FGR group attendances. Excluding the
attendances between 20 and 35 weeks GA, only 15% of IUGR/FGR group attendances plotted below the 10th percentile.

In addition to the common strengths of the nomogram being developed from a strictly selected Australian-based population, analysis using quantile regression and the expression of the results in percentiles, the strengths of the UCV PV measurement, development of the reference ranges, and identification of IUGR/FGR can be summarised as follows:

i. The nomogram was developed using a manual measurement of the PV in a vertical portion of intra-amniotic UCV. This measurement technique is a robust and easy to perform measurement that is available on a wide spectrum of ultrasound machines; inferring this measurement can be undertaken in many clinical settings.

ii. The UCV peak velocities from both the raw data and the 50th percentile predicted from the quantile regression formula were in keeping with previous published data.

iii. The quantile regression linear modelling used in the development of the reference range was a similar trend to the majority of previously published results.

iv. The modelling of the three birthweight categories identified a significant difference between the birthweight groups with different shaped curves, but with overlapping CI curves between 20 and 35 weeks GA. This overlapping of the CI curves may explain the poor adoption of UCV PV reference ranges into clinical practice. If the separation between SGA and AGA fetuses is not obvious, then UCV PV will not allow distinction between normally growing and compromised fetuses.
13.7 Potential Application of the Umbilical Cord Vein Velocity Results

Even though the ICC results for measurement of the UCV PV were excellent in this research, the PV is technically difficult to accurately measure, with both sonographer and fetal aspects contributing to erroneous values. This coupled with the poor detection rate identified in this research and the lack of distinct separation between the birthweight groups, infers that the UCV PV has relatively limited clinical application.

13.8 Conclusion

This research presents the first UCV PV reference range developed from an Australian derived “normal” obstetrics population using quantile regression. The research demonstrated a linear increase in UCV PV with increasing GA and this trend is in keeping with other published works. Using linear mixed modelling, different shaped, overlapping curves were used to describe the UCV peak velocities of the SGA, AGA and LGA birthweight categories and it is this lack of distinction between the groups that may explain the poor detection rate of IUGR/FGR fetuses and the failure of UCV PV to be widely accepted into clinical practice. The reference range correctly identified 27.7% of IUGR/FGR group data points using the 10th percentile as a cut-off and 15% when employing the GA cut-offs suggested by the birthweight category regression curves. In combination, all these factors suggest that UCV PV would be of little use in the diagnosis and monitoring of abnormal fetal growth.
Chapter 14 Umbilical Cord Vein Blood Flow Results

14.1 Introduction

In this chapter, the range and measures of central tendency of $Q_{ucv}$ will be outlined and scatterplots constructed to display the relationship between $Q_{ucv}$ and GA, for the whole research sample and the nAGA group. Statistical modelling will assess the differences between the $Q_{ucv}$ for the three birthweight categories. Further modelling will develop a reference range from the nAGA data. Analysis will determine the nomogram’s ability to identify growth restricted fetuses and specifically the IUGR/FGR group. Lastly, the relationships between UCV diameter and PV will be explored. Continuous variables are reported as means ± $SD$ and discrete variables are reported as numbers or percentages. Where appropriate $p$ values are provided.

14.2 The Umbilical Cord Vein Blood Flow Data and Data Checking

The $Q_{ucv}$ was calculated from the untruncated average UCV diameter and PV measurements for each participant at each attendance. In the whole research sample the $Q_{ucv}$ was calculated in 1,345 (97.9%) examinations and was not calculated in 29 examinations as 15 average UCV diameters and 14 average UCV PV measurements were not available (Appendix K1). The exclusion of the UCV PV outlier (Participant 58/Visit 60d) left 1,344 $Q_{ucv}$ data points available for analyses which were plotted against GA to visually determine the strength and type of relation between the two variables (Figure 14.1a). In the whole research sample the lowest $Q_{ucv}$ was 16.5 ml/min at 138 days (19 weeks and 5 days) and was calculated for a fetus (Participant 608/Visit 13) classified as AGA by birthweight. The highest $Q_{ucv}$ was 516.4 ml/min at 236 days (33 weeks and 5 days) and was calculated for a fetus (Participant 4/Visit 4b) classified
as LGA by birthweight. The whole research sample $Q_{ucv}$ median was 195.6 ml/min and the mean 187.1 ml/min.

In the nAGA group the $Q_{ucv}$ was calculated in 588 (97.5%) examinations (Appendix K2). The $Q_{ucv}$ was not calculated in 15 examinations due to five missing average UCV diameters and 10 missing average UCV PV values. A scatterplot of these measurements against GA was constructed (Figure 14.1b). The lowest $Q_{ucv}$ was 16.5 ml/min at 138 days (19 weeks 5 days) (Participant 608/Visit 13) and the highest was 499.3 ml/min at 268 days (38 weeks 2 days) (Participant 451/Visit 462c). The lowest $Q_{ucv}$ in both the whole research sample and the nAGA group were recorded by the same participant at the same visit. The nAGA group $Q_{ucv}$ median was 185 ml/min and the mean 177.4 ml/min.

The Figure 14.1 scatterplots, of both the whole research sample and the nAGA group, show greater variability after 200 days GA. This was confirmed by boxplots of both samples divided into second (< 26 weeks GA) and third trimesters (≥ 26 weeks GA) (Figure 14.2). These boxplots show greater variability in the $Q_{ucv}$ measurements, as indicated by wider interquartile ranges ($Q_3 - Q_1$) during the third trimester, and data sets from both trimesters were symmetrical with the median central in the interquartile range boxes.

The scatterplots in Figure 14.1 suggest a curvilinear relation between $Q_{ucv}$ and GA. A natural logarithm transformation of the $Q_{ucv}$ values was performed to address the greater variability in the $Q_{ucv}$ data with advancing GA and scatterplots produced for both the whole research sample and the nAGA group (Figure 14.3). Figure 14.3 still shows increased variability with advancing GA, but was less than Figure 14.1. This increased variability with advancing GA was also evident in the scatterplots of the UCV diameter and PV, which are two components of the $Q_{ucv}$ calculation.
Figure 14.1. Scatterplots of $Q_{ucv}$ with advancing GA. (a) whole research sample ($n = 1,344$) and (b) nAGA group ($n = 588$).

Figure 14.2. Boxplots of $Q_{ucv}$ in the second and third trimesters. (a) whole research sample ($n = 1,344$) and (b) nAGA group ($n = 588$).

Figure 14.3. Scatterplot of $\ln(Q_{ucv})$ with advancing GA. (a) whole research sample ($n = 1,344$) and (b) nAGA group ($n = 588$).
14.3 Modelling the Three Birthweight Categories

The differences between $Q_{ucv}$ of the SGA, AGA and LGA birthweight categories will be explored in this section of data analysis (Appendix L4). The previously transformed whole research sample data was colour coded according to birthweight category and visually assessed for differences between the birthweight categories (Figure 14.4). This figure shows that after 200 days GA the SGA birthweight category fetuses tended to have lower $Q_{ucv}$ than the LGA classified fetuses, but there was some overlap with the AGA fetuses across all GA.

![Colour coded scatterplot of ln($Q_{ucv}$) with advancing GA (n = 1,344).](image)

*Figure 14.4. Colour coded scatterplot of ln($Q_{ucv}$) with advancing GA (n = 1,344).*

Figures 14.3 and 14.4 show a curvilinear relationship between ln($Q_{ucv}$) and GA. A linear mixed model was fitted to the transformed $Q_{ucv}$ data using ASReml-R computer program (Butler et al., 2007) to model the relationship between ln($Q_{ucv}$) and
GA. The linear mixed model expressed as a quadratic polynomial regression is symbolically written as:

\[
\ln(Blood \ flow) \sim mean + birthweight \ category + gestational \ age + (gestational \ age)^2 + birthweight \ category: gestational \ age + birthweight \ category: (gestational \ age)^2.
\]

ANOVA (Table 14.1) shows a significant curvilinear relationship between \(\ln(Q_{ucv})\) and GA \((p < 0.001)\). There is also a significant difference between the birthweight categories \((p < 0.001)\), but with similar shaped curves for all three birthweight categories \((p = 0.639)\).

Table 14.1

<table>
<thead>
<tr>
<th></th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>1,3810.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(GA^2)</td>
<td>959.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category</td>
<td>54.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category: GA</td>
<td>13.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birthweight category: (GA^2)</td>
<td>0.45</td>
<td>0.639</td>
</tr>
</tbody>
</table>

Note. GA = gestational age

The model had a coefficient of determination close to one \(R^2 = 0.992\), therefore indicating the regression line was a good fit to the data points. The mean squared error \(0.202\) of the model approached zero, thus also indicating the regression line was a reasonable fit to the data and the variance and bias were small. The residuals were assumed to be independent and the residual plots (Figure 14.5) show an approximate normality of the residuals and constant variance; thereby meeting these model assumptions. The Shapiro-Wilk test of normality \((p = 0.001)\) indicated that the residuals were not drawn from a normally distributed population.
Figure 14.5. Residual plots derived from the linear mixed model.

The quadratic polynomial regression equations for the three birthweight categories are written below. The dependent variable is ln(Quv) and GA in days is the explanatory variable. The birthweight category: GA$^2$ term was not significant and was dropped from the model.

SGA

$$\ln(UCV \ Quv) = -2.34583669 + 0.05856228 \times GA - 0.00011227 \times GA^2$$

AGA

$$\ln(UCV \ Quv) = -2.52446344 + 0.06045165 \times GA - 0.00011227 \times GA^2$$

LGA

$$\ln(UCV \ Quv) = -2.62659149 + 0.06184323 \times GA - 0.00011227 \times GA^2$$
Figure 14.6, plotted from the quadratic regression formulae at the 95% CI, shows three similar shaped curves and marked overlap of all CIs of the three birthweight categories prior to 164 days gestation (23 weeks 3 days). To further investigate this observation (Appendix L5), the LGA and AGA birthweight categories were combined and quadratic polynomial regression equations were developed and plotted in Figure 14.7. This graph shows an overlap of the upper 95% CI of the SGA and the lower 95% CI of the combined category up until a GA of 161 days (23 weeks), after which there is divergence of the two CIs. The most advanced GA of 23 weeks and 3 days was chosen as the cut-off to define the separation between the birthweight curves, as above this GA there was no overlap.

![Graph showing predicted ln(Q_{ucv}) from birthweight categories quadratic polynomial regression formulae. Dashed lines indicate 95% CI. Vertical line indicates GA where separation of the CIs occurs.](image)

*Figure 14.6. Predicted ln(Q_{ucv}) from the birthweight categories quadratic polynomial regression formulae. Dashed lines indicate 95% CI. Vertical line indicates GA where separation of the CIs occurs.*
In summary, linear mixed modelling of the birthweight categories showed a curvilinear relationship between ln(Q_{ucv}) and advancing GA. The quadratic polynomial regression equations demonstrated a significant difference between the three birthweight categories (p < 0.001). The three birthweight category regression curves had a similar shape. Separation of the CIs of the birthweight curves was only evident after a GA of 23 weeks 3 days.
14.4 Developing a Reference Range (Nomogram) using Quantile Regression and the nAGA Group

This portion of data analysis will report on the development a $Q_{ucv}$ reference range from the nAGA data. Visual inspection of Figures 14.1b and 14.3b and previous modelling of the AGA birthweight category suggests a curvilinear relation between $Q_{ucv}$ and GA. A polynomial quantile regression model was applied with backward selection of power and initially a cubic (order 3) model was selected for analysis. The model can be symbolically written as:

\[
\text{Umbilical cord vein blood flow} \sim \text{mean} + GA + GA^2 + GA^3.
\]

The regression equation can be written:

\[
Q_{ucv} = b_0 + b_{1p}GA + b_{2p}GA^2 + b_{3p}GA^3.
\]

Where $b_0$ = intercept

$p$ = percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97th

$GA$ = gestational age in days

However, the 3rd and 97th percentiles were not significant at both cubic (order 3) and quadratic (order 2) powers. Using backward selection it was found that a linear (order 1) quantile regression was required for these two percentiles. The model can be written as:

\[
\text{Umbilical cord vein blood flow} \sim \text{mean} + GA.
\]

The regression equation can be written:

\[
Q_{ucv} = b_{0p} + b_{1p}GA.
\]

Where $b_0$ = intercept

$p$ = percentile value 3rd and 97th

$GA$ = gestational age in days
Quantile regression is a non-parametric model and has no assumptions about the underlying distribution of data. Therefore, no tests for assumptions of normality were required in this form of modelling. The regression coefficients, standard errors of the coefficients, and $p$ values for all percentiles are shown in Table 14.2. The $p$ values indicate the significance of each order of the polynomial in the quantile regression given that $p < 0.05$ was considered statistically significant. The standard error of the coefficients relates to quantile regression analysis and provides an estimate of the range of the errors for coefficient estimation. The predicted values from the model for each percentile at weekly GA intervals are shown in Table 14.3. The percentile curves obtained from quantile regression are plotted in Figure 14.8 with the nAGA group data superimposed.

![Graph of Predicted Qucv vs Gestational Age](image)

**Figure 14.8.** Nomogram of $Q_{ucv}$ with advancing GA derived from cubic and linear quantile regression with the nAGA data plotted in black ($n = 588$). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
### Table 14.2

$Q_{ucv}$ Coefficients

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Parameter</th>
<th>Coefficient Value</th>
<th>Standard Error of the Coefficient</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>$b_0$</td>
<td>-115.4502145</td>
<td>9.591638</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$b_1$</td>
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<td>0.062886</td>
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<td>$b_1$</td>
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<td>GA (days)</td>
<td>GA (weeks)</td>
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<td>5th</td>
<td>10th</td>
</tr>
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<td>----------</td>
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<td>42</td>
<td>192.0</td>
<td>143.5</td>
<td>157.1</td>
</tr>
</tbody>
</table>

Note. GA = gestational age. Q_{ucv} = umbilical cord vein blood flow.
All values rounded to one decimal place.
14.5 Identification of IUGR/FGR Group using the Reference Range

Having determined that there was a significant difference between the $Q_{ucv}$ of the three birthweight categories with advancing GA, further analysis was undertaken using the nomogram and SGA birthweight category data. The SGA birthweight category was stratified using multiple combinations of birthweight and EFW percentiles, AC, UA Doppler indices, AFI, MCA PI, Apgar scores and stillbirths. The data from 15 of these combinations were plotted on the nomogram (Appendix L7). Table 14.4 shows the percentage of data points falling below specified percentiles for fetuses with a birthweight less than the 10th percentile and the IUGR/FGR group. In this table, data points were considered normal if the $Q_{ucv}$ plotted above a specified percentile and at risk of IUGR if the data point plotted below the same specified percentile.

Table 14.4

<table>
<thead>
<tr>
<th>Stratified SGA Birthweight Group</th>
<th>Percent below percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd</td>
</tr>
<tr>
<td>Birthweight &lt; 10th</td>
<td>18.5</td>
</tr>
<tr>
<td>IUGR/FGR group</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age.

The $Q_{ucv}$ data points of the IUGR/FGR group, which consisted of seven participants providing 46 data points, are plotted on the nomogram in Figure 14.9.
Figure 14.9. Nomogram of $Q_{ucv}$ with advancing GA derived from cubic and linear quantile regression with the IUGR/FGR group plotted in red ($n = 46$). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

14.6 Relationships between Umbilical Cord Vein Diameter and Peak Velocity

In this portion of the research the relationship between the UCV PV and diameter will be assessed. There were 1,344 data points available for analysis due to 15 missing average UCV diameter, 14 missing average UCV PV measurements and the exclusion of a UCV PV outlier. A colour coded scatterplot of the average UCV PV plotted against the average UCV diameter for each attendance was constructed (Figure 14.10) and shows that all three birthweight categories had a general trend for the UCV PV to increase as the UCV diameter increased.
Figure 14.10. Colour coded scatterplot of average UCV PV against average UCV diameter \((n = 1,344)\).

Comparison of the slopes of the nomograms for UCV diameter and PV (Figure 14.11) shows the gradient of the UCV diameter median slope had a steeper angle of inclination of 53° from 16 to 28 weeks gestation, then declined to approximately 20° inclination until 37 weeks GA, and then had a negative 6° slope until 42 weeks GA. The UCV PV median gradient had an angle of inclination of approximately 24° from 16 to 42 weeks GA. The rate of increase was greater for the UCV diameter compared to the UCV PV up until 28 weeks gestation, and then both variables increased at approximately the same rate until 37 weeks after which time the UCV PV was the only variable to increase until 42 weeks GA.
Figure 14.11. UCV diameter and PV nomogram slopes. (a) UCV diameter with red lines indicating gradients of the median and (b) UCV peak velocity with red line indicating a 45° gradient.

14.7 Conclusion

The lowest $Q_{ucv}$ calculated in the whole research sample was 16.5 ml/min and the largest was 516.4 ml/min after the elimination of an outlier. The lowest $Q_{ucv}$ calculated in the nAGA group was 16.5 ml/min and the largest was 499.3 ml/min. The lowest $Q_{ucv}$ 50th percentile predicted value from quantile regression was 34.9 ml/min at 16 weeks and the highest was 277.9 ml/min at 39 week.

There was increased variability in the $Q_{ucv}$ measurements with advancing GA and a natural logarithm transformation was undertaken to accommodate for this heterogeneity in the data. Linear mixed modelling was used to develop regression formulae for the three birthweight categories. A significant difference between the birthweight categories was identified and modelling showed that the curves were similar in shape with considerable overlap of the birthweight curves’ CIs at less than 23 weeks 3 days GA.
Both linear and cubic quantile regression was used to model the nAGA data and develop a reference range of the $Q_{ucv}$ with advancing GA. The coefficients for the regression formula and the calculated values for each percentile were presented. Using the stratified SGA data, the percentage of $Q_{ucv}$ measurements plotting below the 3rd, 5th, 10th and 50th percentiles on the reference range were determined and 54.3% of the IUGR/FGR group attendances plotted below the 10th percentile. The UCV PV increased with enlarging UCV diameter; however, the rate of change was constant for the UCV PV and was greatest in the UCV diameter prior to 28 weeks GA.
Chapter 15 Umbilical Cord Vein Blood Flow Discussion

15.1. Introduction

In this chapter, the Q_{ucv} raw data and predicted values will be compared to published results and explanations offered for variations in values. Errors in measuring and calculating Q_{ucv} and variations in techniques will be discussed in the context of the research methods. The nomogram will be compared and contrasted to other published trends. The performance of the nomogram in identifying IUGR/FGR group data will be compared to other research results. In addition, the relationship between the average UCV diameter and PV will be discussed and compared to other publications. The limitations, strengths and potential applications of the Q_{ucv} research will also be discussed.

15.2 Comparison of Umbilical Cord Vein Blood Flow Values

After reviewing relevant literature, I concluded that Q_{ucv} increased with GA, with a flow of approximately 30 ml/min at 19 weeks, 95 ml/min at 22 weeks and 300 ml/min near term, which is in keeping with the results of this research (Table 15.1).

Table 15.1

<table>
<thead>
<tr>
<th></th>
<th>Umbilical cord Q_{ucv} (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest</td>
</tr>
<tr>
<td>Whole research sample</td>
<td>16.5 (19 weeks and 5 days)</td>
</tr>
<tr>
<td>nAGA group</td>
<td>16.5 (19 weeks and 5 days)</td>
</tr>
<tr>
<td>Predicted 50th percentile</td>
<td>34.9 (16 weeks)</td>
</tr>
</tbody>
</table>

Note. nAGA = “normal” appropriate for gestational age
The range of the 50th percentile predicted $Q_{ucv}$ values in this research were comparable to early research. The predicted range was very similar to work by Gill et al. (1981), who found that intra-abdominal $Q_{uv}$ increased from 100 ml/min at 22 weeks to a peak of 300 ml/min at 38 weeks. Gerson et al. (1987) reported a similar range of 92.4 ml/min at 22 weeks to 295.6 ml/min at 38 weeks for intra-abdominal $Q_{uv}$, as did Lees et al. (1999) who reported a $Q_{ucv}$ range from 95.0 ml/min at 23 weeks to 303.3 ml/min at 33 weeks. Two other 20th century research papers identified higher term values than the 50th percentile predicted values, although they were similar to raw data: Chen et al. (1986) reported an intra-abdominal $Q_{uv}$ of 450 ml/min at 41 weeks GA and Barbera et al. (1999) reported a $Q_{ucv}$ of 529.1 ml/min at 38 weeks.

The $Q_{uv}$ values of four recent studies are compared to research values in Table 15.2. These four studies, spanning 17 years of research, were chosen as comparisons as they applied the same formula and spatial velocity profile coefficient as used in this research. There was a consistent trend across all studies for an increasing $Q_{uv}$ with advancing GA and this is in keeping with the concept that $Q_{uv}$ is higher in the term fetus compared to the preterm fetus (Link et al., 2007). At 22 weeks GA, the earliest GA when data was available for all studies in Table 15.2, the predicted research $Q_{ucv}$ values were very similar to the three earliest works, and that were noticeably less than the large recent study by Rizzo et al. (2016). This observation was repeated when the data were compared at 39 weeks GA. Also at 39 weeks GA there was a wider range of the $Q_{uv}$ values, which has been reported previously by Prior et al. (2014, p. 61.e5), who found “considerable variation” in $Q_{uv}$ values in term fetuses.
Table 15.2

*Published and Research Q_{uv} Values*

<table>
<thead>
<tr>
<th>Published Research Papers</th>
<th>Author</th>
<th>Participants</th>
<th>Data points</th>
<th>Velocity</th>
<th>Diameter method</th>
<th>UV Site</th>
<th>Derivation of blood flow</th>
<th>Spatial velocity profile coefficient Model</th>
<th>Acquisition</th>
<th>Gestational age (weeks)</th>
<th>Umbilical vein blood flow (ml/min)</th>
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<td>Boito et al. (2002)</td>
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<td>n = 100</td>
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<td>Intra-anniotic</td>
<td>Calculated from formula</td>
<td>Linear</td>
<td>50th percentile from formula</td>
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<td>n = 511</td>
<td>PV</td>
<td>measure</td>
<td>Intra-abdominal</td>
<td>Calculated from formula</td>
<td>Curvilinear</td>
<td>50th percentile from published charts</td>
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<td>-0.6</td>
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<td>n = 232</td>
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<td>50th percentile from published charts</td>
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<td>10.6</td>
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<td>Rizzo et al. (2016)</td>
<td>n = 852</td>
<td>n = 852</td>
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\( T_{amaxv} = \) time-averaged maximum velocity. \( PV = \) peak velocity. All measurements rounded to one decimal place for comparison.
The predicted $Q_{ucv}$ in this research was very similar to earlier works in Table 15.2, when comparison was made across sample sizes, measurement sites, and model type. Rizzo et al.’s (2016) study, with the largest sample size, predicted larger $Q_{ucv}$ than earlier, smaller studies, and their $Q_{ucv}$ values were larger than comparable intra-abdominal results from Acharya et al. (2005). This finding may reflect the advancements in the quality of ultrasound equipment, refinement of research and statistical analysis techniques, and more robust data from larger sample sizes. The only striking difference in Table 15.2, was that the research values were the only values in this comparison to show a peak prior to the oldest GA; however, this trend has been described by other researchers (Gill et al., 1984; Gill et al., 1981) and will be discussed in more detail later in this chapter.

15.3 Errors and Method Variations in Measuring the $Q_{ucv}$

In a review of the accuracy of $Q_{ucv}$ measurements, Figueras et al. (2008) compared the $Q_{ucv}$ measured in ovine subjects by triplex ultrasound to radionuclide microspheres coupled with electromagnetic flow transducers and steady state diffusion techniques. Figueras et al. concluded that Doppler measurements of $Q_{ucv}$ were accurate when compared to in vivo gold standards. This supported previous work comparing Doppler ultrasound and steady state diffusion techniques in ovine fetuses undertaken by Galan and colleagues (1999).

Irrespective of comparability with gold standards, blood flow studies are complex to perform and require careful attention to measurements, as small errors in any component will result in a substantial error in the calculated $Q_{ucv}$ value. There are several points that deserve interrogation when errors and variations in measurement techniques involved in blood flow calculations are discussed, including: the area of the
UV, averaging diameter measurements, UV measurement sites, coiling of the umbilical cord, fluctuating spectral traces, mean velocity, and spatial velocity profile coefficients.

15.3.1 Area of the umbilical vein.

The UV area is required for $Q_{uv}$ calculations and there are several methods of determining this value. The two most common methods are to measure the diameter ($d$) of the UV and calculate the area of a circle by the formula:

$$\text{area} = \pi r^2 = \pi \left( \frac{d}{2} \right)^2 = \pi \left( \frac{d^2}{4} \right).$$

Or alternatively, trace the UV circumference and calculate the area using ultrasound software or the formula:

$$\text{area} = \frac{\text{circumference}^2}{4\pi}.$$

The effect of diameter measurement errors on $Q_{uv}$ were identified very early in $Q_{uv}$ research. Gill (1979) quantified the effect of UV diameter measurement errors on the calculated intra-abdominal $Q_{uv}$ and found an error of 0.5 mm in the measured diameter of a 2 mm vessel could produce a 50% error in the $Q_{uv}$. This conclusion was supported by Hoskins (2011), who documented that an under-measurement of 0.25 to 0.5 mm in the diameter of a 5 mm vessel diameter transfers a 10 to 20% underestimation of the cross-sectional area.

Figueras et al. (2008) compared the data from research carried out by Barbera et al. (1999) and Boito et al. (2002) who both used free floating UCV. Boito et al. (2002) traced the inner edge of the UCV and utilised ultrasound software to calculate the area, and Barbera et al. (1999) measured the diameter and calculated the area using the area of a circle formula. UCV area values for both authors, calculated from their regression formula, showed that prior to 21 weeks and after 39 weeks GA, the
diameter method predicted a larger area, and between 22 and 38 weeks GA the diameter method predicted a smaller area. The implications of this variation were summarised by Figueras et al. (2008, p. 588) who stated “these methodological differences may account for inconsistent clinical findings”.

Variations in published values may also be due to measurements of inner to inner lumen width, as opposed to leading edge to leading edge or even outer wall to outer wall. In addition, structural differences, known tapering in vein size from placental to fetal end and the assumption that the cross-sectional area of the UCV is circular, may also explain some differences in published Q_{uv} values.

15.3.2 Impact of averaging diameter measurements on Q_{uv}.

The research protocol repeated the UCV diameter measurement three times in order to improve accuracy. This increased accuracy is paramount given that \( \text{radius} = \frac{\text{diameter}}{2} \) and the radius is squared for area calculations, as such, a small error in the diameter measurement results in a large error in the area calculation (Link et al., 2007; Rizzo et al., 2016). Kiserud and Rasmussen (1998) illustrated the effect of repeated diameter measurements on the calculated Q_{uv} in Figure 15.1.

Averaging two measurements of an intra-abdominal UV with a diameter of 3 mm has a corresponding 22% error in the calculated Q_{uv} value, averaging six repeat diameter measurements on the same vessel reduces the error to 12% and repeating the measurement 10 times reduces the error to 9%. Further reduction requires an exponential increase in the number of measurements which is unrealistic in the clinical setting. In a latter publication, Kiserud (2003a, p. 69) concluded that “the accuracy of volume flow estimations is mainly restricted by the error of diameter measurements,
particularly of small vessels", supporting the research method of using the mean of three UCV diameter measurements.

Figure 15.1. Reduction in blood flow calculation errors with increasing number of repeated intra-abdominal UV diameter measurements. Each line represents the upper 95th percentile. Adapted from “How repeat measurements affect the mean diameter of the umbilical vein and the ductus venosus,” by T. Kiserud and S. Rasmussen, 1998, *Ultrasound in Obstetrics and Gynecology*, 11(6), p. 423.

15.3.3 Measurement sites.

The UV is a single vessel and the volume of blood flowing through it should be equal irrespective of the measurement site. Flo et al. (2009) calculated the $Q_{uv}$, both free-floating and intra-abdominally, by measuring the inner diameter and $T_{amaxv}$ on 53 low risk pregnant women. They proposed the intra-abdominal site was the superior measurement site due to a fixed location and known parabolic flow (Flo et al., 2009). In contrast, the intra-amniotic UCV proved difficult for repeat examinations to use the same site, had a spatial velocity profile coefficient increase from the fetus to placenta,
and a diameter reduction along the length of the cord. Flo et al. (2009) concluded that free floating and intra-abdominal $Q_{uv}$ were similar, but were not interchangeable, and adherence to method was crucial in the clinical application of nomograms.

In early studies of intra-abdominal $Q_{uv}$ it was seldom documented that the measurement was undertaken before the origin of the portal vein (Ferrazzi, 2001). The research groups lead by Tchirikov (Tchirikov et al., 2002; Tchirikov et al., 2009) were the only ones who stated that the intra-abdominal $Q_{uv}$ was measured prior of any intra-hepatic branches. Whereas, other researchers simply stated the straight intra-abdominal segment (Acharya et al., 2005; Hebbar, Rubeena, et al., 2015; Kiserud et al., 1994; Liao et al., 2014; Rizzo et al., 2016).

When the UV is measured intra-abdominally there is an assumption that the straight portion between the umbilicus and the liver is the portion under investigation. If this assumption is incorrect, there are multiple factors that can cause erroneous $Q_{uv}$ values. Within the liver the UV joins a confluence of vessels termed the portal sinus which connects the left and right intrahepatic portal veins and from which the DV arises (Mavrides, Moscoso, Carvalho, Campbell, & Thilaganathan, 2001) (Figure 15.2). The intra-abdominal UV must be measured prior to portal vein branches, prior to shunting through the DV to the fetal right atrium, exclude blood contributed by possible reversed flow in the portal vein, and not be measured in the periphery of the liver (Baschat, 2006; Battaglia, 2003; Bellotti et al., 2004; Kiserud, 2005; Kiserud et al., 2000). Skulstad et al. (2004) found that UV velocities were higher at the umbilicus due to constriction of the vessels by the umbilical ring. The velocity rapidly reduces in the intra-abdominal portion, but $Q_{uv}$ based on measuring PV close to the umbilical results in mistakenly increased values. Conversely, Acharya et al. (2005) found minimal effect
of the umbilical ring in their study comparing intra-abdominal to free loop blood flow. The presence of branches, the potential redistribution of blood flow and the possibility of measuring vessels in the periphery of the liver diminishes the reproducibility of blood flow calculations involving the intra-abdominal portion of the UV and supports the choice of imaging the intra-amniotic portion in this research.

![Diagram of blood pathways in the umbilical, portal, and hepatic veins. Arrows indicate the direction of blood movement and the colour, the degree of oxygen content: red high, purple medium and blue low. Adapted from “The anatomy of the umbilical, portal and hepatic venous systems in the human fetus at 14–19 weeks of gestation,” by E. Mavrides, G. Moscoso, J. Carvalho, S. Campbell, and B. Thilaganathan, 2001, Ultrasound in Obstetrics and Gynecology, 18(6), p. 602.](image-url)

RA  right atrium   PS  portal sinus
DV  ductus venosus   LPV  left portal vein
UV  umbilical vein   RPV  right portal vein
HV  hepatic veins   EPV  extrahepatic portal vein
IVC  inferior vena cava   GB  Gallbladder
15.3.4 Coiling of the umbilical cord.

In a study of 45 singleton term pregnancies who were examined within 24 hours of delivery, Degani, Lewinsky, Berger, and Spiegel (1995) found a significant ($p < 0.001$) linear trend between the umbilical cord coiling index (reciprocal of the distance (cm) between two adjacent coils in the UA) and both $T_{\text{ameany}}$ and $Q_{\text{uv}}$ per kilogram of EFW. Degani et al. concluded that decreased coiling was associated with reduced $Q_{\text{uv}}$ and this was supported by Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al. (2001). A subsequent study of 244 singleton pregnancies after 24 weeks gestation found that increased cord coiling correlated with increased $Q_{\text{uv}}$ (El Behery et al., 2011). Both El Behery et al. (2011) and Di Naro, Ghezzi, Raio, Franchi, D’Addario, et al. (2001) proposed that less UA coils reduced $Q_{\text{uv}}$ due to the reduction in small variations in pressure produced by the pumping arteries, which has been referred to as arterial massaging (Todros et al., 2002). Arterial massaging is based on an original hypothesis by Reynolds (1978), who proposed that the umbilical cord was a pistonless pulsometer pumping system. However, using physical models, Waters and Guoit (2002) showed that under physiological conditions the venous flow was unlikely to be enhanced by the umbilical arteries pulse as suggested by Reynolds (1978). Dado, Dobrin, and Mrkvicka (1997) found a similar outcome using ten postpartum segments of cord; they found no difference in the $Q_{\text{uv}}$ in the veins of coiled and noncoiled umbilical cords when exposed to physical stresses. This raises the question as to whether coiling index should be a consideration in the development of velocity and $Q_{\text{uv}}$ nomograms, as there are persuasive arguments from both sides.
15.3.5 Fluctuations in blood flow.

Blood flow has been shown to increase slightly during active phases when the fetus exhibits gross body and eye movements (Boito et al., 2004). In 330 observations of the placental end of the UCV, Nyberg et al. (2010) noted the UCV cross-sectional area increased by 27% and the velocity by 9%, which resulted in a 42% increase in $Q_{ucv}$ between fetal quiescence and fetal breathing states. Boito et al. (2004) and Nyberg et al.'s (2010) research findings reinforce the study protocol that measurements should only be performed during fetal quiescence.

In addition to changes during fetal activity, a recent 3D perfusion study found that healthy second trimester fetuses demonstrated an undulating, cyclical variation in $Q_{ucv}$ resulting from changes in size of the UCV and velocity (Scholbach et al., 2016). These undulations were present in both resting and active states. Scholbach et al.'s (2016) finding has implication for the application of the nomogram, but also reinforces the study protocol of averaging measurements.

15.3.6 Mean velocity.

The advantages and disadvantages of calculating the mean from either the $T_{amaxv}$ or PV, or measuring the mean velocity by $T_{ameanv}$, $V_{mean}$ or intensity-weighted mean frequency have been previously discussed in Chapter 2. Multiplying the automatic $T_{amaxv}$ or the manual PV measurement by the spatial velocity profile coefficient 0.5, is an easy method of determining the $V_{mean}$ assuming parabolic flow throughout the length of the cord, the axes of the cord and the cardiac cycle (Hoskins, 2011). Pennati et al. (2004) thought that the $V_{mean}$ calculated from PV was more robust to small deviations from $0^\circ$ in the insonation angle. Figueras et al. (2008) considered that $V_{mean}$ calculated from PV resulted in less distortion by adjacent arteries and was
less software dependent. Therefore use of $V_{\text{mean}}$ calculated from PV is more likely to be reproducible across machines types and users.

$T_{\text{mean}}$ measured from an automatic trace has several vulnerabilities, including: insonation angle deviating from $0^\circ$ (S. Li et al., 1993), not encompassing the whole vessel within the sample gate (Hoskins, 2011), incorrectly set high pass filters (Lees et al., 1999), effects of noise (Boito et al., 2002), vessel wall movement, interference from adjacent arteries (Kiserud, 2003a), and being extremely software dependent (Figueras et al., 2008). These features make determining the mean velocity automatically less reproducible across the broader ultrasound community.

Acharya et al. (2005) found little difference in the calculations of intra-abdominal $Q_{uv}$ from 515 longitudinal measurements between calculating the mean ($0.5 \times PV$) and an automated $T_{\text{mean}}$ measurement; for example at a GA of 30 weeks the intra-abdominal $Q_{uv}$ calculated from the PV was 157.7 ml/min and from $T_{\text{mean}}$ 158.0 ml/min. This observation concurs with an earlier study by S. Li et al. (1993), who compared various methods of measuring the mean velocity in physiological phantoms with that calculated from Doppler $T_{\text{max}}$ measurements, and found the phantom and Doppler results were comparable. The $V_{\text{mean}}$ calculated from a manually measured PV as applied in this research is the easiest to measure, least susceptible to error and it is easily calculated.

**15.3.7 Spatial velocity profile coefficient.**

The formula for calculating blood flow applies a spatial velocity profile coefficient. A coefficient of 0.5 was used in this research and the formula is written (Acharya et al., 2005; Kiserud, 2003a; Rigano et al., 2008):

$$Q_{uv} = (\pi \times r^2)(PV \times 0.5) \times 60.$$
The spatial velocity profile coefficient is calculated as the ratio between $V_{\text{mean}}$ and PV (Flo et al., 2010; Pennati et al., 2004). A value of 1 corresponds to a flat profile, 0.5 to parabolic flow and values closer to zero relate to a high velocity central flow (Figure 15.3) (Pennati et al., 2004). Several researchers have used a spatial velocity profile coefficient of 0.5 (Acharya et al., 2005; Barbera et al., 1999; Boito et al., 2002; Prior et al., 2014; Rizzo et al., 2016); however, others have disputed use of this value. Pennati et al. (2004) suggested the use of 0.61, as the coefficient in free floating cord as the flow profile changes with vessel curvature and with distance from the placental insertion site. Flo et al. (2009) found that using a spatial velocity profile coefficient of 0.5 instead of 0.61 underestimates the $V_{\text{mean}}$ in free floating cord. In fact, in their own study, Flo et al. (2010) calculated the coefficient as 0.62 with a partially blunted profile.

![Figure 15.3. Blood flow profile in a circular vessel with the shape of the profile corresponding to a spatial velocity profile coefficient of 0.5, 0.7 and 0.97. Adapted from “Fetal venous circulation,” by T. Kiserud, 2003, Fetal and Maternal Medicine Review, 14(01), p. 68.](image)

Other potential sources of errors related to flow profiles included the third dimension of blood flow and coiling. Current 2D Doppler systems are used to quantify
three dimensional blood flow based on an assumption of axial symmetry of the flow distribution in the third plane (Pennati et al., 2004), which is not imaged in standard Doppler. Guiot, Roatta, Piccoli, Saccomandi, and Todros (1999) found an asymmetrical parabolic velocity profile related to vessel coiling and avoiding Doppler sampling near vessel curvatures has been recommended (S. Li et al., 1993). Selection of a vertical segment of cord in this research optimised the flow profile within the vein; however, reliability of this technique can be diminished if a hypercoiled segment of cord is sampled or if the blood flow profile is not symmetrically parabolic in all dimensions.

15.4 Comparison of the Nomogram with Published Reference Ranges

Quantile regression showed a linear relation between $Q_{ucv}$ and advancing GA for the 3rd and 97th percentiles, whereas a curvilinear relation described the remaining percentiles. The linear and cubic quantile regression formulae can be written:

\[
Q_{ucv} = b_{0p} + b_{1p} GA
\]

\[
Q_{ucv} = b_{0p} + b_{1p} GA + b_{2p} GA^2 + b_{3p} GA^3.
\]

Where $p$ = percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th 95th and 97th

\[\text{b}_0 = \text{intercept}\]

\[\text{GA} = \text{gestational age in days}\]

Using the coefficients provided in Table 14.2, the normal reference ranges are plotted in Figures 15.4 and 15.5, with the latter showing the nAGA data points.

Omission of the 3rd and 97th percentile eliminated the visually confusing superimposed linear and curvilinear percentile lines, and was consistent with other published reference ranges.
Figure 15.4. Nomogram of $Q_{ucv}$ with advancing GA derived from cubic quantile regression. Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

Figure 15.5. Nomogram of $Q_{ucv}$ with advancing GA derived from cubic quantile regression with the nAGA data points plotted in black ($n = 588$). Colour coded percentile curves: 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
Three relationships between $Q_{uv}$ and GA were identified in published research papers. Some authors demonstrated a linear increase in $Q_{uv}$ with advancing GA, others describe a curvilinear relationship with either $Q_{uv}$ continuing to increase until term, or declining after a mid-third trimester peak. As linear and curvilinear trends were modelled in this research, the following discussion includes comparison with both trends.

15.4.1 Linear relation with gestational age.

A positive linear trend has been used to describe the relationship between $Q_{uv}$ and advancing GA by several researchers including Chen et al. (1986). Chen et al. (1986) found a steadily increasing linear relationship with the intra-abdominal $Q_{uv}$ and GA. Gerson et al. (1987) described a linear relation between intra-abdominal $Q_{uv}$ and GA with the formula: $Q_{uv} = -187.02 + 12.7(GA)$. In a cross-sectional study, Lees et al. (1999) identified a significant increase in $Q_{ucv}$ ($p < 0.001$) between 23 to 33 weeks GA and described the increase as linear. Boito et al. (2002) also modelled a linear response to their data with the formula: $Q_{ucv}(ml/min) = 0.000328 \times GA^3 + 10.9443931 \times GA - 188.288068$. Linear modelling confirms a positive trend of increasing UV blood flow with advancing GA, similar to that modelled in this research for the 3rd and 97th percentiles.

15.4.2 Curvilinear relation with gestational age.

The relationship between $Q_{uv}$ and GA has also been described by curvilinear models. Some researchers have described a continued increase in $Q_{uv}$ with advancing GA and others have shown an increase and then a declined near term, similar to this research. Pioneering researchers, Gill et al. (1981, 1984) observed that the intra-abdominal $Q_{uv}$ increased steadily with GA until 36 weeks, reached a peak at 37 to 38
weeks, and then declined towards 40 weeks, in 47 normal fetuses. This work supported Gill and colleagues’ earlier research from 1979, in which the intra-abdominal $Q_{uv}$ recorded in 12 patients was described as having a curvilinear relation with GA.

Sutton et al. (1990) were some of the first researchers to visualise the cord and align the Doppler angle. Sutton et al. (1990) found that $Q_{ucv}$ increased exponentially with GA with no tendency to decrease near term as described by the formula:

$$\log_{10} Q_{ucv} = 1.09 + 0.04 \times GA.$$  Barbera et al. (1999) proposed that $Q_{ucv}$ increased exponentially throughout pregnancy and they described the curvilinear relation between $Q_{ucv}$ and GA with the equation:

$$Q_{ucv} = 14.811e^{0.0941 \times GA}.$$  In addition, Kiserud et al. (2000) found intra-abdominal $Q_{uv}$ increased throughout pregnancy and described the curve with the formula:

$$Q_{uv} = -10.85 + 4.97 \ln(GA) - 0.00125GA^2.$$  Kiserud et al. also found that the variability of blood flow values increased with GA, which was also one of the features of the research data.

Acharya et al. (2005) found that the intra-abdominal $Q_{uv}$ had a curvilinear increased throughout pregnancy with no reduction nearer term. Flo et al. (2010) published reference ranges in 2010 that showed a similar curvature as that described by Acharya et al. (2005), but it was at a lesser gradient and with lower maximum values at term. The latest research by Rizzo and colleagues (2016) showed a steady, continual increase of the intra-abdominal $Q_{uv}$ with advancing GA, and these researchers described their data by the cubic regression formula for the 50th percentile as

$$Q_{uv} = 176.7367 - 28.1074GA + 1.3857GA^2 - 0.0132GA^3.$$  The majority of previous studies describe an exponential increase in UV blood flow throughout pregnancy; there is some variation in opinion as to whether this
exponential increase continues up and past 40 weeks or whether there is a decline in the later weeks of pregnancy. The final research nomogram, which omitted the 3rd and 97th percentiles, showed an exponential increase up to a peak at 39 weeks and then a slight decline to post term. The trend or shape of the $Q_{ucv}$ reference range curve reflects the trends of its components, in that the diameter followed this trend. The research nomogram is very similar to that produced by Gill and colleagues in the late 1980s.

The rate of blood flow increase slowed slightly towards term in several studies (Acharya et al., 2005; Flo et al., 2010; Rizzo et al., 2016), but the volume did not reduce as found in this research. The reduction in $Q_{ucv}$ after 39 weeks modelled in this research may reflect the low number of data points above this GA, as there were only six observations greater than 280 days (40 weeks) of gestation. This trend also highlights the upper limit of gestational ages over which other researchers collected data. In this research data collection extended to 42 weeks GA, as did both Acharya et al. (2005) and Sutton et al. (1990); whereas, others collected to 36 weeks (Boito et al., 2002), 38 weeks (Barbera et al., 1999) and 40 weeks GA (Flo et al., 2010; Gill et al., 1984; Rizzo et al., 2016). Although there were limited data points after 40 weeks GA, if other researchers did not collect data in this region then there are a limited number of comparisons based on raw data and not an extrapolation.

The actual trend of $Q_{ucv}$ later in the pregnancy may be an irrelevant debate as current RCOG (2014a) guidelines recommend growth restricted fetuses detected prior to 32 weeks, with absent UA end diastolic flow, are delivered by 32 weeks GA; and, growth compromised fetuses detected after 32 weeks, with or without abnormal UA Doppler, should be offered delivery by 37 weeks GA. If these guidelines are followed
the reference range is only useful prior to 37 weeks GA, then whether the $Q_{ucv}$ decreases after this time has no clinical relevance.

15.5 Identifying IUGR/FGR Group using the Reference Range

Figure 15.6 displays the calculations from Table 14.4, in that 47.8% of data points fell below the 5th percentile, 54.3% below the 10th percentile and 95.7% below the median. Using the 10th percentile line as an arbitrary cut-off and assuming IUGR fetuses have reduced $Q_{ucv}$, the nomogram correctly detected 54.3% of the IUGR/FGR group attendances and erroneously classified 45.7% of these attendances as normal. The CIs of the birthweight curves in Figures 14.6 and 14.7 showed marked overlap of all three birthweight categories prior to 164 days gestation (23 weeks 3 days) and this defined the earliest GA when $Q_{ucv}$ could be reliably differentiated between the categories. The GA at the time of scanning the IUGR/FGR group ranged from 139 (19 weeks and 6 days) to 278 days (39 weeks and 5 days). Elimination of the single IUGR/FGR group data point collected prior to 23 weeks and 3 days GA left 45 $Q_{ucv}$ data points for evaluation. Of the remaining 45 data points, 28 plotted below the 10th percentile giving a detection rate of 62.2%. This is a useful GA at which monitoring for growth restriction could be initiated and at which other ultrasound parameters are also available to identify and monitor poor intrauterine growth.
Clinical application of the nomogram necessitates a low false negative rate to facilitate correct classification of fetuses. Studies of ovine pregnancies determined that transfer of oxygen and nutrients to the lamb was not affected until $Q_{uv}$ was reduced by approximately 50% (Battaglia, 2003). This finding has implications for the clinical applications of the reference range as it infers that $Q_{uv}$ would have to be well below normal ranges if growth restriction was to be predicted with certainty, and it supports the choice of the 10th percentile as a cut-off.

The consensus of research is that $Q_{uv}$ is reduced in IUGR fetuses compared to AGA fetuses. Results have been published detecting greater than three quarters of IUGR fetuses on reference ranges, including the following research. Jouppila and
Kirkinen (1984) found that 100% of IUGR$^{43}$ fetuses had a last attendance intra-abdominal $Q_{ucv}$ corrected for EFW, below the 10th percentile on their reference ranges, but four of these fetuses had an undetectable velocity at their last attendance which was presumed to be low. If the IUGR detection rate is calculated on an attendance basis and the four attendances with no flow were eliminated, then 92% of IUGR fetuses were correctly identified. In a study of 38 IUGR$^{44}$ fetuses involving 11 fetal or neonatal deaths, Kiserud et al. (1994) reported 76% of these fetuses had a reduced value $Q_{ucv}$ and the reduction was more frequent before 31 weeks GA. Ferrazzi et al. (2000) found that 28 of 37 IUGR$^{45}$ fetuses plotted wholly below the 10th percentile, thereby correctly identifying 92% of IUGR fetuses; however, the physical size of the data points on the nomogram made interpretation difficult. Boito et al. (2002) also showed a high level of detection with 94% of IUGR$^{46}$ fetuses plotting below their normal $Q_{ucv}$ range. In summary, these studies quote a higher rate of IUGR identification compared to the research. They also used the 5th or 10th percentile as the cut-off, and reduced biometry and birthweight defined IUGR, thereby eliminating these possible reasons for variations in the results. The research detection rate of 62.2% compared favourably with other methods of IUGR identification, with similar rates of IUGR detection quoted for third trimester ultrasound biometry (67 %) (Hammad et al., 2016) fetal weight (65.8%), AC (62.2%) and UA S/D (66.7%) (Ott, 2005).

In this research, the mean $Q_{ucv}$ of the nAGA group was 177.4 ml/min (mean GA = 207 days) and was greater than the mean of the IUGR/FGR group at 154.5 ml/min (mean GA = 235 days). Rigano et al. (2001) reported a similar observation with the

$^{43}$ Birthweight < 10th %, reduced fetal growth, Apgar < 7 at 1 and 5 minutes, pathological CTG
$^{44}$ BPD, abdominal diameter and birthweight < 2.5th %
$^{45}$ AC < 2 SD below mean and birthweight < 10th %
$^{46}$ AC and birthweight < 5th %
mean $Q_{ucv}$ per kilogram significantly ($p < 0.001$) higher in the age matched control group (121 ml/min/kg) than in the IUGR group (66 ml/min/kg). Tchirikov et al. (2009) found the mean intra-abdominal $Q_{ucv}$ in a control group (253.3 ml/min) of fetuses with a good pregnancy outcomes was significantly higher ($p = 0.0054$) than in non-age matched, compromised fetuses (160.2 ml/min). Direct comparison of the mean $Q_{uv}$ between normal control fetuses and IUGR fetuses does show a difference between the groups, but fails to consider the natural increase in $Q_{uv}$ with advancing GA, which is accentuated if the groups are not age matched.

15.6 Diameter and Velocity Effects on Blood Flow

The scatterplot of average UCV PV plotted against average UCV diameter (Figure 14.10) showed that all three birthweight categories had a general trend of increasing UCV PV with increasing UCV diameter. The nomograms in Figure 14.11 showed the gradient of the UCV diameter median slope had an angle of inclination of 53° from 16 to 28 weeks gestation, then 20° inclination until 37 weeks GA and a declining 6° slope until 42 weeks GA. The UCV PV nomogram median gradient had a constant 24° positive angle of inclination from 16 to 42 weeks GA. These changes in the gradients of the nomograms infers that at different GA, the magnitude of the contribution to increasing $Q_{ucv}$ switches between widening diameter and PV increase, with diameter contributing mostly to increasing blood flow up until 28 weeks GA. Both UCV PV and diameter contribute equally between 28 and 33 weeks, and increased PV is the major component after 37 weeks GA. Although Acharya et al. (2005) imaged the intra-abdominal UV and demonstrated that PV declined towards term, their research

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47 AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
48 Poor outcome based on the cord blood pH, Apgar score, birthweight percentile, length of pregnancy, requirement of respiratory support and transfer of the newborn to a specialist ward
also showed the gradient of the UV diameter graph was steeper than the rise in PV nomogram.

In normal pregnancies, the size of the umbilical vein has been found to be the major contributor to the increase in $Q_{uv}$, in four publications. Lees et al. (1999) proposed a positive linear relation between UV area, $T_{amaxv}$ and advancing GA, and concluded that increasing blood flow throughout pregnancy had a greater dependency on increasing UV area than on velocity. Barbera et al. (1999) proposed a positive linear relation between UV diameter and velocity with increasing GA, and concluded the slope was greater for the UCV diameter and that growth of the vein accounted for most of the increase in $Q_{uv}$. Boito et al. (2002) found that the five-fold increase in the UV area far outstripped the increase in the $T_{amaxv}$. In a study of preterm and term fetuses, Link et al. (2007) found that the growth of the UV was responsible for increasing $Q_{uv}$ with advancing GA. Rizzo et al. (2016) found that the increase in intra-abdominal $Q_{uv}$ was due to an increase in both the UV diameter and $T_{amaxv}$, which is similar to the conclusion formed in this research; however, different degrees of contribution were attributed at different stages of the pregnancy.

Early research by Jouppila and Kirkinen (1984) attributed reduced umbilical blood flow in compromised fetuses entirely to constriction of the vein. In some cases of growth restriction, a reduction in UV velocity has been cited as the major cause of reduced blood flow: Rigano et al. (2001) found that the UV diameter normalised to body size was unaffected by IUGR and that reduced UV velocity was the major cause of reduced $Q_{uv}$ in IUGR fetuses. However, Skulstad et al. (2004) proposed that to maintain $Q_{uv}$, the UV velocity increased to compensate for a reduction in diameter; however, several researchers found that blood flow is not maintained in cases of IUGR (Bellotti
et al., 2004; Di Naro et al., 2002; Ferrazzi et al., 2000; Gill et al., 1984; Kiserud et al., 1994).

Rigano et al. (2008) found that both diameter and velocity were the cause of reduced blood flow. Raio et al. (2003) also found that both velocity and diameter contributed to a reduced $Q_{ucv}$ in IUGR and speculated that “abnormal placentation might cause an early reduction of both the umbilical vein blood flow and velocity. A persistent reduction of umbilical vein velocity with increasing fetoplacental impedance might induce remodeling (sic) of the umbilical vessels and, in particular, the umbilical vein” (p. 1345).

Numerous researchers have proposed that either, or both, UCV diameter and PV contribute to increasing blood flow in the normal fetus and that either, or both, may contribute to reduced flow in growth compromised fetuses. Differing contributions of UCV diameter and PV to increasing $Q_{uv}$ with advancing GA, found in this research concurs with previous studies.

15.7 Limitation and Advantages of the Umbilical Cord Vein Blood Flow

In this research there were several weaknesses in the calculated $Q_{ucv}$ value. These weaknesses are in addition to; the sparsity of data in the late second and third trimesters, the IUGR/FGR sample of moderately compromised fetuses, and whole weeks used to define birthweight categories as previously discussed. These weaknesses can be summarised as follows:

i. The $Q_{ucv}$ is extremely dependent on the accuracy of both the diameter and PV measurements.

ii. A $Q_{ucv}$ value requires a calculation or additional software techniques that are dependent on the sonographer for correct application.
iii. The formula for the blood flow assumes a parabolic flow in three dimensions and uses a spatial velocity profile coefficient of 0.5. Although this is accepted within the research community, some researchers have found different coefficient values better describe the blood flow along the length of the cord and consideration should be given to this factor if the UCV is measured at different points.

iv. Fluctuations in $Q_{uv}$ have been demonstrated over a five minute time frame. Further investigation of the effects of these fluctuations on $Q_{uv}$ provide an area for future research and consideration in the application of this nomogram; provided implications of prolonging fetal exposure to ultrasound are considered.

In addition to the common strengths of the nomogram being developed from a strictly selected Australian based “normal” population, analysis using quantile regression and the expression of the results in percentiles, the strengths of the $Q_{ucv}$ measurement, development of the reference ranges and identification of IUGR can be summarised as follows:

i. The nomogram was developed using the simplest possible measurement techniques for the PV and diameter and a commonly used blood flow calculation. Using this combination means that $Q_{ucv}$ can be calculated and the nomogram utilized by all clinicians irrespective of their location or the complexity of their ultrasound machine.

ii. The $Q_{ucv}$ from both the raw data and the 50th percentile predicted from the quantile regression formula were in keeping with previous published data.
iii. The modelling of the three birthweight categories identified a significant
difference between the groups with overlapping CI curves prior to 23
weeks 3 days GA. This adds further support to the hypothesis that blood
flow is reduced in IUGR fetuses and indicates that measurements
undertaken before this cut-off will have little diagnostic value.

iv. Quantile regression with backward selection of powers modelled both
linear and curvilinear lines to describe the nAGA data. Both trends have
been described previously in the literature. Modelling both trends
highlights recent advances in statistical analysis which allow the true
relation between the variables to be explored, as this may be different at
various percentiles.

v. The reference range correctly identified 54.3% of IUGR/FGR group
attendances and detection rate increased to 62.2% when the cut-off of 23
weeks and 3 days GA was applied which compared favourably with other
current ultrasound methods used for the identification of IUGR.

15.8 Potential Applications of the Umbilical Cord Vein Blood Flow Results

Q_{ucv} is technically difficult to accurately measure or calculate due to the errors
associated with components of the formula. Given this, Q_{ucv} is well established in other
facets of ultrasound and its use in obstetrics should be encouraged. Further research
could be undertaken to compare software calculation versus manual calculation, and
determine if the reference ranges developed in this research are compatible with
different methods of acquiring Q_{ucv} values.

The detection rate of 54.3%, or 62.2% when the cut-off of 23 weeks and 3 days
GA was applied, is not sufficient to support the use of Q_{ucv} as a stand-alone diagnostic
tool for IUGR. However, in combination with other established ultrasound parameters, $Q_{ucv}$ may prove to be another useful tool in the arsenal of ultrasound measurements that can be used to monitor and diagnose IUGR.

Recent research has found that in a low risk obstetric population a $Q_{ucv}$ below the 20th percentile increased the risk of intrapartum fetal distress, necessitating emergency delivery (Prior et al., 2014). This finding may have beneficial repercussions for obstetric management of patients in regional and rural area where timely transfer to larger facilities may be appropriate.

### 15.9 Conclusion

This research presents the first $Q_{ucv}$ reference range developed from an Australian based “normal” obstetrics population using quantile regression. Both linear and curvilinear relationships between $Q_{ucv}$ with increasing GA have been demonstrated and these trends are in keeping with other published works. Linear mixed modelling found a significance difference between the birthweight categories, with similar shaped curves and overlapping of the CIs of the three categories prior to 23 weeks 3 days GA.

The reference range correctly identified 62.2% of IUGR/FGR group attendances using the 10th percentile and 23 weeks and 3 days as cut-offs, suggesting that $Q_{ucv}$ has a role in the identification of growth restricted fetuses. Analysis of the relationship between the average UCV PV and diameter showed that there was a simultaneous increase in both, but their contribution to increasing blood flow varied with GA.
Chapter 16 Ratios and Longitudinal Trends Results

16.1 Introduction

In this chapter, the ratio of the UCV diameter:PV and the UCV PV:diameter, calculated from the nAGA group data, will be investigated. Statistical modelling will construct reference ranges for both ratios and analysis will determine each nomogram’s ability to identify IUGR/FGR group data. Sequential plotting of UCV diameter, PV, and Q_{ucv} data points will visually assess longitudinal trends and explore differences in slopes.

16.2 Developing Umbilical Cord Vein Ratios Reference Ranges and Identification of the IUGR/FGR Group

There were 588 ratios available for analysis as five average UCV diameter measurements and 10 average PV measurements were not recorded in the nAGA group (Appendix K4). Quantile regression was used to model the relationship between the ratios and GA (Appendix L8). A polynomial quantile regression model was applied with backward selection of power, and a cubic (order 3) model was selected for analysis of both ratios. The regression equation is written below:

\[
\text{Ratio} = b_{0_p} + b_{1_p} GA + b_{2_p} GA^2 + b_{3_p} GA^3.
\]

Where \(\text{Ratio} = \) UCV diameter:PV ratio or UCV PV:diameter ratio

- \(b_{0_p} = \) intercept
- \(p = \) percentile value 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 97th
- \(GA = \) gestational age in days
The coefficients and predicted values for each percentile are provided in Appendix L8. The quantile regression curves for both ratios, with the nAGA data superimposed, are shown in Figures 16.1 and 16.2. These figures show curvilinear relationships between the ratios and advancing GA. The UCV diameter:PV ratio increased until the third trimester and then declined, whereas the UCV PV:diameter ratio declined with GA until the third trimester and then stabilised.

Figure 16.1. Nomogram of UCV diameter:PV ratio derived cubic quantile regression with the nAGA data points plotted in black (n = 588). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
**Figure 16.2.** Nomogram of UCV PV:diameter ratio derived cubic quantile regression with the nAGA data points plotted in black ($n = 588$). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

Table 16.1 shows the percentage of IUGR/FGR group data points falling below specified percentiles. The IUGR/FGR group ratios are plotted on the nomograms in Figures 16.3 and 16.4.

Table 16.1

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Percent below percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd</td>
</tr>
<tr>
<td>UCV diameter: PV</td>
<td>17.4</td>
</tr>
<tr>
<td>UCV PV: diameter</td>
<td>0</td>
</tr>
</tbody>
</table>

*Note. UCV = umbilical cord vein. PV = peak velocity.*
Figure 16.3. Nomogram of UCV diameter:PV ratio with IUGR/FGR group data in red \((n = 46)\). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).

Figure 16.4. Nomogram of UCV PV:diameter ratio with the IUGR/FGR group data in red \((n = 46)\). Colour coded percentile curves: 3rd and 97th (green), 5th and 95th (blue), 10th and 90th (gold), and 50th (black).
16.3 Analysis of Longitudinal Data

All average UCV diameter, average UCV PV, and \( Q_{ucv} \) data points for SGA participants with more than two scans \((n = 38)\), with more than three scans in the LGA category \((n = 11)\), and with more than five scans in the AGA birthweight category \((n = 11)\) were plotted against GA (Appendix L9). Visual assessment of these longitudinal plots demonstrated an increasing trend of the UCV variables with advancing GA; however, the graphs were chaotic and no clear distinction between the birthweight categories was identifiable. In order to declutter these graphs the first and last UCV variables values from subgroups of the SGA \((n = 38)\), nAGA \((n = 19)\), and LGA categories \((n = 19)\) were constructed (Appendix K5) and plotted against GA (Appendix L8). The decluttered graphs, plotting only the first and last scan UCV variables values, are shown in Figures 16.5, 16.6 and 16.7.

![Graph showing average UCV diameters against GA](image)

Figure 16.5. First and last average UCV diameters against GA \((n = 76)\).
Figure 16.6. First and last average UCV PV against GA ($n = 76$).

Figure 16.7. First and last $Q_{ucv}$ against GA ($n = 76$).
The data used in the construction of the UCV diameter, PV and $Q_{ucv}$ decluttered graphs, plus similar data from the IUGR/FGR group ($n = 7$) (Appendix K5), was used to calculate the slope of the line connecting the first and last attendances (Appendix L10). Comparison of the UCV diameter slope between these four groups showed that the IUGR/FGR subgroup had the lowest mean and median slope values and that the SGA subgroup had the second lowest mean and median slope value; both IUGR/FGR and SGA subgroups were less than the nAGA and LGA subgroups. Weighted least squares analysis was used to account for different variances associated with each group and the slope of the UCV diameter was dependent ($p < 0.001$) on the groups. Figure 16.8 graphs the predicted means of the UCV diameter slope, with the IUGR/FGR subgroup having the only negative slope.

![Graph showing predicted means of UCV diameter slope](image)

*Figure 16.8. Graph of the predicted means of the UCV diameter slope.*
Comparison of the UCV PV slope between the four groups showed that the IUGR/FGR and SGA subgroup had lower mean and median slope values than the nAGA and LGA subgroups. Weighted least squares analysis showed no significant difference ($p = 0.0990$) existed between the UCV PV slopes of the subgroup and as such no further analysis was undertaken.

A single outlier was removed from the SGA subgroup before comparison of the $Q_{ucv}$ slopes was undertaken. The IUGR/FGR subgroup had the lowest mean and median slope values and the SGA subgroup had the second lowest mean and median slope values; mean and median slope values for both groups were less than the nAGA and LGA subgroups. Weighted least squares analysis showed that the $Q_{ucv}$ slopes were significantly different for the groups ($p < 0.001$). The predicted mean slope value was positive for all four groups (Figure 16.9).

![Graph of the predicted means of the $Q_{ucv}$ slope.](image)

*Figure 16.9. Graph of the predicted means of the $Q_{ucv}$ slope.*
16.4 Conclusion

The ratios of UCV diameter:PV and PV:diameter were calculated and quantile regression modelled cubic curvilinear curves. Using the 10th percentile as a cut-off, the UCV diameter:PV ratio correctly identified 4.3% and the UCV PV:diameter ratio 19.6% of the IUGR/FGR group data. The IUGR/FGR subgroup had the lowest mean and median slope values for all three UCV parameters. There was a significant difference between the UCV diameter and $Q_{ucv}$ slope depending on the four subgroups. The only negatively predicted slope was the IUGR/FGR subgroup UCV diameter.
Chapter 17 Ratios and Longitudinal Trends Discussion

17.1 Introduction

In this chapter, the reference ranges developed from quantile regression analysis of the UCV ratios and their performance in identifying IUGR fetuses will be discussed. Longitudinal data will be reviewed in the context of identifying trends associated with poor intrauterine growth and compared to other published results.

17.2 Ratios and the Identification of the IUGR/FGR Group

Reference ranges for UCV diameter and PV ratios against advancing GA have not been presented previously. The regression curves of both ratios were curvilinear and highlight one of the benefits of using percentiles, which is that a majority of the percentile curves have a unique shape tailored to the response of each variable at various gestational ages.

The UCV diameter:PV ratio reference range had a non-normal distribution and exhibited increased variability with advancing GA, but did perform better at correctly classifying IUGR/FGR group data. This ratio correctly identified 63% of IUGR/FGR group attendances using the median as a cut-off and 19.6% using the 10th percentile. The denominator in the ratio UCV PV:diameter was the most stable variable and therefore ideally the reference range developed from this ratio would be the most robust. However, the reference range only correctly identified 37% of IUGR/FGR group attendances using the median as a cut-off and 4.3% using the 10th percentile. These detection rates were less than the 62.2% rate achieved by both the UCV diameter and \( Q_{UCV} \).
17.3 Longitudinal Data and the Identification of the IUGR/FGR Group

17.3.1 Umbilical cord vein diameter.

Figure 16.5 shows a predominantly positive UCV diameter slope with advancing GA for the nAGA and LGA subgroups and the slope appeared less in the SGA subgroup. Only the IUGR/FGR subgroup had a negatively predicted slope (Figure 16.8) inferring that this group did not experience a significant increase in UCV diameter throughout the pregnancy. The SGA subgroup had a small positive predicted mean value inferring that the slope of the UCV diameter was positive, but at a lesser gradient than the nAGA and LGA subgroups. The UCV diameter of the IUGR/FGR and SGA subgroups showed an overall tendency to not increase, or not increase rapidly, with advancing GA, compared to the nAGA and LGA subgroups. This finding is similar to data published by Di Naro et al. (2002), who compared the UCV cross-sectional area of 15 IUGR to 30 AGA fetuses at ultrasound scans completed two weeks apart. The mean UCV cross-sectional area reduced between the first examination and second examination for IUGR fetuses (35.5 to 34.2 mm$^2$) compared to an increase in the AGA mean value (43.1 to 45.3 mm$^2$).

17.3.2 Umbilical cord vein peak velocity.

The decluttered PV longitudinal graph (Figure 16.6) shows a predominant trend of increasing UCV PV with advancing GA for the nAGA and LGA subgroups and the slope appeared less in the SGA subgroup; however, statistically there was no association between the groups and the UCV PV slope. This finding was at odds with a

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49 AC < 5th % and birthweight < 10th %
study of 11 compromised pregnancies\textsuperscript{50} in which the UV $V_{\text{mean}}$ declined in three of the four women undergoing serial scans (Jouppila & Kirkinen, 1984).

### 17.3.3 Umbilical cord vein blood flow.

The decluttered $Q_{\text{ucv}}$ longitudinal graph shown in Figure 16.7 displayed a predominant trend of increasing $Q_{\text{ucv}}$ with advancing GA, between the first and last scan measurements. There was a significant difference between the slopes of the four subgroups; however, all predicted $Q_{\text{ucv}}$ slopes were positive, making it visually difficult to distinguish between the groups on the basis of trend alone.

The predicted mean of the slope for the SGA and IUGR/FGR subgroups were positive, but were less than the nAGA and LGA subgroups. This is in keeping with a longitudinal study of $Q_{\text{ucv}}$ corrected for AC and EFW undertaken by Rigano et al. (2001), who reported that $Q_{\text{ucv}}$ was reduced in IUGR\textsuperscript{51} fetuses at first diagnosis of IUGR and that the reduction persisted throughout the pregnancy. Both Rigano et al.’s and the research findings differ from the trend described by Di Naro et al. (2002), in which a progressive reduction in $Q_{\text{ucv}}$ for IUGR\textsuperscript{52} fetuses was identified between fortnightly scans, even in the presence of normal UA Dopplers.

### 17.4 Limitation and Advantages of the Umbilical Cord Vein Ratios and Longitudinal Results

The limitations for making a robust analysis of the detection of fetal growth restriction was the sparsity of data between 23 to 28 weeks and after 39 weeks GA, and the absence of severely growth restricted data. The collection of larger data sets would overcome these limitations and is an avenue for further research.

\textsuperscript{50} Birthweight < 10th %, reduced fetal growth, Apgar < 7 at 1 and 5 minutes, pathological CTG
\textsuperscript{51} AC < 2 SD below mean, UA PI > 2 SD above mean, abnormal uterine artery and birthweight < 10th %
\textsuperscript{52} AC < 5th % and birthweight < 10th %
This portion of the research presents the first analysis undertaken on ratios between the UCV diameter and PV. The ratio reference ranges did not correctly classify the IUGR/FGR group attendances to a clinically useful level; however, further data collection including fetuses with severe IUGR could be undertaken to thoroughly assess these reference ranges. This analysis provides supporting evidence for the use of the UCV diameter in the diagnosis of IUGR fetuses as the SGA and IUGR/FGR subgroup had little or no increase in the diameters on serial plotting compared to increasing UCV diameters seen in the nAGA and LGA subgroups.

17.5 Potential Applications of the Umbilical Cord Vein Ratios and Slopes

The reference ranges developed from the ratios of the UCV diameter and PV have limited clinical applications as they involved an additional calculation and proved to have a low detection rate of IUGR/FGR group data. The diameter of the IUGR/FGR group was the only UCV feature to exhibit a negative slope when longitudinal data was examined over several weeks of gestation. Therefore in clinical practice when sequential plotting of the UCV diameter is undertaken over several weeks of the pregnancy, the presence of a negative slope provides supporting evidence of compromised growth.

17.6 Conclusion

The reference ranges developed from the ratios of the UCV diameter and PV performed poorly in the correct identification of IUGR/FGR fetuses using the 10th percentile as a cut-off, inferring that their clinical application may be limited. Analysis of the slope of the UCV diameter, PV and $Q_{ucv}$ against advancing GA showed that the UCV diameter for IUGR/FGR subgroup fetuses had the only negative slope. Given that
the UCV diameter is a simple measurement to undertake, serial plotting of this measurement may provide supporting evidence for the diagnosis of IUGR.
SECTION VI CONCLUSION AND CLINICAL IMPLICATIONS

Chapter 18 Conclusions and Clinical Implications

18.1 Introduction

This exegesis presents umbilical cord vein diameter, PV and $Q_{ucv}$ reference ranges that were developed using a Central West NSW “normal” obstetrics population and quantile regression. In addition, this research assessed the proficiency of these reference ranges in the identification of moderately growth restricted fetuses, documented measurement and calculation methods, investigated other relations between the UCV parameters and analysed maternal and pregnancy outcome characteristics of the samples. The research had several advantageous design features; however, data analysis was restricted by referral patterns to regional hospitals and categorical boundaries. All research aims were achieved and the outcomes have contributed to the expansion of knowledge, potentially influenced clinical practice and identified several areas for further research.

18.2 Research Outcomes

The maternal and pregnancy outcome characteristics of the whole research sample and the nAGA group were analysed and compared to local, state and national populations. The whole research sample was typical of the Australian population; except mothers were younger, more mothers smoked at some point in their pregnancy, and neonates were lighter than comparable Australian data. The characteristics of the nAGA group were reasonably typical of the whole research
sample and the nAGA group was similar to the 2012 Australian population, except that mothers were younger, parity was higher, and fewer male babies were born.

Linear mixed modelling showed a significant difference between the SGA, AGA and LGA birthweight categories for each of the UCV features; however, there was overlap of the CIs of the three birthweight regression curves at certain gestational ages. The three birthweight curves for the UCV diameter and $Q_{ucv}$ had similar shapes and their CIs overlapped until 25 weeks and 6 days GA and 23 weeks and 3 days, respectively. The UCV PV had different shaped curves for each birthweight category and the CIs overlapped between 20 weeks and 4 days and 35 weeks and 1 day GA.

The reference ranges developed from quantile regression demonstrated a curvilinear increase in the UCV diameter with advancing GA up until 37 weeks, followed by a plateau lasting 4 weeks, and then a slight decline to 42 weeks GA. There was a positive linear increase in UCV PV with increasing GA and an exponential increase in $Q_{ucv}$ up to a peak at 39 weeks followed by a slight decline until 42 weeks GA. The UCV diameter, PV and $Q_{ucv}$ values that were measured, calculated or predicted from modelling were comparable to previously published values. The reference ranges for all three UCV measurements increased with advancing GA and the trends described in this research are in keeping with previously published outcomes. A peer reviewed article detailing these reference ranges is contained in Appendix N.

Using the 10th percentile and the GA cut-offs defined by the CIs of the birthweight categories, the reference ranges correctly identified 15% of IUGR/FGR group attendances for UCV PV, and 62.2% for both the UCV diameter and $Q_{ucv}$. Reference ranges for ratios of the UCV diameter and PV had curvilinear trends with advancing GA and identified less than 20% of the IUGR/FGR group attendances.
Analysis of the slope of the UCV diameter, PV and $Q_{ucv}$ showed that the UCV diameter for IUGR/FGR subgroup fetuses had the only negative slope.

### 18.3 Advantages and Limitations of the Research Methods

The research methods employed population appropriate data, strict selection criteria, familiar measurement protocols, and preeminent statistical analyses. Data collection and analyses were restricted by referral patterns to regional hospitals, the absence of severely growth compromised fetuses, rounding the GA at delivery to whole weeks, and a Doppler sample gate that was wider than the UCV at early gestational ages.

The advantages of the research methods included:

i. Australian, gender and GA specific birthweights percentiles, instead of ultrasound criteria, were used to retrospectively classify intrauterine growth. This was a superior method based on a tangible outcome as opposed to ultrasound biometry and EFW that incur errors in measurement and formulae.

ii. The exclusion criteria used to sequester the nAGA group was strict, resulting in data collected from “normal” pregnancies without recruiting clients with no medical or clinical need for an obstetric ultrasound, which would have had financial, ethical and safety issues beyond the scope of this research.

iii. Comparison of the maternal characteristic and pregnancy outcomes of the whole research sample and the nAGA group indicated: the nAGA group was typical of the whole research sample and that both research samples were reasonable typical of NSW and Australian populations. Inferring that the
data was representative and the reference ranges developed from the data were applicable to the broader population.

iv. Measuring the intra-amniotic UCV and utilising the nuchal translucency measurement protocol meant that the research sonographers were familiar with the required technical aspects of data collection and the measurement protocol should be readily transportable to the wider sonography profession.

v. The excellent ICC results demonstrated that the measurement methods for the UCV diameter and PV were simple, reproducible and provided high quality data for the construction of the reference ranges.

vi. Quantile regression was used in the development of the reference ranges and has several features that overcome assumptions of parametric analysis. Quantile regression is more robust to outliers, does not assume a normal distribution of the data, or that the residuals have a mean of zero and constant variance.

vii. The use of percentiles to describe the data trends when developing the reference ranges permitted a different fitted function for each percentile.

The interpretation and assessment of the results were subject to several limitations including:

i. A sparsity of data between 23 to 28 weeks and after 39 weeks GA reflecting the referral patterns to the research hospitals. The reference ranges may have been more robust if the modelling did not rely on interpolation between available data.
ii. The IUGR/FGR group definition identified moderately compromised fetuses, as severely growth restricted fetuses were not available within the sample population. The IUGR/FGR group contained seven participants who provided less than 50 data points for evaluation.

iii. Birthweight categories were assigned using the GA at delivery rounded to whole weeks, which may have inadvertently skewed the classification of the birthweights; however, this was the most relevant, population appropriate data available.

iv. The Doppler sample gate covered the entire umbilical cord in early pregnancy. However, modelling of the three birthweight categories demonstrated that the PV reference range had limited clinical usefulness and the $Q_{ucv}$ reference range which incorporated the PV value, was not appropriate until late second trimester, when this limitation is no longer be relevant.

18.4 Contributions to Knowledge

The exegesis and portfolio have made the following contributions to knowledge:

i. UCV diameter, PV and $Q_{ucv}$ reference ranges derived from a cohort of low risk, singleton pregnancies in Central West NSW were developed and disseminated (Appendix N).

ii. Simple measurement and calculation protocols for the UCV diameter, PV and $Q_{ucv}$ were documented and disseminated (Appendix N).

iii. Mixed linear modelling of the SGA, AGA and LGA birthweight categories indicated there was a significant difference between the birthweight
groups for all three UCV parameters. The differences between the birthweight categories were not identifiable during the gestational ages when the CI curves overlapped. The CI curves for the UCV diameter and $Q_{ucv}$ overlapped until the late second trimester, but the overlapping was the most significant for the UCV PV as this occurred during the crucial period of fetal growth from 20 to 35 weeks GA.

iv. UCV diameter and $Q_{ucv}$ reference ranges were used to correctly identify more than half of IUGR/FGR group attendance using a 10th percentile cut-off. This detection rate increased to 62.2% when the GA cut-offs identified in the linear mixed modelling of the birthweight categories was applied. The UCV PV reference range detected only 27.7% of IUGR/FGR group attendances using a 10th percentile cut-off. This detection rate reduced to 15% when the GA cut-offs identified in the linear mixed modelling of the birthweight categories was applied.

v. Reference ranges for ratios of the UCV diameter and PV were developed using quantile regression and found to have a detection rate of less than 20% for IUGR/FGR group data using a 10th percentile cut-off.

vi. Analysis of the slope of the UCV diameter, PV and $Q_{ucv}$ against advancing GA showed that the UCV diameter for IUGR/FGR subgroup fetuses had the only negative slope.

vii. Analysis of the slopes of the UCV and PV nomograms showed that UCV PV increased with enlarging UCV diameter; however, the rate of increase was constant for the UCV PV and was greatest in the UCV diameter prior to 28 weeks GA. This finding was in keeping with previous research and implied
that both UCV diameter and PV contribute to increasing $Q_{ucv}$, but increasing UCV diameter contributes most to rising $Q_{ucv}$ before 28 weeks GA.

viii. The literature review highlighted an absence of a comprehensive review of the anatomy and development of the umbilical vein and a review article was published to address this gap in knowledge (Appendix A).

ix. An article comparing maternal and pregnancy outcome of the whole research sample to local, state and national data was published and provided a detailed review of the local obstetric population (Appendix M).

### 18.5 Implications for Clinical Practice

The exegesis and portfolio provided six significant implications for clinical practice:

i. The UCV diameter, PV and $Q_{ucv}$ reference ranges provide a useful addition to the assemblage of ultrasound parameters currently employed for the diagnosis and monitoring of fetal growth restriction.

ii. The UCV diameter was easy to measure and required only B-mode imaging. It was used to identify 62.2% of attendances of the IUGR/FGR fetuses after 25 weeks and 6 days and demonstrated a negative slope with sequential plotting for fetuses classified as growth restricted. These features make the UCV diameter measurement and reference range useful across a wide array of ultrasound machine designs and to a diverse range of user expertise.

iii. Analysis of the three birthweight categories showed overlap of the confidence intervals, most significantly for the UCV PV during an important 15 week period of fetal growth. The inability of the UCV PV to distinguish
between fetuses in different birthweight categories during the gestational ages of 20 and 35 weeks may explain the historical neglect of this measurement in clinical practice and indicates that UCV PV would be of little use in the diagnosis and monitoring of abnormal fetal growth.

iv. The $Q_{ucv}$ reference range identified 62.2% of IUGR/FGR data, but the errors in measuring the components, the additional calculation, and the assumptions in the formula inhibit clinical application.

v. Reference ranges of the ratios between UCV diameter and PV were developed and had a low detection rate of the IUGR/FGR data implying that these ranges would have restricted clinical use in the identification of growth restriction.

vi. The ultrasound safety portion of the literature review formed the basis of a Local Health District Operating Protocol to inform sonographers of the current bioeffect indices and their limits, a means of complying with the guideline limits, and current opinions regarding the bioeffects of fetal ultrasound exposure (Portfolio Appendix H).

18.6 Further Research

On the basis of the literature review and research results, suggestions for future studies include:

i. Continue data collection to provide a larger sample and data covering the gestational ages that currently have limited information and reanalyse.

ii. Assess the reference ranges with a larger IUGR/FGR sample including severely growth compromised fetuses.
iii. Undertake a multicentre study to assess the utility of the reference ranges to the broader Australian population.

iv. Compare the UCV diameter, PV and $Q_{ucv}$ data collection techniques to automated methods to determine if these reference ranges remain relevant with different acquisition methods.

v. Examine the possible effects of umbilical vein fluctuations on the calculation or measurement of $Q_{ucv}$.

vi. Examine the possible effects of coiling on PV and $Q_{ucv}$ values.

vii. Investigate possible interrelations between the UCV diameter and UA indices given that the fetus is considered a closed system and that UCV diameter decreases in the presence of IUGR and UA indices increase.

viii. Determine if there is a significant difference between the UCV diameter, PV and $Q_{ucv}$ of males and females fetuses.

18.7 Conclusion

In this exegesis and portfolio, I have presented reference ranges for the UCV diameter, PV and $Q_{ucv}$ that were developed from a Central West NSW population. Simple methods of measuring or calculating these values have been documented. In conjunction with cut-offs identified from modelling three birthweight categories, these reference ranges have been assessed for their ability to identify IUGR. Due to the simplicity of measurement, availability of B-mode imaging across a wide range of machines and localities, and a negative slope with longitudinal plotting, the UCV diameter was found to be the superior parameter. The UCV PV was less successful in identifying IUGR attendances and I proposed that the overlap of the birthweight
categorises between 20 and 35 weeks GA may explain the poor acceptance of the UCV PV in clinical practice.
References


Quinton, A., Cook, C., & Peek, M. (2015). The prediction of the small for gestational age fetus with the head circumference to abdominal circumference (HC/AC) ratio: a
doi:10.1002/sono.12022


Appendices (CD)


Appendix B  Greater Western HREC approval letter

Appendix C  Greater Western HREC Site Specific approval letter

Appendix D  CSU Board of Graduate Studies approval letter

Appendix E  CSU HREC approval letter

Appendix F  Participant Information Sheet

Appendix G  Consent Form

Appendix H  Participant Characteristics Sheet

Appendix I  Quantitative Consulting Unit estimated minimum sample size

Appendix J  Obstetric pre-sets

Appendix K  Raw data spreadsheets

  Appendix K1  Whole research sample

  Appendix K2  nAGA group

  Appendix K3  Sonographer reliability

  Appendix K4  Ratios and SGA stratification

  Appendix K5  Longitudinal data

Appendix L  Statistical analyses

  Appendix L1  Whole research sample

  Appendix L2  nAGA group

  Appendix L3  Sonographer reliability
Appendix L4  Linear mixed modelling – 3 birthweight categories
Appendix L5  Linear mixed modelling – 2 birthweight categories
Appendix L6  Quantile regression nAGA group
Appendix L7  Stratified SGA groups
Appendix L8  Ratios and longitudinal data analysis
Appendix L9  Longitudinal data analysis – all attendances
Appendix L10 Slope analysis


CD in sleeve