A FETAL WEIGHT REFERENCE FOR TWINS BASED ON ULTRASOUND MEASUREMENTS

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August, 2011

Thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Abstract

Background and Objective

Fetal growth and size are known indicators of perinatal health, although the etiology of this relationship is unclear, and twins are known to differ from singletons with respect to fetal growth trajectories over the course of pregnancy. With a lack of research on ultrasound-based fetal weight references in twin populations, this study aimed to construct ultrasound-based *in utero* fetal weight references for each gestational age for a twin population.

Methods

Twins delivered at a tertiary care hospital in Montreal were used in this study. Fetal weight was calculated using a published formula that was validated in this population. Fetal growth was then modeled in twins using serial ultrasound measurements of fetal weight, and adjusting for sex and chorionicity. Linear mixed models were used to adjust for the correlation between twins from the same pregnancy, and for the use of multiple ultrasound measurements for each fetus. Restricted cubic splines were used to account for the non-linear growth of fetuses over the course of pregnancy. Predictions were made from this regression model for the 1^{st}, 10^{th}, 50^{th}, 90^{th} and 99^{th} fetal weight percentiles for the gestational period between weeks 22 and 37.

Results

Median gestational age at birth was 37 weeks, with a predicted median birth weight of 2686 g. The rate of change in fetal weight was observed to be S-shaped over the course of pregnancy, with a period of accelerated growth in the second trimester, and slower growth in the third trimester. Ultrasound-based fetal weight references constructed from this population corroborated other published ultrasound-based fetal weight references in twin populations. As expected, fetal weight in twins was consistently lower than singletons over the course of pregnancy, when compared with other published fetal weight references.

Conclusions

The ultrasound-based fetal weight reference and predicts fetal weight at each gestational age, estimates which could be used to assess fetal size at each gestational age in twin pregnancies. This study additionally validates the use of formulae to estimate fetal weight in a twin population, and adds to literature published in this field.

Résumé

Considérations préliminaires et objectif

La croissance *in utero* des jumeaux diffère de celle des enfants uniques. La croissance et la taille foetale sont des indicateurs communs de santé périnatale, bien que la nature exacte du lien de causalité soit encore incertaine. La surveillance de la taille foetale s'inscrit naturellement dans le suivi des grossesses. L'imagerie par ultrasons a permis l'élaboration de normes pondérales ultrasonographiques *in utero* pour les enfants uniques. Moins d'efforts ont été déployés pour quantifier les variations de taille des jumeaux au cours de la grossesse. Le présent ouvrage cherche à proposer une norme pondérale *in utero* pour les jumeaux.

Méthode

Le poids foetal a été estimé grâce à une formule validée pour la population retenue. La croissance intrautérine des jumeaux a été modélisée à l'aide d'estimés pondéraux ultrasonographiques prenant compte du sexe et de la chorionicité. Des splines cubiques naturels ont été utilisés pour rendre compte de la non-linéarité de la croissance foetale. Le modèle de régression a permis d'obtenir des prédictions pour les 1er, 10e, 50e, 90e et 99e centiles du poids foetal de la 22e à la 37e semaine de grossesse.

Résultats

La norme pondérale ultrasonographique élaborée à partir de la population retenue était compatible avec des normes de même nature publiées précédemment. Tel que prévu, le modèle a révélé que le poids *in utero* des jumeaux était systématiquement plus bas que celui des enfants uniques, les valeurs de référence pour ce dernier étant tirées d'une norme pondérale reconnue.

Conclusion

La norme pondérale ultrasonographique *in utero* pour les jumeaux tirée de cette étude complémente la littérature traitant de la croissance intrautérine des jumeaux, peu étoffée en comparaison avec celle portant sur la croissance intrautérine des enfants uniques. Après validation externe, elle pourrait se révéler utile en pratique clinique.

Preface

The current thesis focuses on the construction of a fetal weight reference over the course of pregnancy in a twin population. First, an introduction is provided to the project in Chapter 1, outlining the rationale behind a fetal weight reference for twins. Then, in Chapter 2, an outline of the objectives of this project are provided. Chapter 3 provides a detailed explanation of the epidemiology of twinning, concepts central to twin pregnancies and fetal growth, as well as a detailed summary of the literature on the estimation of fetal weight, and assessment of fetal growth to date. Then, details of the study methodology used are provided in Chapter 4. Chapter 5 consists of the results from the analysis, presented as two manuscripts. Finally, Chapter 6, includes an interpretation of the study findings, the strengths, limitations and scope of this work.

The following thesis has been prepared according to the guidelines for a manuscript-based thesis. The results are outlined in two manuscripts:

A Comparison of Five Commonly Used Formulae to Estimate Fetal Weight in Twins
Sushmita Shivkumar, BA. BSc., Robert Platt, PhD.

An Ultrasound-Based Fetal Weight Reference for Twins
Sushmita Shivkumar, BA. BSc., Robert Platt, PhD.

Details of authors' contributions are provided on page vi.

Contributions of Authors

The original research idea was developed by Dr. Robert Platt. Specific study objectives and study design were outlined by Ms. Sushmita Shivkumar. The study design and objectives were presented to Dr. Olga Basso and Dr. Robert Gagnon, who offered input regarding study design, and other issues specific to the conduct of the study.

Ms. Shivkumar wrote the study protocol, which was subsequently revised and approved by Dr. Platt.

The merging of the databases necessary for the study, data cleaning, and data analysis was conducted by Ms. Shivkumar with input from Dr. Platt. Complete drafts of the two manuscripts: "A Comparison of Five Commonly Used Formulae to Estimate Fetal Weight in Twins" and "An Ultrasound-Based Fetal Weight Reference for Twins" were written by Ms. Shivkumar, and subsequently reviewed and revised by Dr.Platt.

Acknowledgements

Financial support for this research project was provided by CIHR grant MOP-84379. Financial support for my Master's thesis was provided by a Studentship from the Foundation of Stars/Montreal Children's Hospital Research Institute. The Montreal Children's Hospital Research Institute also receives funds from the Fonds de la recherche en Santé du Québec. I would like to extend my sincere gratitude to these organizations for their financial support.

I would like to thank Dr. Robert Platt, my supervisor, for all the help, and advice provided during my Master's thesis. Also, I thank Dr. Olga Basso and Dr. Robert Gagnon for providing their expertise on subject matter in the field of perinatal epidemiology. Specifically, Dr. Basso was willing to talk about the strengths and weaknesses of the methodology. I would also like to thank the members of the perinatal epidemiology research group at the Montreal Children's Hospital Research Institute, in particular Dr. Jennifer Hutcheon, who was so helpful in the beginning stages of planning this thesis, and provided valuable perspective from her years of working in this field, and Luc Villandré, who provided so much informal statistical consulting, and feedback.

Finally, I would like to thank my family, my parents and my sister, who have been my anchor over the years. And, all my friends, old ones, made over my years living in Montreal, and new ones I have been lucky to meet over the past two years.

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List of Acronyms

AC: Abdominal Circumference

AD: Abdominal Diameter

AGA: Appropriate-for-gestational age

AIC: Akaike Information Criteria

ART: Artificial Reproductive Technologies

BPD: Biparietal Diameter

BMI: Body Mass Index

CI: Confidence Interval

cm: Centimetre

CRL: Crown-Rump Length

EFW: Estimated Fetal Weight

FAA: Fetal Abdominal Area

FL: Femur Length

GA: Gestational age

g: Grams

HC: Head Circumference

HELLP: Hemolysis, elevated liver enzymes, low platelet count

IQR: Inter-quartile Range

IVH: Intra-ventricular hemorrhage

IUGR: Intrauterine Growth Restriction

kg: Kilogram

LBW: Low Birth weight

LMM: Linear Mixed Model

LNMP: Last Normal Menstrual Period

m: Metre

MOND: McGill Obstetrical and Neonatal Database

MRN: Medical Record Number

NEC: Necrotizing Enterocolitis

OR: Odds Ratio

RDS: Respiratory Distress Syndrome

ROC: Receiver Operating Curve

RVH: Royal Victoria Hospital

SGA: Small-for-gestational age

Chapter 1: Introduction

Although the occurrence of twin pregnancies in humans has been recognized throughout history, with the advent of infertility treatments and changing demographics over time, the importance of the study and characterization of twin pregnancies and multiple pregnancies in general, has increased.[1] After a decrease in the incidence of multiple pregnancies between the 1930s and the mid 1970s, the rate of multiple pregnancies has since been rising after the 1980s, in particular in Western Europe and North America.[2] For instance, in Canada, the number of live twin births increased by 40% between 1974 and 1990.[3] Factors such as delayed childbearing, increased use of ovulation induction, and other artificial reproductive technologies (ART) have been suggested as contributing to the observed increase in the incidence of twinning and multiple pregnancies in general.[2]

Twin pregnancies, like all multiple pregnancies, are known to be associated with a higher risk of adverse outcomes when compared to singletons. Twins have been shown to be born at lower gestational ages and have significantly higher rates of perinatal mortality and morbidity when compared to singletons.[4] For instance, by 1999, multiple births were found to represent 3% of all live births in the United States, up from 2% in 1980, and simultaneously represent 14% of all infant deaths, up from 11% in the earlier time period.[5] This is within the context of an overall decrease in infant mortality of 42% between 1980 and 1997, in the United States.[6] Moreover, the growth patterns of twin fetuses are different from that of singletons, characterized by a slow-down in fetal growth in the third trimester, which is in turn, related to factors such as limited uterine capacity or uteroplacental insufficiency.[7]

Fetal growth, in general, *in utero* is influenced by a number of physiological and pathological factors including their genetic growth potential, maternal characteristics and placental function.[8] Maternal factors such as smoking, advanced age, placental function, presence of disease, namely chronic hypertension, preeclampsia, eclampsia, HELLP syndrome, and diabetes are all known to be associated with intrauterine growth restriction

(IUGR), as are fetal characteristics such as the presence of chromosomal or congenital anomalies, fetal infections, and multifetal gestations.[9]

IUGR, specifically, when a fetus fails to reach its own growth potential during gestation, is associated with a higher risk of adverse perinatal outcomes. These include intrauterine or neonatal death,[8, 10] respiratory distress syndrome (RDS), chronic lung disease, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), low Apgar scores at birth, umbilical cord pH< 7.0, a higher likelihood of requiring resuscitation, hypoglycemia, hypocalcemia, polycythemia, compromised immune function and hepatocellular dysfunction.[8] Moreover, infants that experienced growth restriction *in utero* are also considered to be at risk of long-term effects on growth into young adulthood, neurologic outcome, metabolic disease,[11] and other developmental outcomes, although the etiology of this is unclear.[8]

Given the adverse perinatal and long-term outcomes associated with IUGR, there has been much research focusing on the earliest possible detection of deviations from an optimal growth trajectory, one that is associated with the least risk of adverse perinatal outcome. This has implications for the clinical management and monitoring of pregnancies, especially in higher-risk ones such as twin pregnancies. Over the years, a number of approaches and definitions have been considered to appropriately detect when a fetus experiences IUGR.

IUGR refers to a longitudinal process of fetal growth restriction, in which there is a downward deviation from a steady growth trajectory. However, historically, this term has often been used interchangeably with “small-for-gestational age” (SGA), which is an assignment based on a cross-sectional comparison to a distribution of fetal weight or birth weight at a given gestational age.[12] Increasingly, there is a recognition that SGA fetuses or infants are not a homogenous group, and include both those fetuses that are physiologically small but growing normally, as well as those fetuses that experience pathological growth restriction.[9]

Despite this caveat, however, there is value in comparing either the birth weight or fetal weight estimates calculated using *in utero* biometric measurements such as biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL),[13] with cross-sectional references.[9] A number of formulae have been developed and tested in different populations using combinations of these biometric measures in order to accurately estimate fetal weight. Fetal weight estimates or birth weight of the fetus or infant are then usually compared against population-based birth weight references,[14] or estimated fetal weight references based on one or multiple serial ultrasounds.[15-16]A fetus or infant is usually classified as SGA when it falls under the 10th percentile of weight for its gestational age, but other thresholds can be used.[8] Alternatively, customized fetal growth standards have been proposed, which are specific to maternal characteristics such as height, weight, parity and ethnicity.[17] The rationale behind customized growth standards is to distinguish between those fetuses that are physiologically small but attaining their growth potential from those fetuses that are pathologically growth restricted.[18] While customized percentiles for fetal growth have been shown to be superior to birth weight reference charts, there is evidence that their performance is not different relative to intrauterine ultrasound-based references.[18] In fact, their enhanced performance relative to birth weight references may arise from the fact that customized standards, in addition to adjusting for maternal characteristics, use an intrauterine growth standard to calculate an infant's "optimal weight".[18]

This may, in turn, be related to the fact that the use of birth weight references for each gestational age is known to be associated with bias, especially at lower gestational ages.[19] At early gestational ages, the number of fetuses remaining *in utero* will be much higher than those that are delivered. Therefore, substantial data are missing when references are constructed based on the birth weights of those infants that are born preterm.[19] Moreover, preterm delivery due to spontaneous labour, or medical intervention is presumably linked to poor fetal health or impaired fetal growth. This creates a selection bias whereby infants whose birth weights are used to construct fetal weight references are not a random sample of the entire cohort of fetuses at preterm gestational ages.[19] Using growth references constructed from the birth weights of these infants, who are probably less healthy than their counterparts that remain *in utero*, is thus inappropriate for the purposes of comparison.[19]

Given these findings, and in the context of routine ultrasound monitoring of pregnancies, there has been a general recognition of the value of fetal weight references, constructed from *in utero* estimations of fetal weight. However, while there have been a number of fetal weight references constructed for singletons,[20-23] and much research conducted in charting the growth trajectory of individual biometric measurements in twins,[24-27] there has not been as much focus on creating ultrasound-based fetal weight references for twins. This is the case despite the recognition, as early as 1978, that comparing the fetal size of twins to references constructed from the fetal weight of singletons is inappropriate.[28] Overall, twins are smaller than singletons over the course of pregnancy, with significant differences in fetal growth patterns manifesting after approximately week 30 of gestation.[29] Moreover, there is evidence that clinically optimal birth weights for twins associated with a minimal risk of adverse perinatal outcomes may be lower than those for singletons.[30] Therefore, there is a need for ultrasound-based fetal weight references specific to twins that can be used for comparison and monitoring of fetal weight over the course of gestation.

Chapter 2: Study Objectives

2.1 Primary Objective

The primary objective of this study was to construct fetal weight references for twins based on *in utero* measurements from the second and third trimester, for each completed week of gestation between weeks 22 and 37. Separate fetal weight references were constructed by sex of the fetus and based on the placentation (chorionicity) of the pregnancy, both of which are known to affect fetal weight over the course of pregnancy.

2.2 Secondary Objective

In order to inform the choice of formula to estimate fetal weight in the construction of fetal weight references for twins, the accuracy and precision of five commonly used formulae were assessed in a sub-sample of this hospital-based twin population. Specifically, the performance of two Hadlock formulae, the Shepard formula, the Ong formula, and the Combs formula were assessed for their accuracy and precision.

Chapter 3: Background and Review of Literature

3.1 The Epidemiology of Twinning

3.1.1 Twinning

Although the occurrence of twinning in humans has been recognized throughout history, with the recent advent of biotechnology and with changing demographic trends, the study of twin pregnancies has greatly expanded.[1] Blickstein proposes a definition of twin pregnancies as the "result of intracorporeal development of more than one zygote and/or the intracorporeal development of a split zygote, which was produced in the same or in a different ovulatory cycle."[1] This is a broad enough definition to include those pregnancies that begin as multiple pregnancies but result in the birth of only one live fetus due to spontaneous or iatrogenic fetal reduction; those twin pregnancies resulting from ART, and surrogate pregnancies.[1]

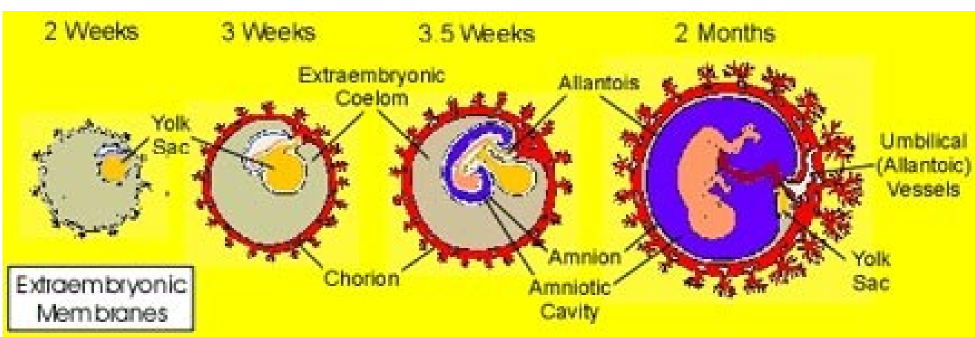
In humans, twinning can occur either by the splitting of one fertilized ovum or from two fertilized ova. Monozygous twins are the result of the splitting of one embryo into two genetically identical halves, usually between fertilization and around day eight after conception, while dizygotic twins result from the separate fertilization of two ova by two different sperms.[31] Monozygotic twins are of the same sex, while dizygotic twins may be of like or unlike sex.[32]

A short aside on embryonic development is useful to understanding placentation in twins. After fertilization, the embryo undergoes rapid cell division until it is made up of a mass of cells, which, by the sixth day, forms a hollow fluid-filled cavity called the blastocyst. Around the seventh day, the blastocyst implants in the uterus.[33] A mass of cells in the blastocyst known as the trophoblasts are involved in implantation of the blastocyst into the uterus, and in subsequent placentation.[34] These cells form part of the chorion, a membrane that surrounds the fetus, and that forms the fetal contribution to the placenta. Interior to this, in the second week of the developmental process, the amniotic membrane forms, which directly surrounds and protects the fetus, bathing it in amniotic fluid.[35]

Figure 1 is an illustration of the general arrangement of fetal membranes and the placenta.

Dizygotic twins have separate placentas, and separate placental membranes, including the chorion and amnion. Monozygotic twins, on the other hand, may have different arrangements of placental membranes, with separate membranes for each fetus, namely, dichorionic diamniotic, or common membranes surrounding both fetuses, namely monochorionic diamniotic (one chorion but two amnions for each fetus), or monochorionic monoamniotic (only one chorion and amnion surrounding both fetuses).[33] Monochorionic twins share one placenta, while each dichorionic twin usually has its own placenta.[33] In monozygotic twins with separate membranes and placentas (dichorionic diamniotic), splitting of the zygote is thought to occur 0-3 days after fertilization, while monochrionic diamniotic twins are thought to result from splitting of the zygote between day 4 and 7, and monochorionic monoamniotic twins thought to arise from splitting of the zygote between days 6 and 8.[32]

Figure 1 : An Illustration of the Chorionic and Amniotic Membranes[36]



The prevalence of dizygotic twinning is reported to be 1 in 100 live births in North America and Britain, with the risk of dizygotic twinning thought to vary by race, use of artificial reproductive technologies, season, parity, and levels of the follicle stimulating hormone.[32]

Monozygotic twinning has been found to have a prevalence of 1 in 330 live births,[32] with 20-30% of monozygous twins being dichorionic diamniotic, 60-70% being monochorionic diamniotic, and a rare 1% being monochorionic monoamniotic and facing the highest risk of adverse perinatal outcomes.[37]

3.1.2 The Global Prevalence of Twinning and Trends over Time

The incidence of twin births has been on the rise globally since the mid-1970s, related to changes in biotechnology such as the increasing use of ART over time, and demographic changes, primarily delayed childbearing.[38] Prior to this, and related to a decrease in average maternal age, the incidence of twinning had been observed to fall between 1930 and the mid 1970s.[2] The United Kingdom reported incidence rates of between 9.9 and 12.3 twin births per 1000 pregnancies between 1971-1975, with an increase to between 14.6 and 15.6 twin births per 1000 pregnancies in 2001-2002.[39] In 2001-2002, other Western European countries reported similar incidences, with the lowest reported incidence of 10.55 twin births per 1000 live births from Luxembourg and the highest incidence of 20.05 twin births per 1000 pregnancies reported from Greece.[39] Nordic countries reported somewhat higher incidence of twin births, ranging from 15.1 per 1000 live births in Finland in 2002 to 22.1 per 1000 live births in Denmark in 2001. These countries have also experienced increases in the incidence of twin births since the 1980s.[39] Between the mid 1970s and 1996, the incidence of twinning increased in Australia by 30%, from a reported rate of 5.8 per 1000 live births in 1974 to 8.9 per 1000 live births in 1996.[40]

Japan, Hong Kong and Singapore have all demonstrated increases since the 1970s, although they report lower incidence of twinning compared to European countries, with 9.9 twin births per 1000 pregnancies, 8.7 twin births per 1000 pregnancies and 9.7 per 1000 pregnancies, respectively, in the 1990s and 2000s.[39, 41]Figure 2 shows the trend in incidence of twinning per1000 live births for ten developed countries, including Canada, between 1972 and 1996.

Figure 2: Trends in Incidence of Twinning for Developed Countries (1972-1996)[42]

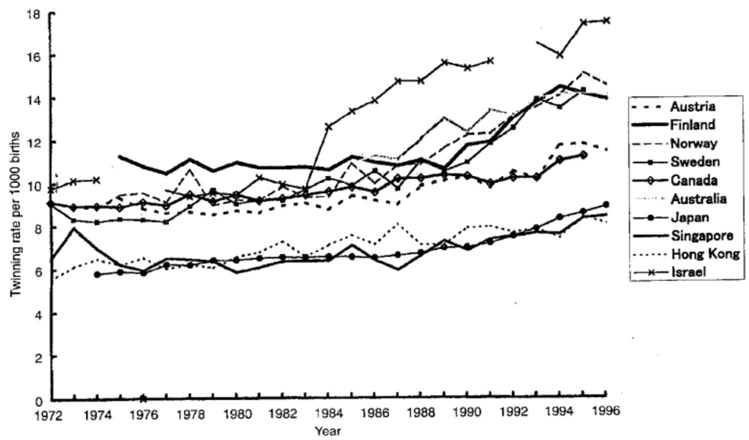


Fig. 1 - Secular changes in twinning rates in ten countries during the period from 1972 to 1996

In South Asia, the incidence of twin births was reported to be 8.7 per 1000 births (including live and still births) for the period between 1969 and 1975 in South India.[43 44] Researchers in the North-west of India reported higher twinning incidence rates of 10.70-19.2 per 1000 deliveries for the period between 1987 and 1993,[45] while researchers based in Bangladesh reported twinning incidence between 7.9 and 11 per 1000 deliveries between 1979 and 1983. Finally, a study from Nepal reported a twinning incidence of 16.1 per 1000 deliveries between 1992 and 1996.[46]

Countries in Africa have consistently reported a higher incidence of twinning compared with Western countries. Specifically, Nigeria has been reported to have the highest incidence of twinning in the world. For instance, as early as 1979, the twinning incidence in Nigeria was found to be 45-50 twin births per 1000 deliveries, four times that found in Europe and the United States.[47] However, two recent studies have found the incidence of twinning to be 29.5 per 1000 deliveries in Nigeria between 1998 and 2004,[48] and 36 per 1000 deliveries in 2005.[49] Factors such as diet, environment, and levels of the follicle stimulating hormone in Nigerian women have been proposed as being associated with this higher prevalence of twinning.[47]

In North America, as early as 1935, Hamlett reported that the prevalence of twinning was 1 in 88.2 pregnancies in the white population of the United States.[50] Most recently, the prevalence of twin births in the United States has been reported to be around 32 per 1000 live births.[51] Finally, in a study conducted for Statistics Canada, researchers found a 35% increase in the incidence of twin pregnancies between 1974 and 1990, and an increase of 40% in live twin births, with a reported twin birth rate of around 10 per 1000 live births in 1990.[3]

3.1.3 Correlates of Twinning

A number of factors have been proposed as being associated with the observed temporal trends in twinning. Increased maternal age,[38, 52] and use of ART have been associated with a general increase in the incidence of twinning over time. Specifically, older age at conception is associated with a higher risk of dizygotic twinning, which results from double ovulation. This, in turn, may be linked to age-related hormonal changes and the subsequent growth of multiple follicles, the precursor to oocytes, in any given menstrual cycle.[53] Moreover, ovulation induction and ART such as in vitro fertilization, and their increased use over the years have been shown to be associated with a large increase in the rates of dizygotic twinning specifically, and multiple pregnancies, in general, at a population level over the years.[54] Other factors proposed to be associated with an increase in the frequency of dizygotic twinning are increased folic acid intake, peri-conceptional multivitamin intake, diet and environmental factors.[40, 47, 55]

Additionally, parity,[38, 46] race of the mother, and diet[55] have also been associated with a higher risk of twinning in general.

Consistent with the fact that the above-mentioned factors are all related to the risk of dizygotic twinning and have changed over time, the incidence of monozygotic twinning appears to have remained unchanged over the same time period that the overall twinning incidence has increased.[42]

3.1.4 Outcomes Associated with Twinning

Twin pregnancies are known to be associated with higher perinatal morbidity and mortality when compared to singletons,[38, 56-59] although there is a general acknowledgement that this may be linked with conditions that specifically affect twin pregnancies, rather than inherent to being a twin.[60-62]

For instance, twins tend to be born at earlier gestational ages than singletons, with a mean age at delivery of 35 weeks compared with 39 weeks in singletons, some proportion of which can be attributed to medical intervention.[29, 58, 63-66] As is the case in singletons, delivery at earlier gestational ages is associated with higher rates of perinatal morbidity in twins as well.[67]

Related to their tendency to be born earlier than singletons is the fact they are generally of lower birth weight, a known predictor of adverse perinatal outcome.[63] Twins are also known to be at higher risk for intrauterine growth restriction,[59] and are vulnerable to complications unique to them such as growth discordance,[59] and twin-to-twin transfusion syndrome.

Growth Discordance

Some researchers consider small differences or discordance in the fetal growth of twins over the course of pregnancy to be an adaptive mechanism that compensates for the mother's uterine capacity and promotes gestational age, allowing the mother to carry the fetuses for longer.[68-70] However, it is well recognized that when the fetal growth trajectory of each twin from the same pair begins to diverge severely, a pathological condition arises. Factors associated with growth discordance between twins include maternal disease (hypertension, pre-eclampsia, eclampsia),[71-72] the presence of congenital anomalies,[73] monochorionicity,[74] and discordance in placental territory between twins,[75-79] while there is some conflicting evidence on the role played by conception by ART,[80-83] and reduction of multi-fetal pregnancies.[80-81, 84-86]

Increasing levels of birth weight discordance, generally quantified as the percentage difference between birth weights of twins from the same pair, are associated with higher rates of perinatal mortality, preterm birth, lower APGAR scores, lower cord pH at birth, more frequent delivery by cesarean section, a higher risk of admission to intensive care, longer hospital stays, higher incidence of RDS, and NEC.[73, 81, 87-96] Increasingly, there is a recognition that in the case of growth discordance, the smaller twin or the twin that is SGA is at increased risk,[72, 94, 96-97] a factor compounded by the fact that the risk of at least one twin being SGA is higher in growth discordant pairs compared with concordant pairs.[71, 73, 92, 96]

Twin-to-Twin Transfusion Syndrome

Twin-to-twin transfusion syndrome (TTTS) is a condition that develops predominantly in monochorionic twins. The formation of anastomoses between the arteries and veins of co-twins, as well as an imbalance in the blood pressure of either of the twins, can lead to a transfusion of blood from the donor twin to the recipient twin.[39] A more severe form of this is a condition called twin reversed arterial perfusion (TRAP).[39] As the severity of TTTS increases, fetal growth is affected, and fetal demise of the donor twin is a possibility, with the surviving co-twin then also at higher risk for adverse outcomes, or death.[39]

Perinatal Outcomes Associated with Zygosity and Chorionicity

The association between zygosity and the risk of adverse perinatal outcome is still somewhat controversial, with a growing tendency for the chorionicity of twin pregnancies to be considered a more accurate predictor of adverse perinatal and neonatal outcome than zygosity.[98-101] Consistently, monochorionic twins have been shown to be at higher risk for adverse outcomes such as perinatal mortality, IUGR, and preterm birth than dichorionic twins.[102-106]

When twins are dizygous, due to their differing genetic makeup, they may experience chromosomal abnormalities discordantly, with the possibility of one twin from the pair remaining unaffected.[59] However, the presence of a fetal anomaly in at least one twin has been shown to have an influence on gestational age at delivery, on the mean birth weight of twins from the affected pregnancy, and on the risk of perinatal mortality.[107-112] Additionally, fetal death of one twin, especially after the first trimester, has been shown to be associated with IUGR, preterm birth, and higher perinatal mortality for the other twin.[45, 59]

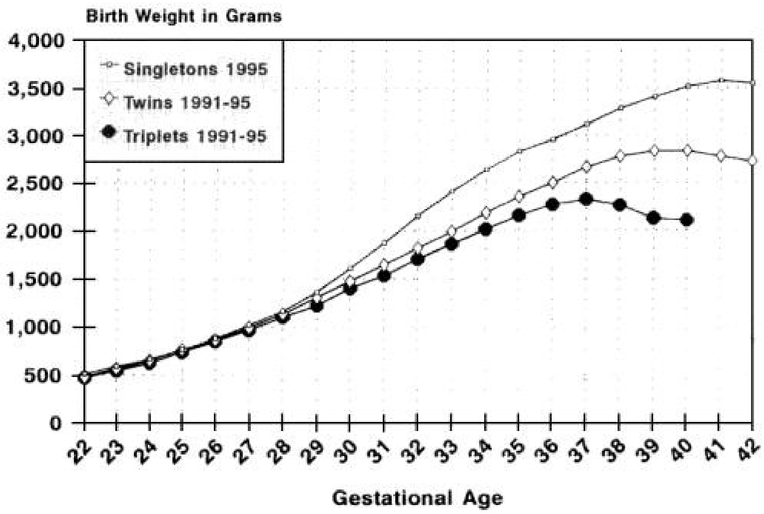
Outcomes in Twins Conceived by ART

While some studies report no differences in outcomes between twins that are spontaneously conceived and those that are conceived by ART,[113-114] or even a lower risk of adverse outcomes in those conceived using ART,[115] some report a higher frequency of premature deliveries, lower birth weights and a higher risk of IUGR.[116-119] Specifically, twins conceived by intracytoplasmic sperm injection appear to fare worse, with a higher risk of stillbirth relative to those conceived by in vitro fertilization.[38] Moreover, when twins conceived by ART undergo fetal reduction from higher-order pregnancies to twins, they appear to be at higher risk for preterm birth and lower birth weights compared with non-reduced or spontaneously conceived twins.[120-123]

3.2 Fetal Growth Patterns in Twins

In addition to experiencing a higher risk of adverse perinatal outcomes than singletons, the *in utero* growth of twins has been shown to be different from that of singletons. For instance, Alexander et al., in a population-based study in the United States, reported that while fetal growth was similar for singletons and twins at early gestational ages, by 32 weeks, there was a 300g difference in the median birth weight between twins and singletons; this difference widened till it reached 500g by week 38. Moreover, the median birth weight of twins was highest at 39 weeks of gestation compared with singletons, whose median birth weight peaked at 41 weeks.[29]

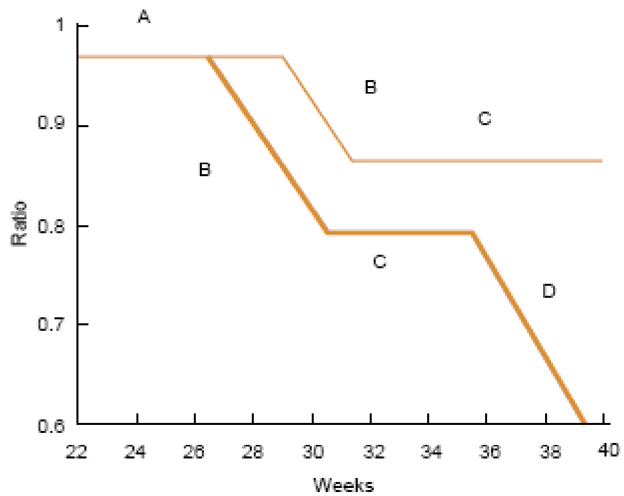
Figure 3: Median Birth Weights for Singletons, Twins, and Triplets (United States) [29]



These findings were in agreement with those from other research groups, who reported similar fetal growth patterns till the late second trimester,[124] with divergent growth between twins and singletons setting in around weeks 30-33.[27, 58, 125-127] One research group has reported significant differences between the size of twins and singletons only at around 35-36 weeks of gestation.[128-129] This difference persists until birth, with twins being classified SGA more often than singletons when compared to singleton-based weight references.[130]

Blickstein explained this divergence in fetal growth as being physiological, and related to uterine capacity.[7, 97, 131] With increasing fetal demands, he reasoned, the growth rate in multiple pregnancies tends to slow down earlier than in singletons. He described fetal growth in twins as being of three phases. Phase A, up to 28-30 weeks is one in which twins grow at the same rate as singletons, and the uterus is able to adequately contain their growth. Until this time, although, if born, they will be of low birth weight (LBW), twins are not considered to be restricted in their growth. After this, phase B is one in which growth slows in twins with as much as a 15-20% difference in the birth weights of twins and singletons. Finally, phase C is characterized by a period of maintenance of this difference in weights between twins and singletons. Phases A and C are considered to be phases where fetal maturity is promoted, and size is maintained, while phase B is thought to promote maturity at the cost of fetal size.[7, 97, 131] Twin-to-singleton fetal weight ratios over the course of pregnancy are illustrated in Figure 4.

Figure 4: Phases of Fetal Growth in Triplets and Twins Relative to Singletons[97]
(Thick line – Triplet to Singleton Birth Weight Ratio.
Thin line – Twin to Singleton Birth Weight Ratio.)



3.3 Intrauterine Growth Restriction (IUGR)

3.3.1 Etiology

Fetal growth, the change in size and weight of the fetus over the course of pregnancy, is largely influenced by the nutrients and uterine space available to the fetus.[132] In this, the placenta plays a pivotal role, mediating the transfer of nutrients, metabolites, and oxygen from the mother to the fetus.[132] Thus, IUGR of the fetus is a manifestation of numerous placental, maternal and fetal factors and not a disease in and of itself.

The placenta acts as an interface for maternal and fetal oxygen and nutrient exchange[133] The size of the placenta, specifically the placental surface area available, is thought to influence fetal growth. Of importance are the placental disk shape, the location of insertion of the umbilical cord relative to the edge of the placental disk, placental disk diameter, placental thickness and weight.[133]

Maternal factors that may lead to compromised placental function, such as reduced uteroplacental blood flow, reduced blood volume, or reduced oxygen-carrying capacity have all been linked with IUGR.[134] Conditions that are associated IUGR include living at high altitudes, maternal disorders such as pre-eclampsia, chronic hypertension with or without superimposed pre-eclampsia, autoimmune disorders, chronic severe nephropathy, and pregestational diabetes complicated by vasculopathy.[135] Other maternal behavioural and environmental factors that are associated with a higher risk of IUGR include cigarette smoking, alcohol consumption, cocaine use, use of anticonvulsants, and chemotherapeutic drugs.[136] Moreover, severe malnutrition and some environmental pollutants have been implicated as risk factors for IUGR.[136]

Fetal factors that influence fetal growth include the presence of genetic and structural anomalies, presence of umbilical cord abnormalities, infection with cytomegalovirus, rubella, malaria or toxoplasmosis, and multiple gestation.[134] With respect to multiple gestations, overall reduced substrate availability can lead to IUGR in both fetuses, while unequal proportions of placental mass per fetus, or in extreme cases, occurrence of TTTS or TRAP may be responsible for the selective growth restriction of one of the twins.[10]

3.3.2 Perinatal and Long-Term Outcomes Associated with IUGR

Although IUGR may be reflective of upstream factors, regardless of its etiology, it is associated with a number of perinatal and longer-term adverse outcomes. For instance, an IUGR fetus is at higher risk for fetal death and neonatal mortality,[137] a risk directly proportional to the severity of IUGR, in both term and preterm fetuses.[135] The incidence of perinatal morbidity is also higher in IUGR fetuses, with respiratory, cardiac, neurological, gastrointestinal, renal, immune and metabolic consequences.[138] Specifically, IUGR infants are at higher risk for RDS, perinatal asphyxia, neonatal encephalopathy and seizures, NEC, hypothermia, hypoglycaemia, polycythemia, and culture-proven sepsis.[138]

Long-term consequences have been reported with regard to growth, academic achievement, and neurodevelopment although the evidence on this is not definitive.[138]

Moreover, research by Barker and colleagues suggests that IUGR occurring during so called "critical" periods in fetal growth and development may place adults at later risk for diseases such as coronary heart disease, high blood pressure, insulin resistance and diabetes.[11]

Thus, the assessment of fetal growth and detection of a downward trend in a fetus' growth trajectory are important in the clinical monitoring and management of pregnancies.

3.3.3 Cross-sectional and Longitudinal Assessment of Fetal Growth

Historically, birth weight, rather than gestational age, has been used as a proxy for maturity of the fetus, due to the relative unreliability of the latter.[139] A fetus with birth weight of less than 2500 g is generally considered to be of LBW. While this classification is associated with a higher risk of perinatal mortality and morbidity, it does not take into account the gestational age at birth.[140] Thus, very early on, there was a shift towards assessing size given gestational age, with the 10^{th} percentile of the weight distribution at each gestational age considered indicative of a fetus being SGA, although other thresholds have been suggested as well.[141]

Although this is still arguably the most common method used to detect IUGR, and SGA is known to be a predictor of adverse perinatal outcome, it is not reflective of longitudinal growth in individual fetuses. Comparing a particular fetus to weight distributions at a given gestational age is only a comparison of cross-sectional size, and is thus not an effective manner to assess growth in a particular fetus.[12] Ideally, fetal growth would be assessed longitudinally, and by distinguishing fetuses that are physiologically small but growing normally from those that experience pathological IUGR.

However, for the purposes of comparison in research and clinical settings, a number of birth weight and fetal weight references have been elucidated over the years.

3. 4 Birth Weight References

In 1963, one of the first studies on the distribution of birth weights at each gestational age was published by Lubchenco et al. for 5,635 live-born Caucasian and Hispanic infants. The authors presented the 10^{th}, 50^{th} and 90^{th} percentiles of birth weight for each week of gestation.[142] Even in this early study, researchers recognised the deviation in fetal growth of twins relative to singletons by the 35^{th} week of gestation, with the median weight of twins matching the 10^{th} percentile of the singleton weight distribution at this time. While of considerable value to perinatal literature, the fact that this reference was constructed from data based on infants born at a higher altitude, which is known to affect infants' birth weight, means that it may not be applicable to other geographic areas.[142]

Since then, a considerable number of birth weight references and standards have been published on singletons. These tend to be population-based,[29, 143-146] and have adjusted for fetal sex,[143-150] race,[143, 145, 150] parity,[143-145] and even maternal age[150] and size.[143]

While there has been less research conducted on twins or higher order multiples, there has been an increasing recognition for the need for separate references for this group.[30] Comparisons of "optimal" birth weights that are associated with the lowest risk of adverse perinatal outcome, have found that twins fare better at lower birth weights compared to singletons at the same gestational age.[30]

3.4.1 Birth Weight References in Twins

In one of the earliest studies conducted on twins, Naeye et al. constructed a birth weight reference for all twins delivered between 1957 and 1963 in a New York and a Boston Hospital. Infants with congenital anomalies and those with hemolytic disease of the newborn were excluded from the study.[127] They found a difference between birth weights of twins and singletons as early as 33 weeks, when they compared their findings with those of Lubchenco et al.[142]

In 1982, Leroy and colleagues charted the height, weight, cranial circumference and thoracic circumference of 1,049 twin infants born at gestational ages between 28 and 42 weeks.[151] Again, weights of twins were found to be less than singletons as early as week 30 of gestation, with a difference at the 50th centile of up to 610 g between twins and singletons at week 40.[151] Both research groups found that dichorionic twins were heavier than monochorionic twins throughout pregnancy.[127, 151]

Arbuckle and colleagues conducted two studies in Canada examining birth weight distributions by gestational age in singletons and in twins.[149, 152] The first compared the distributions in 1972 and 1986.[152] Overall, the mean birth weight was found to increase by 5.7% between 1972 and 1986, with 33.8% of babies born preterm in 1972 and 41% born preterm in 1986. As expected, birth weights at each gestational age were consistently lower for twins compared to singletons, and lower for females compared to males.[152] In a follow-up study covering the years between 1986 and 1988, the previous findings were corroborated.[149] Moreover, a bimodal peak at 30 weeks and 42 weeks in birth weight observed in the 1989 study was observed again in the 1993 study. These peaks were thought to be due to errors in dating of pregnancies by last normal menstrual period (LNMP).[149]

Alexander et al. constructed birth weight references for singletons, twins, and triplets using United States Natality data files for the years between 1991 and 1995.[29] In the 463,856 twins included in the study, they found a difference in median birth weights of 300g between twins and singletons by 32 weeks of gestation, a difference that increased to 500g by term. Moreover, similar to Lubchenco's findings, by 38 weeks of gestation, the 50th percentile of twin birth weights was similar to the 10th percentile of singleton birth weights. Both race and fetal sex were found to influence weight at each gestational age, with African-American mothers delivering lighter babies than Caucasians, and male infants heavier than female infants at every gestational age.[29]

In 1998, Ananth et al. constructed birth weight references for live-born twins stratified by placental chorionicity, a known predictor of birth weight in twin gestations.[153] As expected, dichorionic twins were heavier than monochorionic twins between 30 and 40 weeks of gestation with respect to the 10th, 50th and 90th percentiles. Results from this study agreed well with those of Naeye et al.[153] Reflective of the change in practice over time, and in contrast with previous studies where dating was done by LNMP only, pregnancies were dated in this study by LNMP and confirmed by ultrasound.[153] Unlike other groups, in this study, Ananth et al. accounted for the correlation between twin fetuses in each pregnancy.

3.4.2 Limitations of Birth Weight References

The historical use of birth weight as an indicator of adverse outcome included its use as an implicit indicator of maturity.[154] However, the use of birth weight as a marker of perinatal risk does not consider the contribution of gestational length to fetal development. This recognition has led to the development of birth weight references adjusted for gestational age.

While they are the most commonly used references to compare fetal or infant weight, birth weight references are associated with bias. Hutcheon and Platt illustrated this in a recent paper by showing that, at lower gestational ages, the 10th percentile of birth weight references only captured the smallest 1% of the total population of fetuses *in utero*.[19] Hutcheon and Platt reasoned that, at any given gestational age, all those fetuses that remain *in utero* are not considered in the creation of birth weight references.[19] At preterm gestational ages, the percentage of fetuses remaining *in utero* will be much higher than those who are born, with birth weight references then based on the weights of a small (and selected) portion of the original cohort of conceptions, thus creating a situation of missing data.[19] The bias that is created by this depends on the nature of the missing data. At preterm ages, birth weights of infants are not a random sample of all fetal weights for the same gestational age.[19]

In fact, a number of other research groups have also found that, at lower gestational ages, *in utero* estimates of fetal weight are higher than birth weights.[155-158] Moreover, fetal growth patterns are different in preterm infants, with IUGR occurring more frequently in preterm infants than in infants born at term.[158-161] Among those fetuses that go on to be born preterm, evidence of IUGR appears as early as week 32.[161] This association is marked in medically induced preterm births with a reported OR of 6.4 (95% CI: 5.53, 7.43),[159] which is reasonable given that IUGR is one of the most common indications for preterm delivery.[162] However, even in the case of spontaneous preterm delivery, this association, although milder (OR 1.61, 95% CI: 1.43,1.82), persists.[159] Using birth weight for gestational age references derived from this population for comparison purposes is likely to result in an under-estimation of the number of fetuses that are, in fact, SGA.

Within this context, and with a shift towards the routine use of ultrasound in the clinical management of pregnancies, it is relevant to examine evidence on the use of ultrasound to monitor fetal growth.

3. 5 Use of ultrasound to monitor fetal growth

Over the years, ultrasound has begun to play an ever-increasing role in the clinical management of pregnancies, especially in the care of twins and higher-order multiple pregnancies. Ultrasound is now used in the early detection of twin pregnancies, congenital abnormalities, assessment of chorionicity, fetal demise, fetal malpresentation, TTTS, and in guiding more invasive treatments required in the case of complications.[163-164] The monitoring of fetal growth, including assessment of fetal size and estimation of gestational length, is arguably among the most routine uses of ultrasound.

3.5.1 Estimation of Gestational Age

An important component in the monitoring of fetal growth is the assessment of gestational age of the fetus or infant. The use of ultrasound in the estimation of gestational age has gained increasing acceptance over time against the backdrop of errors associated with the use of LNMP as the means of estimating gestational age.[165] Inaccurate recall of the date of LNMP, early bleeding in pregnancy mistaken for menstrual bleeding, and irregular or delayed menstruation all lead to bias in the estimation of gestational age using LNMP.[165-166] The use of LNMP has been found to over-estimate gestational age (and, hence, under-estimate rates of preterm birth) relative to ultrasound estimates of gestational age.[167] This may be due to the fact that, in general, ovulation tends to occur later than the traditionally assumed day 14 in a normal menstrual cycle, more often than it occurs earlier than day 14.[167]

Much of the evidence shows that ultrasound-based estimation of gestational age is more accurate than LNMP-based estimates of gestational age in predicting the actual day of delivery, and when compared in pregnancies conceived by ART where the actual date of conception is known,[168-169] with narrower margins of error.[168-169] However, there is evidence that a majority of LNMP estimates and ultrasounds estimates (~80%) agree within 10 days of their estimate of gestational age.[170]

The concern with ultrasound-based estimates relates to the estimation of gestational age by comparing biometric measurements such as CRL and BPD to published reference values; errors in dating arise when true early variations in fetal size are interpreted as differences in gestational age.[170] Also, ultrasound-based estimates of gestational age have been shown to be lower than those of LNMP, and thus to over-estimate the rate of preterm births.[170]

3.5.2 Estimation of Fetal Weight

Formulae Used to Estimate Fetal Weight

Over the years, a number of research groups have developed methods to estimate fetal weight using biometric measurements of fetuses obtained by ultrasound. Table 1 lists some of the formulae that have been developed over the years to estimate fetal weight. This is not a complete list, but includes the major formulae that have been researched over the years. In contrast, to date, only one research group has developed a formula in a twin population, which uses AC and FL to estimate fetal weight. This is listed in Table 2.

Table 1: Formulae Developed in Singletons (or unspecified populations) to Estimate Fetal Weight

Biometric measures used	Authors	Formula
AC	Campbell and Wilkin. 1975[171]	$Ln (EFW)=-4.564+0.0282(AC)-0.00331(AC)^2$
	Higginbottom et al. 1975[172]	$EFW=0.0816(AC)^3$
	Warsof et al. 1977[173]	$Log(EFW)=-1.8367+0.092(AC)-0.000019(AC)^3$
	Hadlock et al. 1984[174]	$Ln(EFW)2.695+0.253(AC)-0.00275(AC)^2$
	Jordaan. 1983[175]	$Log(EFW)0.6328+0.1881(AC)-0.0043(AC)^2+0.000036239(AC)^3$
FL	Warsof et al. 1986[176]	$Ln(EFW)=4.6914+0.00151(FL)^2 – 0.0000119(FL)^3$
AC, FL	Hadlock et al. 1985[177]	$Log (EFW)=1.304+0.05281(AC)+0.1938 (FL)-0.004 (AC)(FL)$
	Woo et al. 1986[178]	$Log (EFW)=0.59+0.08(AC)+0.28(FL)-0.00716(AC)(FL)$
	Warsof et al. 1986[176]	$Ln (EFW)=2.792+0.108(FL)+0.0036(AC)^2 – 0.0027(FL)(AC)$
	Roberts et al. 1985[179]	$Log (EFW)=1.7942-0.27244(FL)-0.00052(AC)^2-0.015491(FL)^2$

Table 1: Formulae Developed in Singletons (or unspecified populations) to Estimate Fetal Weight (Contd.)

Biometric Measures Used	Authors	Formula
AC, BPD	Warsof et al. 1977[173]	Log (EFW)=-1.599+0.144 (BPD)+0.032(AC)-0.000111(BPD)^2 (AC)
	Shepard et al. 1982[180]	Log (EFW)= -1.7492+0.166(BPD)+0.046(AC)-0.002546(AC)(BPD)
	Jordaan. 1983[175]	Log (EFW)=-1.1683+0.0377(AC)+0.0950(BPD)-0.0015(BPD)(AC)
	Hadlock et al. 1984[174]	Log (EFW)=1.1134+0.05845(AC)-0.000604(AC)^2-0.007365(BPD)^2 + 0.000595(BPD)(AC)+0.1694(BPD)
	Woo et al.1986[178]	Log (EFW)=1.63+0.16 (BPD)+0.00111(AC)^2 – 0.0000859(BPD)(AC)^2
	Secher et al. 1987[181]	EFW=0.0108(AD)$^{1.72}$ x (BPD)$^{0.99}$
HC, AC	Jordaan. 1983[175]	Log (EFW) = 0.9119 + 0.0488(HC) + 0.0824(AC) -0.001599(HC)(AC)
	Hadlock et al. 1984[174]	Log(EFW) = 1.182 + 0.0273(HC) + 0.07057(AC) -0.00063(AC)^2 - 0.0002184(HC)(AC)
	Roberts et al. 1985[179]	Log (EFW)=1.5050-0.02660(AC0-0.03514(HC)
AC, BPD, FL	Hadlock et al. 1985[182]	Log(EFW) = 1.335 - 0.0034(AC)(FL) +0.0316(BPD) +0.0457(AC) + 0.1623(FL)
	Woo et al. 1986[178]	Log(EFW) = 1.54 + 0.15(BPD) + 0.00111(AC)^2 - 0.0000764 (BPD)(AC)^2 + 0.05(FL) - 0.000992(FL)(AC)
	Roberts et al. 1985[179]	Log (EFW)=1.7918-0.24557(FL)-1.66999(BPD)^2 – 0.00058(HC)(AC)-0.00319(AC)(FL)
	Siemer et al. 2009[183]	EFW=-5948.336+2101.261(ln(AC))+15.613 (FL)^2 +0.577(BPD)^3
	Rose and McCallum. 1987[184]	Ln (EFW)=0.143(BPD+AD+FL) + 4.198
AC, HC, FL	Hadlock et al. 1985[182]	Log(EFW) = 1.5662-0.0108 (HC) +0.0468 (AC)+0.171 (FL)+0.00034 (HC)^2-(0.00386 (AC)(FL)
	Combs et al. 1993[185]	EFW = 0.23718(AC)^2 (FL) +0.03312(HC)^3
HC, AC, FL, BPD	Jordaan et al.1983[175]	Log(EFW) = 2.3231 +0.02904(AC) + 0.0079(HC) -0.0058(BPD)

	Hadlock et al. 1985[182]	Log(EFW) = 1.3596 + 0.0064(HC) +0.0424(AC) + 0.174(FL) +0.00061(BPD)(AC) -0.00386(AC)(FL)
	Roberts et al. 1985[179]	Log(EFW)=1.6758-0.01707(AC)-0.42478(BPD)-0.05216(FL)-0.01604(HC)

*EFW: Estimated Fetal Weight; HC: Head Circumference; AC: Abdominal Circumference; FL: Femur Length; BPD: Biparietal Diameter

Table 2: Formula Developed in Twins to Estimate Fetal Weight

Authors	Formula
Ong et al. 1999[186]	Log(EFW)= 0.0259(AC)+0.6720(FL)-0.0475(FL)^2-2.7606

*BW: Birth Weight; AC: Abdominal Circumferene; FL: Femur Length

3.5.3 Accuracy of Estimation of Fetal Weight

Ultrasound-Based Estimation of Fetal Weight Compared to Clinical Methods

Several research groups have found ultrasound estimations of fetal weight to be superior to clinical methods, such as abdominal palpation or maternal estimation of fetal weight, especially in cases of maternal obesity.[187-189] In contrast, other researchers have reported no difference in the ability of clinical methods and ultrasound to estimate fetal weight, although these studies have been limited by small sample sizes and the exclusion of preterm births in the studied sample.[190-194]

While it would be reasonable to assume that studies conducted in later years would reveal higher accuracy in estimated fetal weight (EFW) compared to clinical estimates, as ultrasound technology has improved over the years, surprisingly, this has not been the case. Studies that have investigated the influence of gestational age at birth have found that ultrasound is superior to clinical estimates of fetal weight in the case of preterm births (<37 weeks), but are comparable after this time.[195-196] This finding was validated by a randomized clinical trial that included 758 term singletons, and found that, after the age of 37 weeks, clinical estimates of fetal weight were more likely to be within 10% of birth weight than ultrasound estimates (58% vs 32%, $p<0.0001$). Moreover, this study found that both methods were equally accurate in detecting birth weight ≤ 2500 g or ≥

4000 g,[197] in contrast with another study that reported ultrasound estimates to be significantly more accurate than clinical estimates when birth weight was less than 2500 g.[194]

Estimation of Fetal Weight in Singletons

In singletons, despite their generally high accuracy in predicting birth weight, there has been a tendency for most formulae to perform poorly at the extremes of birth weight. For instance, there have been reports of systematic under-estimation of fetal weight at lower birth weights, by the Hadlock formulae, the Warsof formulae, the Rose and McCallum formula, the Woo formula, the Campbell and Wilkin formula, and others.[35, 198-200] Among all the formulae, the Hadlock formulae have been shown to have the smallest systematic error in singletons weighing <2500g,[183, 201] although one study reported the Schild formula as having 100% sensitivity and 99.4% specificity in detecting singleton infants that were SGA at birth.[202] Overall, there is a general tendency in clinical practice towards using the Hadlock formulae to estimate fetal weight across ethnicities and birth weight ranges.[200, 203]

Estimation of Fetal Weight in Twins

It appears that the only formula developed in a twin population, so far, is the Ong formula that incorporates AC and FL to estimate fetal weight.[186] When compared with the Hadlock (HC, AC, FL),[182] Shepard (AC, BPD)[180] and Campbell formulae (AC),[171] the formula developed by Ong et al. performed the best, with 71.4% of EFWs by the Ong formula within 10% of birth weight, compared to 57.6% of EFWs for the Campbell formula, 65% of EFWs for the Shepard formula and 65.5% of EFWs for the Hadlock formula. However, when birth weights were below 2000 g, the Hadlock formula was found to out-perform the Ong formula.[186]

Since most of the formulae currently in use have been developed using measurements from singletons, it is relevant to question their accuracy in twin fetuses. Studies that have compared the accuracy of EFW in twins compared to singletons have found some differences in the errors associated with estimating fetal weight between the two groups.

Ott and colleagues found that with an interval between ultrasound and delivery of less than 3 days, the Warsof formula (BPD, AC)[173] had an overall mean error of 8.2%, with 66.3% of EFWs falling within 10% of birth weight.[204] However, only four sets of twins were included in the 101 infants studied, and the authors did not differentiate between accuracy in singletons and twins. Other researchers found that with an interval between ultrasound and delivery of four days, the Warsof formula[173] performed comparably in twins compared to singletons, with 77.9% of EFWs in twins within 10% of birth weight compared to 79.3% in singletons.[205]

The Hadlock formulae (HC, AC, FL; AC, FL)[182] and the Shepard formula (BPD, AC)[180] have been found to over-estimate fetal weight in twins under 2500 g of birth weight although, above 2500 g, these formulae performed equally well in twins as in singletons.[206] The Hadlock (AC, FL, BPD)[182] formula has also been found to have a higher mean absolute percentage error in twins (8.9%, 95% CI: 8.2,9.6) than in singletons (6.8%, 95% CI: 6.4, 7.2), with a lower accuracy in second-born twins compared to first-born twins.[203]

In contrast, a number of other studies have found that the Hadlock formulae perform well in twins. For instance, Edwards et al. found that the formulae by Hadlock (AC, FL, BPD; HC, AC, FL, BPD),[182] Shepard (BPD, AC)[180], and the Combs formula (HC, AC, FL)[185] showed similar systematic errors over a study sample of twins and singletons, with no differences in accuracy observed between the two groups.[207] The Combs formula was found to perform poorly at the extremes of birth weight, over-estimating the weight of smaller babies and under-estimating the weight of larger babies, a finding in conflict with Combs et al.'s assessment of their own formula.[185]

In another study based only on twins that assessed the accuracy of the Hadlock formula (AC, BPD),[174] researchers found that the degree of correlation between EFW and actual birth weight was high, at 0.954, that 41% of the EFWs were within 5% of the actual birth weight, and that 72% of the EFWs were within 10% of the actual birth weight.[208] Authors found that this formula tended to over-estimate the EFW of smaller fetuses.[208]

Diaz-Garcia et al. compared the accuracy of two Hadlock formulae (AC, BPD, FL; HC, AC, BPD, FL),[182] the Shepard formula (AC, FL),[180] the Ong formula (AC, FL),[186] and the Warsof formula (AC, FL)[176] in twins, and found that the Hadlock formula (HC, AC, BPD and FL)[182] had the lowest systematic error and highest precision. A significantly higher percentage, 62% of the study population, had EFWs within 10% of birth weight when using this formula compared with 41.9% for Shepard's formula, 52.5% for Ong's formula, and 49.6% for Warsof's formula.[209]

In 1989, Secher et al. tested a formula that integrated BPD and abdominal diameter (AD),[181]and had originally been developed in singletons, to estimate fetal weight specifically in twins.[210] When ultrasound-to-delivery intervals were within four days, the accuracy of this formula in twins was found comparable to what had been reported in the literature for singletons, with 60% of birth weights within 10% of EFW.[210]

Ability to Detect SGA and Growth Discordance in Twins

In a study assessing the ability of the Secher formula[181] to detect SGA in twins, Henriksen et al. found that EFW was more predictive of SGA compared with just AD or BPD alone, with an area under the receiver operating curve (ROC) of 0.85 compared with 0.69 and 0.65 respectively.[211]

The Hadlock formula (AC, FL, BPD)[182] has been found to have a comparable ability to detect fetal growth restriction in twins as in singletons, with a low overall sensitivity of 48.9% in twins and 47.5% in singletons, but with a higher specificity of 95.7% in twins and 97.7% in singletons.[203] Moreover, when stratifying for chorionicity, the same Hadlock formula[182] had a similar ability to detect SGA in first- and second-born twins of a dichorionic pair, with sensitivities of 40% and 46% respectively, and specificities of

94% and 95% respectively. This was found in monochorionic twins as well, with sensitivity and specificity of detecting SGA for the first-born twin at 50% and 97% respectively, and, 67% and 96% respectively for the second-born twin.[212] Therefore, birth order and chorionicity do not appear to influence the ability of the Hadlock formula to detect fetal growth restriction.

When HC was incorporated in addition to AC, FL and BPD, the Hadlock formula[182] had a higher ability to detect growth discordance between twins of >15%, >20% or 25%, compared with the Hadlock formula that only used AC, FL and BPD, the Shepard formula,[180] the Ong formula[186] or the Warsof formula.[176, 209]

The ability of the Hadlock formula to detect growth discordance between twins was found to be lower by other researchers, with a sensitivity of 11.1% to detect >25% discordance, 16.7% to detect growth discordance >20%, and 17.8% to detect discordance of >15% between twins, with much higher specificities of 94.9% to detect growth discordance>25%, 91.5% to detect growth discordance>20% and 94.2% to detect discordance >15%.[213] The levels of discordance tended to be under-estimated by ultrasound, with authors explaining this as being due to a systematic under-estimation of the weight of the larger twin. Specifically, this under-estimation was found to occur more frequently in dichorionic twin pairs compared with monochorionic twins (OR 3.4, 95% CI: 1.4, 8.4).[213] However, the authors of this study did not specify which Hadlock formula was used.

Factors Affecting the Accuracy of EFW

Difficulty in obtaining biometric measures such as HC and BPD in twins due to uterine over-crowding, and more variable fetal positions has been suggested by some authors as influencing the accuracy of EFW in twins.[26, 208]

Additionally, studies on the growth velocity of singletons have reported approximate weight gains of 25-28g/day in the late third trimester.[15, 35] Although fetal growth in twins is expected to be slower closer to term,[29] it is possible that not accounting for this daily change in weight, especially in studies that use longer intervals-to-delivery, affects the accuracy of EFW when compared to birth weight.[212, 214] However, there is some evidence that the actual interval between ultrasound and delivery has a minimal influence on the accuracy of EFW, when the maximum interval between ultrasound and delivery examined is seven days.[215]

In a systematic review of the accuracy of different formulae used to estimate fetal weight, Dudley observed that the magnitude of systematic and random errors of ultrasound EFWs tended to cluster by study site, and suggested that factors specific to each study, including ultrasound protocols, study population, and equipment could explain this.[214]

Other factors that have been suggested to affect the accuracy of EFW are inter- and intra-observer variability in obtaining measurements,[35, 216-217] and operator experience,[195] while there is conflicting evidence on the effect of maternal body mass index (BMI),[187, 215] chorionicity,[213, 218] and birth order.[203, 218] Fetal sex has been suggested to have no or minimal influence on EFW accuracy.[214-215, 218] The presence of IUGR in any one twin increases the error associated with estimating its fetal weight, as does the presence of severe growth discordance (≥ 25%) between twins.[218]

Finally, the accuracy reported when comparing the ability of different formulae to detect SGA by different research groups may have been influenced by the use of different growth references to classify infants as SGA at birth. For instance, while Danon et al. used the Dolzberg curves specific to Israeli populations,[203] Chauhan et al. used an American reference from Ananth et al. that was stratified by chorionicity.[212]

3.5.4 Ultrasound growth references

Singletons

There has been much research investigating antenatal fetal growth using ultrasound measurements in singletons. Weight at each gestational age has been charted using individual biometric measurements,[15, 21, 219-221] as well as EFW.[16, 20-23, 35, 157, 222-223]

Consistently, ultrasound-based fetal weight references in singletons have been found to have higher EFWs than previously published birth weight curves, especially at lower gestational ages.[16, 23, 35, 157, 222] Dating of pregnancies in these studies has been performed by ultrasound only,[15, 35] LNMP only,[21, 23, 220-221] or LNMP confirmed by ultrasound measurements.[16, 157, 222] A significant limitation of studies conducted in this field is the lack of statistical adjustment for the use of serial measurements in the construction of fetal growth references. Only one group used hierarchical models in their analysis,[23] while another calculated fetal growth for each fetus and constructed references for fetal growth velocity, thus taking into account the correlation between serial measurements.[15] None of the other studies considered correlation between serial ultrasound measurements on the same fetuses.

Twins

As early as 1978, Grennert et al. charted the growth of BPD measurements for 119 pairs of twins with two ultrasound measurements over the course of pregnancy, and compared their growth trajectory with that of singletons from the same population.[25] Growth in BPD was found to slow down in twins from around the 32nd week relative to singletons, and the variability of mean BPD values was also found to be higher for twins. Moreover, the authors found that the second-born twin consistently had lower BPD values than the first-born twin.[25]

Similarly, in 1979, Leveno and colleagues constructed BPD standards using 123 twin pregnancies with 340 ultrasound examinations on a sample of uncomplicated pregnancies with live-born twins and a discordance in BPD of less than 4mm between twins, comparing these values with those of singletons.[26] They found that the BPD of twins were consistently smaller in twins than in singletons, with the difference increasing over the course of pregnancy.[26]

In 1980, Crane et al. modeled BPD measurements for eighteen twin pairs between 15 and 40 weeks of gestation, whose mothers had a history of regular menses, no oral contraceptive use in the three months prior to conception, a high correlation of the pediatrician's estimated gestational age at birth with the LNMP estimate, and who were not growth discordant at birth.[224] The pattern of growth for the BPD observed in growth concordant twins was found to be similar to that observed in appropriate-for-gestational age (AGA) singletons, with similar mean BPD values and standard deviations throughout the course of pregnancy. This finding was confirmed at birth by comparing HC measurements from twins with AGA singletons born at the same institution, and suggested no slowing of growth in twins, at least with respect to head size.[224]

Socol et al. investigated growth patterns in BPD and AC in 43 twin pregnancies between 17 and 37 weeks of gestation, with reliable menstrual dates confirmed by neonatal examination, no obstetric or medical complications, delivered after 34 weeks of gestation, and with what they deemed to be concordant birth weights (<25% difference in weight).[27] They found that the 50^{th} percentile values of twin BPD fell below previously published singleton values for the 50^{th} percentile after 32 weeks, with 50^{th} percentile AC values for twins falling below that of singletons after 34 weeks.[27] The authors attempted to account for pregnancy-level clustering of twins by comparing these results with those when an average of the BPD and AC were taken for each twin pair. However, this may not have been appropriate, given the high threshold of growth discordance that was accepted as the inclusion criteria for this study (up to 25%). Additionally, the singleton comparisons used were from a high-altitude reference, not the ideal comparator for their study population.[27]

In 1986, Gilstrap et al. charted the growth of twins based on BPD and FL measurements between 18 and 38 weeks for 120 twins, who had 327 BPD and 107 FL measurements.[24] Women were recruited from the military and were included in the study if they had known normal LNMP history, if their estimated dates of confinement were in concordance with BPD estimates, and if they did not have chronic hypertension, diabetes, or other metabolic or connective tissue diseases.[24] Both BPD and FL had a positive and strong correlation with gestational age, the correlation of BPD was higher (0.997 vs. 0.97), and, as expected, the rate of growth was slower in twins than that reported for singletons up to 36 weeks of age.[24] These findings were limited by the fact that researchers averaged measurements taken over each week of gestation.

Taylor et al. calculated growth velocities of fetal abdominal area (FAA) and BPD for 130 twin pairs in Scotland for each week of gestation between 30 and 37 weeks.[225] They were unable to study the period after this due to inadequate sample sizes. They found a lower velocity of growth in both BPD and FAA between 30 and 37 weeks of gestation. Sex, chorionicity, and birth order were found to have no influence on growth velocity.[225] This group adjusted for serial measurements by investigating growth for each fetus. Moreover, since the authors did not model growth velocity using linear regression, accounting for the correlation between twins was unnecessary. However, the authors did not provide reference values of weight that might have served as a comparison for assessing weight at each gestational age.

In 2002, Ong et al. charted standards for AC, BPD and FL for 884 twin pairs between 24 and 39 weeks of gestation after excluding any pregnancies where at least one fetus died *in utero*, and cases of suspected IUGR.[226] They only used one ultrasound measurement from each twin in their analysis, and dated pregnancies using the LNMP estimate, unless it differed from the ultrasound estimate by ten days, in which case the ultrasound estimate was used. They found that, in twins, growth patterns in AC and BPD were similar to those previously reported in singletons up to 32 weeks, with BPD in twins even larger than singletons in early third trimester, after which there was a decline. On the other hand, FL values remained the same throughout pregnancy for twins and singletons.[226] While this study benefited from a large sample size, researchers acknowledged that, although care was taken during ultrasound to correctly identify each twin, misclassification of fetuses may have occurred.[226]

In one of the few studies conducted so far on EFW changes in twins over the course of pregnancy, Yarkoni et al. studied 35 healthy women who had what they termed "normal" pregnancies and no fetal deaths, and charted both weight at each gestational age and growth velocity after the 15th week of pregnancy.[227] Changes in AC remained constant throughout pregnancy, while BPD changes showed a linear increase in the second trimester, and a flattening in the third trimester. The function of EFW against gestational age was found to be curvilinear after the 15th week of pregnancy. The rate of fetal weight gain increased until 33 weeks of gestation, after which it slowed down. EFW differences between co-twins were found to increase over the course of pregnancy, although the difference was not statistically significant.[227]

In 1998, Min et al. constructed fetal weight references using a sample of 1,831 American women who gave birth to live-born twins after 28 weeks of gestation, with no major congenital anomalies.[228] They used at least two ultrasound-based EFWs for each fetus, as well as birth weight as an additional data point, to model weight at two-week gestational age intervals, stratifying their sample by fetal sex, race, and chorionicity. As expected, males were heavier than female fetuses, dichorionic twins were heavier (although not significantly so) than monochorionic twins throughout pregnancy, and black twins were lighter than Hispanic and non-Hispanic white twins. Twin fetal weights were found to deviate from singleton fetal weights by 30 weeks, and peak fetal weight gain was found to be between 34 and 36 weeks in twins, as opposed to between 36 and 38 weeks in singletons.[228]

3.5.5 Limitations of Ultrasound-Based References

Ultrasound growth references are limited by the fact that just as birth weight references exclude *in utero* measurements, ultrasound-based *in utero* growth references exclude data from live born infants, which has the potential to create bias.[19] Min et al. circumvented this somewhat by using birth weight in addition to EFWs to model fetal growth.[228] There have been suggestions to move towards "hybrid" references, which either average birth weight and fetal weight references,[229] or switch from fetal weight to birth weight references after 37 weeks.[156] Ideally, growth references would be able to simultaneously assess weight in live born infants and *in utero* fetuses, at each gestational age.[9]

Typical of studies that have created ultrasound-based growth references for twins is their small sample size, with one study constructing growth references with as small a sample size as 18 twin pairs.[224] This affects the generalizability and standard deviation of the estimates at each gestational age. Moreover, those studies that have used longitudinal measures have not accounted for the two levels of clustering inherent in this population, namely, the correlation between serial measurements on each fetus, and the correlation between twins born to the same mother.

Despite these limitations, however, the greatest clinical value of ultrasound growth references and standards lie in their ability to detect deviations from a particular trajectory of fetal growth, which takes on added relevance in higher-risk pregnancies such as twin pregnancies. Moreover, as outlined above, ultrasound-based weight references are able to describe growth at earlier gestational ages more appropriately than references and standards based on birth weight.

While there has been research investigating the growth patterns of individual ultrasound biometric measurements, there has not been as much research on changes in EFW in twins.

Chapter 4: Methods

4.1 Study Design and Setting

The present study was a retrospective cohort study. Data for this study were obtained from the McGill Obstetrical and Neonatal Database (MOND), and the ultrasound records maintained at the Royal Victoria Hospital (RVH) for the periods between 1996 and 2006. A detailed description of each database is provided in Section 4.3.

4.2 Population

4.2.1 Inclusion Criteria

Women who gave birth to live twins at the RVH in the period between 1996 and 2006, with at least one ultrasound conducted during pregnancy at the RVH, and with complete information on HC, AC, FL and BPD were included. Since many of these measurements, and fetal covariates such as sex are only available after the second trimester, only ultrasounds from the second and third trimester were included in this study for construction of growth references.

4.2.2 Exclusion Criteria

Pregnancies in which either infant had a congenital anomaly were excluded from the study, as were those with intrauterine fetal demise (either spontaneous or iatrogenic fetal reduction). Presumably, if performed early enough in pregnancy, iatrogenic fetal reduction would have no impact on the surviving twin. However, since the gestational age at the time of reduction was unknown, pregnancies in which iatrogenic fetal reduction had occurred were excluded.

4.3 Data

4.3.1 Databases used in the study

McGill Obstetrical and Neonatal Database (MOND)

The MOND collects routine information on all women who give birth at the RVH, and their infants. Routine entries are coded by an administrative clerk while a nurse, obstetrician, and neonatologist are involved with coding items that require professional judgement.[230] Information is collected on both the mother and infant, including prenatal and postnatal data.

Although data have been collected in this format since 1978, for the purposes of this study only data collected between 1996 and 2006 were used. Data for the periods between April 1, 1997 and March 31, 1998, and between April 1, 2000 and March 31, 2001 were missing due to administrative reasons (Ms Danielle Vallerand, personal communication).

Ultrasound Records

Ultrasound data for this study came from routinely conducted ultrasounds at the RVH for the study period under consideration.

Six ultrasound machines were used at the RVH during the study period: three Acuson Sequoias (Siemens Medical Solutions USA, Inc., Malvern, PA, USA), two Toshiba Aplios (Toshiba America Medical Systems, Inc., Tustin, CA, USA) and one GE Voluson (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). An Aloka SSD-2000 (Aloka America, Wallingford, CT, USA) was used in the early study period, with the newer machines used from 1999.[215]

Ultrasounds were performed by certified technologists with subspecialty training in obstetrical ultrasound imaging or maternal-fetal medicine physicians with fellowship training in obstetric ultrasound examination.[215] Measurements taken during ultrasound observations are automatically recorded in a database, along with ultrasound technician and physician observations.

Merging of the MOND and Ultrasound Records

The MOND and ultrasound records were available separately from the RVH and, were therefore merged for the purposes of this study. The merging of these databases is explained in detail in Appendix 1.

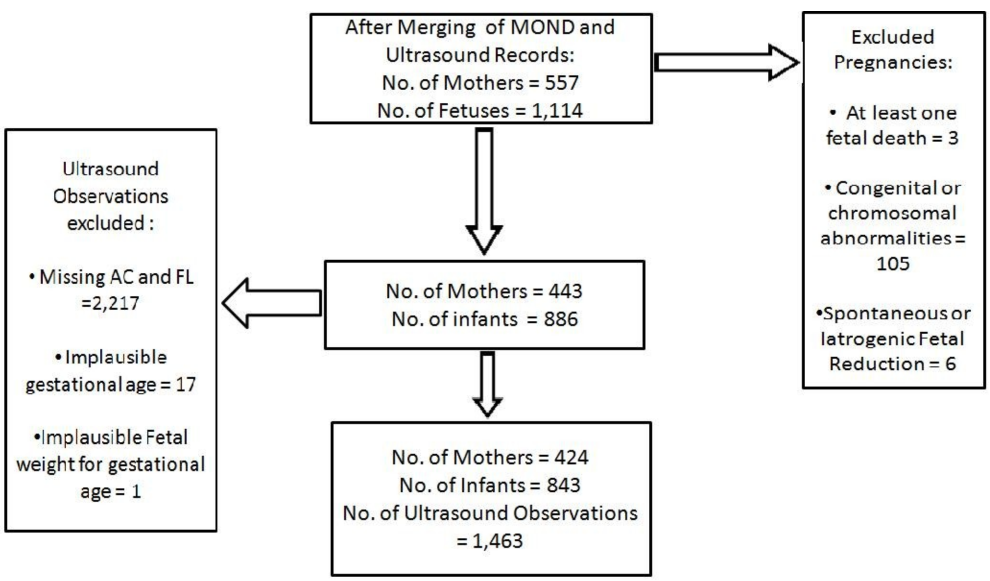
Briefly, in the ultrasound records, a letter assignment (“A” or “B”) is made at the earliest ultrasound to identify each fetus. This letter is then retained by that fetus as an identifier throughout pregnancy, regardless of movement within the uterus, and is recorded in the ultrasound records as the only unique identifier of each fetus along with the mother’s medical record number (MRN).

At the time of birth, a letter is assigned separately in the MOND based on birth order (first-born: “A”; second-born: “B”). Since these two databases are independent, and the letter assignment is made early in pregnancy, it was impossible to ascertain whether the letter assigned in each database did, in fact, correspond with the letter assignment from the other. Therefore, it was not possible to match the serial ultrasound records for each fetus with its birth record. However, since analysis for this project only required fetus-specific variables from each ultrasound, and pregnancy-specific covariates from the birth records, an exact match between the ultrasound and birth record for each fetus was not necessary. Instead, the ultrasound and MOND datasets were merged based on the mother’s MRN.

In a smaller subset of the data where it was possible to match the ultrasound and birth records of each fetus based on fetal sex (in twin pairs of discordant sex) and another variable (last EFW recorded by ultrasound), the choice of formula to estimate fetal weight was validated.

After the two databases were merged, all observations with missing MRNs were dropped, as were all observations with implausible dates, i.e., if the birth date was before the date of an ultrasound, if the gestational age estimate was larger at the time of ultrasound than at the time of birth, or if the difference between the birth date and date of ultrasound was greater than 44 weeks. Also, those fetuses missing biometric measurements (AC, FL) in both the second and third trimester were excluded. Thus, it was possible to have fewer fetuses included in the study than mothers. Finally, a scatter plot of EFW against gestational age was constructed, and combinations of EFW and gestational age judged implausible by visual inspection were excluded. The details of this are provided in Figure 1.

Figure 1: Inclusion of Study Subjects for Primary Objective



4.3.2 Outcome

Birth Weight

Birth weight was used as the reference when assessing the accuracy and precision of formulae used to estimate fetal weight. Birth weight information was taken from the

MOND. At the RVH, newborn infants are weighed nude on an electronic scale by the attending physician or resident, and weight is recorded to the nearest gram.

Ultrasound Biometric Measurements and Estimated Fetal Weight

According to internal protocols, at the time of ultrasound, AC is measured at the skin level from a true transverse view of the fetal abdomen at the level of the junction of the umbilical vein, portal sinus and fetal stomach (when visible). The outline of the abdomen should be as circular as possible. The measurement can be taken as either a direct circumference measurement or interpolated from anteroposterior and transverse diameter measurements. The FL is measured with the beam of insonation perpendicular to the long axis of the femoral shaft, excluding the distal femoral epiphysis. The diaphysis is measured from the greater trochanter above to the lateral condyle below.[185] HC and BPD measurements are taken according to the protocols outlined by Doubilet and Greenes.[231]

Based on findings from the secondary objective (i.e., the comparison of the five formulae used to estimate fetal weight), Ong’s formula was used to estimate fetal weight for the construction of fetal weight references. The Ong formula estimates fetal weight using AC and FL from the following formula:[186]

$$\textbf{Log (Fetal Weight)} = (0.0259 \times AC) + (0.6720 \times FL) - (0.0475 \times FL^2) - 2.7606$$

When constructing the fetal growth reference, fetal weight was modeled as a log-transformed response variable. This was done to ensure that the assumptions of homoscedasticity of model residuals and normality of the outcome variable were not violated.

4.3.3 Assessment of Exposure and Covariates

Estimation of Gestational Age

At the RVH, dating of pregnancies is based on the LNMP as long as there is a difference of less than ten days between the LNMP and ultrasound estimate. In the case of a discrepancy of greater than ten days, the ultrasound estimate of gestational age is used. We used the same method to date pregnancies, as it has been validated in the literature.[232] In the case of either the LNMP estimate or ultrasound estimate being missing, the other estimate was used.

At the RVH, until 13 weeks, ultrasound measurements of CRL are used to date the pregnancy. After the first trimester, however, ultrasound-based BPD measurements are used.[215] At each ultrasound visit, an estimate of gestational age was assigned based on ultrasound, as well as based on the LNMP estimate. Based on the above-mentioned algorithm, either estimate of gestational age was used, under the assumption that ultrasound dating is superior to LNMP estimates of gestational age.

If, at the time of ultrasound, a particular estimate of gestational age (LNMP or ultrasound) was missing, then the other estimate was used.

Chorionicity

As per the recommendations of the Society of Obstetricians and Gynecologists of Canada, determination of chorionicity is ideally done by ultrasound between 10-14 weeks of gestation.[233] The presence of a single placental mass in the absence of a lambda sign at the inter-twin membrane-placental junction as seen on ultrasound is interpreted as indicative of monochorionicity, while twins are classified as dichorionic if a single placental mass is viewed on ultrasound, and the lambda sign is also present.[234] The determination of chorionicity was assigned in the database according to these guidelines during the study period.

Maternal Obesity

Maternal obesity was defined as a pre-pregnancy BMI greater than 30 kg/m^2. This was calculated from maternal self-reported pre-pregnancy weight and height. If either height or pre-pregnancy weight were missing, these observations were excluded from the analysis for the validation of the formulae used to estimate fetal weight.

Pregnancy-Induced Hypertension

Data on pregnancy-induced hypertension were derived from the MOND, and were represented as a binary variable. This variable included pregnancy-induced hypertension ranging in severity from mild pre-eclampsia to HELLP syndrome. Since this was intended for preliminary assessment of EFW formulae, the wide range of disease severity was not considered to be an issue.

Gestational Diabetes

Data on gestational diabetes was derived from the MOND, and was represented as a binary variable, representing a range of severity of disease.

4.4 Statistical Analysis

4.4.1 Primary Objective: Construction of Fetal Weight Reference

Multi-level linear regression was used to model the relationship between EFW and gestational age. Fetal growth is known to be non-linear over the course of pregnancy, and has been modeled using higher degree polynomials. Also, fetal growth is not constant over the course of pregnancy, with periods of accelerated growth in the second trimester, and slower growth in the third trimester.[15] In order to account for this, fetal growth was modeled using restricted cubic splines, which are described in more detail below.

Restricted Cubic Splines

Splines are piecewise polynomial functions that are specific to a particular interval of the predictor variable, X, and are connected over each interval of X.[235] The simplest form, a linear spline, is a piecewise linear function that is connected at the knots, which delimit each interval of X from the other.

Since fetal growth over the course of pregnancy does not follow a linear function, a cubic spline incorporating a piecewise cubic polynomial function is necessary to model fetal growth.[15] Cubic splines are smoothed at each knot by forcing the first and second derivatives of the function to be the same at the knots.[235] Moreover, due to poor behavior of the cubic spline in the extreme tails (before the first knot and after the last), the restricted cubic spline function is generally used, which constrains the function to be linear at the extreme tails.[235] Five knots, as per the recommendations of Harrell, were used in modeling fetal growth.[223, 235]

Clustering of Data

This dataset was hierarchical in nature and was composed of three levels, namely, each mother's pregnancy, twins born to the same mother, and serial measurements of EFW for each fetus. In the cases whereby a mother had multiple twin pregnancies within the study period, all her pregnancies were analysed together without accounting for clustering by mother.

Twins born to the same mother are likely to be more similar than any other randomly selected twins with respect to variables such as birth weight, resulting in a correlation in outcomes between twins born to the same mother.[236] Failure to account for the correlation between twins has been shown to affect the precision of effects of both cluster-constant and cluster-varying covariates.[236] Moreover, serial measurements of EFW in the same fetus also tend to be correlated, and it is thus necessary to take this into account in the analysis.[237] In order to adjust for the twin-level clustering and longitudinal nature of the data, conditional (subject-specific) models were used in the present analysis.

Conditional Linear Regression

Linear mixed models (LMMs) take into account random effects or latent variables, in addition to fixed effects that are constant across clusters, to model correlation between observations in a cluster.[238] A LMM models continuous mean response variables, conditional on measured covariates and on unobserved variables.[238] Effectively, regression coefficients are allowed to vary from one individual to another by introducing random effects that are assumed to follow a probability distribution ~ $N(0, \sigma^2)$.[237]This has implications for the interpretation of regression coefficients, which can then be understood as the effect of the covariate on the response variable for an individual with a particular value of the random effect.[237]

The linear mixed model in its basic form can be represented as:

$$\mathbf{Y_{ij} = \beta_0 + \beta_1 X_{ij} + u_i + \varepsilon_{ij}}$$

where:

Y_{ij} : Outcome of the jth individual in the ith cluster

β_0: Intercept common to all individuals regardless of cluster

β_1 : Effect of X on Y, common to all individuals regardless of cluster

X_{ij} : Value of independent variable for the jth individual in the ith cluster

u_i : Random effect specific to the ith cluster

ε_{ij}: Residual error for jth individual relative to the fitted values for the ith cluster

and:

u_i are independent and follow $N(0, \sigma^2)$

ε_{ij} are independent and follow $N(0, \sigma^2)$

u_i and ε_{ij} are independent of each other

In the case of the present study, the above model would be modified by the addition of individual spline terms and an additional level of clustering k, altering the equation specified above to the following:

$$\mathbf{Y_{ijk} = \beta_0 + \beta_1 (spline\ term\ 1)_{ijk} + \beta_2 (spline\ term\ 2)_{ijk} + \beta_3 (spline\ term\ 3)_{ijk} + \beta_4 (spline\ term\ 4)_{ijk} + u_i + u_k + \varepsilon_{ijk}}$$

where:

Y_{ijk}: Log(Fetal Weight) at the jth ultrasound observation of the ith fetus for the kth mother

β_0: Intercept common to all fetuses regardless of cluster

β_1, β_2 , β_3 , β_4: Effect of each spline term on Y, common to all fetuses regardless of cluster

u_i: Random effect specific to each fetus

u_k: Random effect specific to each mother

ε_{ijk}: Log (Residual error of the EFW) at the jth ultrasound for the ith fetus born to the kth mother relative to the EFW predicted by the model for the ith fetus born to the kth mother.

Covariates Included in Model

As per the review of the literature, fetal sex and chorionicity are known to influence fetal weight at any given gestational age. Therefore, the influence of sex and chorionicity on fetal growth were assessed as per the convention in this field.

Selection of best-fit model

Models were fit including random intercepts and random slopes for each mother, and each fetus respectively, and different combinations of these were explored.

Akaike Information Criterion (AIC) values were compared between each fitted model, with lower values implying better fit.[239] AIC takes into account both the model fit and parsimony, penalizing models with more parameters. Moreover, residual errors were compared between models when deciding on the models with best fit, from which to derive the fetal growth chart. If there was no difference between any two models with respect to the AIC value and the residual error value, the most parsimonious model was selected.

Prediction of Fetal Weight

The mixed linear regression models, chosen as per the criteria specified in the above step, were used to predict log median fetal weights for each week of gestational age between 22 and 37 weeks, as well as the 1st, 10^{th}, 90^{th}, and 99^{th} percentiles. Predictions were not made past 37 weeks due to a lack of sample size.

The predicted values were on the log scale, as fetal weight was log-transformed for the purposes of modeling fetal growth. Assuming that the predicted log fetal weight was normally distributed, the mean predicted value of fetal weight from this scale would equal the median predicted value of fetal weight. Since the median value of a log transformed variable can be back-transformed to the median value on the original scale without bias,[240] the predicted median fetal weight value for each gestational age was calculated instead of the mean on the original scale in grams. Similarly, 1^{st}, 10^{th}, 90^{th} and 99^{th} percentile values were calculated on the log scale and then back-transformed to the original scale in grams. This was done for each completed week of gestation between weeks 22 and 37.

Sensitivity Analysis

In order to verify the results from this study, given the method used to date pregnancies, sensitivity analysis was performed, estimating gestational age either by LNMP only, or by ultrasound only. Predicted median fetal weights at each gestational age for the overall study sample dated by the algorithm used at the RVH were compared with predicted median fetal weights when dating was done by LNMP only, or by ultrasound only. Results from this analysis are provided in Appendix 2.

4.4.2 Secondary Objective: Assessment of Estimated Fetal Weight Formulae

The following five commonly used formulae that are used to estimate fetal weight were compared for their overall precision and accuracy.

Table 1: EFW Formulae Assessed for Accuracy and Precision

	Formula
Hadlock 1	Log (Fetal Weight)=1.5662-(0.0108 x HC)+ (0.0468 x AC)+(0.171 x FL)+(0.00034 x HC^2)-(0.00386 x AC x FL)
Hadlock 2	Log (Fetal Weight)=1.304+(0.05281 x AC)+(0.1938 x FL)-(0.004 x AC x FL)
Shepard	Log (Fetal Weight)=(0.166 x BPD)+(0.046 x AC)-(0.002546 x AC X BPD)-1.7492
Ong	Log (Fetal Weight)=(0.0259 x AC)+(0.6720 x FL)-(0.0475 x FL^2)-2.7606
Combs	Fetal Weight=(0.23718 x AC^2 x FL)+(0.03312 x HC^3)

*HC: Head Circumference; AC: Abdominal Circumference; FL: Femur Length; BPD: Biparietal Diameter. All Biometric measurements are in cm.

Estimates of Accuracy and Precision

Correlation coefficients were calculated for each formula to assess correlation between EFW and birth weight. The difference between EFW and birth weight as a percentage of birth weight was calculated for each formula. The mean percentage difference was thus calculated as a measure of accuracy, which allowed assessment of whether, on average, a formula over- or under-estimated birth weight. Moreover, the percentage of observations that were within 5% and within 10% of birth weight was reported for each formula as a measure of precision.

Time-to-delivery

Analyses were performed on observations in which the ultrasound exam occurred within seven days of delivery. As a secondary analysis, the overall precision and accuracy were calculated for ultrasounds performed within two days of delivery.

Covariates

Stratified analyses were conducted to assess the accuracy and precision of the formulae within strata of the following covariates: fetal sex, chorionicity, birth order, each 500g birth weight category (<2500g, 2500-2999g, 3000-3499g,3500-3999g), two-week strata of gestational age (<30 weeks, 30-31 weeks, 32-33 weeks, 34-35 weeks, 36-37 weeks, ≥ 38 weeks), and in the case of maternal obesity, hypertension and diabetes.

The Student's t-test was used to assess whether the mean percentage difference was significantly different for each formula depending on the group being considered (eg., male vs. female; monochorionic twins vs. dichorionic twins, etc)

Chapter 5: Results

5.1 Preface to Manuscript 1

The results from this study are presented in the form of two manuscripts. The first manuscript addresses the primary objective of this thesis, the construction of an ultrasound-based fetal weight reference for twin fetuses. This includes the modeling of fetal growth, and the subsequent prediction of the 1st, 10th, 50th, 90th, and 99th percentile fetal weight values for each gestational week. Fetal growth was modeled using multi-level models adjusting for clustering between fetuses, and longitudinal measurements on each fetus, and restricted cubic splines, which accounted for non-linear growth over the course of pregnancy. Findings from the secondary objective of this study were used to inform the methods used to study the primary objective. Fetal growth in twins was found to be S-shaped as expected, and growth references for each week of gestation between weeks 22 and 37 were predicted from the model.

5.2 Manuscript 1: An Ultrasound-Based Fetal Weight Reference for Twins

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ABSTRACT

Background: Fetal weight, and growth over the course of pregnancy are known predictors of perinatal health. Therefore, the monitoring of fetal weight, and its comparison with weight distributions for each gestational age are important aspects of the clinical management of pregnancies. Fetal growth trajectories of twin fetuses are known to differ from singletons, with marked differences appearing in the early third trimester of pregnancy. Therefore, it is necessary to compare the fetal weight of twins with a distribution that is derived from a twin population rather than singletons.

Objective: To construct an ultrasound-based fetal weight reference for twins between the gestational ages of weeks 22 and 37, stratified by fetal sex and chorionicity.

Methods: Live-born non-anomalous twins were included in this retrospective cohort study. Twin pregnancies resulting from the reduction of higher-order pregnancies were excluded. Multi-level linear regression models were used to adjust for clustering by each twin pregnancy, and to account for serial measurements taken on each fetus. Moreover, since fetal growth over the course of pregnancy is not linear, restricted cubic splines were used to model fetal growth. Based on this model, predictions of fetal weight were made for the 1^{st}, 10^{th}, 50^{th}, 90^{th}, and 99^{th} percentiles of the fetal weight distribution. Fetal weight references were stratified by fetal sex and chorionicity.

Results: Fetal growth was found to follow an S-shaped pattern over the course of pregnancy, with accelerated growth in the second trimester, and a slowing of growth in the third trimester. Female fetuses were lighter than male fetuses over the course of pregnancy, as expected, although females showed catch-up growth closer to term. Monochorionic twins remained lighter than dichorionic twins throughout pregnancy.

Conclusion: Fetal weights predicted for each week of gestational age from our study agreed well with those from other studies conducted in twins. Also, as expected, fetal weights in this population were consistently lower than those published for singletons.

Introduction

Since the 1980s, the rate of multiple pregnancies has been rising, specifically in Western Europe and North America.[1] For instance, in England and Wales, France, and the United States, the rate of twin pregnancies has risen by 50-60%.[1] Factors such as delayed childbearing, increased use of ovulation induction, artificial reproductive technologies and, more recently, increased folic acid and use of multivitamins have all been suggested as contributing to the observed increase in the incidence of twinning and multiple pregnancies, in general.[1] Twin pregnancies are at higher risk of adverse perinatal outcome than singleton pregnancies. For instance, twins are generally born at lower gestational ages and have significantly higher rates of perinatal mortality and morbidity when compared to singletons.[2-9]

Moreover, in twins, as in singletons, *in utero* growth restriction (IUGR) is associated with increased perinatal mortality and morbidity, [10-12] although the association is likely not causal. [13-14] Thus, the assessment of fetal growth over the course of pregnancy, and any deviations from an "optimal" growth trajectory that is associated with the lowest risk of adverse perinatal outcomes, are especially important in higher-risk pregnancies such as those of twins.

Additionally, twins are known to experience different fetal growth trajectories than singletons, as both uterine capacity and uteroplacental insufficiency influence their growth.[15-16] The median fetal weight of twins has been found to be markedly lower than singletons beginning around week 30.[2] Moreover, there is evidence that "optimal" birth weights are lower for twins than for singletons.[17]

Over the years, a number of approaches and definitions have been considered to appropriately detect when a fetus experiences IUGR. While longitudinal approaches, which assess each fetus' own growth trajectory, are theoretically ideal to assess fetal growth, there is some evidence that they may not be of increased clinical value than cross-sectional references.[18] Moreover, cross-sectional fetal weight references are more practical and easy-to-use in the context of general clinical care and assessment. A number of population-based birth weight references have been generated for twins that chart birth weight for each completed week of gestation.[2, 19-22]

However, there is considerable bias associated with these references, especially at lower gestational ages. Specifically, infants born at lower gestational ages are smaller, and presumably less healthy, than their counterparts that remain *in utero*.[23] These factors point to the utility of ultrasound-based fetal weight references, which chart fetal weight distributions for each completed week of gestation. Since the routine use of ultrasound in the clinical management of pregnancies began, a number of research groups have integrated biometric measurements, such as head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD) and femur length (FL), in different combinations to calculate estimated fetal weight (EFW) *in utero*, with varying accuracy and precision.[24-27] One group, in particular, has developed a formula specifically in a twin population.[28]

Finally, although there have been a number of ultrasound-based fetal weight references constructed for singletons, there are very few studies conducted charting fetal weight distributions in twins.[29-30]The study by Yarkoni et al. was limited by a small sample size of only 35 healthy pregnancies,[29] while the study by Min et al. only provided reference for two-week intervals, which may not adequately characterize fetal growth, especially in the second trimester.[30]

Within this context, our objective was to construct ultrasound-based fetal weight references for twins from a tertiary care centre in Montreal, at each completed week of gestation, stratified by sex and chorionicity, between weeks 22 and 37 of pregnancy.

Methods

This was a retrospective cohort study based on data from a tertiary-care hospital in Montreal, Canada. The McGill Obstetrical and Neonatal Database (MOND) records prenatal and postnatal clinical information on women delivering at the Royal Victoria

Hospital (RVH) and their infants. Independent of this database, ultrasound information is recorded on all women who receive ultrasound scans at the same hospital.

Population

We included women delivering live-born twins at the RVH between 1996 and 2006, and who had ultrasounds conducted at the RVH. Data for the periods between April 1, 1997 and March 31, 1998, and between April 1, 2000 and March 31, 2001 were missing due to administrative reasons (Ms Danielle Vallerand, personal communication).

Pregnancies in which at least one fetus had a congenital anomaly or died, underwent spontaneous or iatrogenic fetal reduction, or had missing information on AC or FL at ultrasounds in both the second and third trimester were excluded from further analysis. In cases of congenital anomaly or *in utero* death of at least one fetus, the entire pregnancy had to be excluded due to the nature of the merging of the databases in which an exact match of the MOND and ultrasound record was not possible for each fetus. This is explained in more detail in the section “Merging of Databases”. Prematurely born infants and those who were sick or died after birth were included in analysis as this growth reference was meant to be of a general population, and not a growth standard of only healthy pregnancies and infants.

For each included fetus, we selected data from the first ultrasound scan performed in the second and third trimesters, as long as they had at least one ultrasound performed in each trimester, and it did not have missing or implausible values.

Merging of databases

We merged the MOND and ultrasound records based on the mother`s medical record number as a unique identifier of pregnancies. However, the identity of each twin at the time of ultrasound could not be matched to their identity at the time of birth. Twin fetuses are labelled as “A” or “B” at the first ultrasound, but while this identifier follows them throughout pregnancy regardless of their movement within the uterus, an independent "A" or "B' label is assigned at the time of birth, depending on their order at birth. Thus, while we were able to match the ultrasound and birth record for each pregnancy, we were unable to ascertain whether there was an exact match for each fetus' record. However, since we only included pregnancy-specific covariates, such as chorionicity (from the MOND) and fetus-specific covariates, such as fetal sex and EFW from the ultrasound records, this did not affect our analysis.

Biometric Measurements

According to internal protocols, AC is measured at the skin level from a true transverse view of the fetal abdomen at the level of the junction of the umbilical vein, portal sinus and fetal stomach (when visible). The outline of the abdomen should be as circular as possible. The measurement can be taken as either a direct circumference measurement or interpolated from anteroposterior and transverse diameter measurements. The FL is measured with the beam of insonation perpendicular to the long axis of the femoral shaft, excluding the distal femoral epiphysis. The diaphysis is measured from the greater trochanter above to the lateral condyle below.[31]

Estimated Fetal Weight

Based on a validation study of five EFW formulae in a subset of this population (results published elsewhere), we selected the Ong formula to estimate fetal weight at each ultrasound visit. This formula was specifically developed in a population of twins and uses AC and FL to estimate fetal weight.[28]

$$\mathbf{Log\ (EFW) = (0.0259 \times AC) + (0.6720 \times FL) - (0.0475 \times FL^2) - 2.7606}$$

Gestational Age

Gestational age at the time of ultrasound was calculated using the algorithm that is routinely used at the RVH. The last normal menstrual period (LNMP) estimate is used as long as it is in agreement within ten days of the ultrasound estimate of gestational age. At the RVH, ultrasound measurements of crown-rump length are used to date the pregnancy until 13 weeks. After the first trimester, however, BPD measurements are used. If either the LNMP or ultrasound estimate of gestational age was missing at the time of ultrasound, then the available estimate was used. In order to further validate this algorithm, we conducted sensitivity analyses using only the LNMP estimate or only the ultrasound estimate of gestational age.

Although results are provided by completed weeks of gestation, gestational age was included in the model in days. This takes into account changes in fetal weight for each day, instead of averaging them over the week.

Chorionicity

As per the recommendations of the Society of Obstetricians and Gynecologists of Canada, determination of chorionicity is ideally done by ultrasound between 10-14 weeks of gestation.[32] The presence of a single placental mass in the absence of a lambda sign at the inter-twin membrane-placental junction as seen on ultrasound is interpreted as indicative of monochorionicity, while twins are classified as dichorionic if a single placental mass is viewed on ultrasound, and the lambda sign is also present.[33] The determination of chorionicity was conducted according to these guidelines during the study period.

Statistical Analysis

Our dataset was hierarchical in nature and was composed of three levels, namely, each mother's pregnancy, twins born to the same mother, and serial measurements of EFW for each fetus. In the four cases where a mother gave birth to two sets of twins during the study period, both pregnancies were analysed together, and we did not account for clustering by each pregnancy for the same mother.

Twins born to the same mother are likely to be more similar than any other randomly selected twins with respect to covariates such as birth weight, thus resulting in correlated outcomes between twins born to the same mother.[34] Failure to account for the correlation between twins has been shown to affect the precision (variance) of effects of cluster-constant and cluster-varying covariates.[34] Moreover, serial measurements of EFW in the same fetus tend to be correlated, and it is thus necessary to take this into account at the analysis stage.[35] Therefore, we used linear mixed models to model the relationship between gestational age and EFW. Prior to this, we examined the intraclass correlation coefficient to assess correlation due to clustering.

Fetal growth is known to be non-linear over the course of pregnancy, and has been modeled using higher degree polynomials.[36] Furthermore, fetal growth is not constant over the course of pregnancy, with periods of accelerated growth in the second trimester and slower growth in the third trimesters.[37] Restricted cubic splines are piecewise segments of cubic polynomial functions, which provide a flexible tool to model smooth curves.[38] To account for the above issues, we used restricted cubic splines with five knots, as recommended by Harrell, to model fetal growth.[38]

The fetal weight variable was log-transformed to ensure that the assumptions of homoscedasticity of residual errors, and normality of distribution of response variable were not violated.[39] The model to be used for prediction was chosen based on the Akaike Information Criteria (AIC) value, and the residual standard errors, with lower values for each implying better fit. The predictions of the 1st, 10^{th}, 50^{th} (median), 90^{th} and 99^{th} percentile fetal weight values were made on the log scale, and then back-transformed to the original fetal weight scale in grams. This was done under the assumption that the distribution of log fetal weight values were normal at each gestational age.[39]

We were able to predict fetal weight values only for the gestational period between 22 and 37 weeks due to a lack of sample size at gestational ages after week 37. All statistical analyses were conducted in Stata Version 10.

Results

The merged databases included 557 mothers and 1,114 infants. We excluded pregnancies in those instances where at least one infant had congenital or chromosomal abnormalities (N=105), at least one fetus died (N=3), and where spontaneous or iatrogenic reduction of pregnancy had occurred (N=6). Overall, 2,217 ultrasound observations were missing information on AC or FL, primarily from ultrasounds conducted in the first trimester. These were excluded from further analysis, and resulted in the exclusion of some pregnancies or individual fetuses from the sample. The first ultrasound conducted for each fetus from the second and third trimester was selected to retain for further analysis as long as it had complete information. From this sub-sample, we used a preliminary scatter plot of fetal weight against gestational age and excluded one observation that was deemed implausible by visual inspection. This was for an infant who had a birth weight of 2925g and was born at 37.28 weeks but who had been recorded as having a fetal weight of 177.39g at 35.14 weeks. Furthermore, 17 observations were dropped because the gestational age estimate at the time of ultrasound was higher than the gestational age estimate at the time of birth.

The final sample size for analysis contained 424 mothers and 843 fetuses, with a total of 1,463 ultrasound observations. The imbalance in number of mothers and fetuses resulted due to the exclusion of fetuses missing AC or FL measurements from both the second and third trimester ultrasounds. Of these, 666 observations were from an ultrasound conducted in the second trimester, while 797 were from a scan conducted in the third trimester. The LNMP estimate of gestational age at the time of ultrasound was missing in 496 observations. In these cases, the ultrasound estimate was used. The ultrasound estimate was missing for 2 observations; in these cases, the LNMP estimate was used.

Maternal and fetal characteristics are displayed in Table 1. The mean maternal age of mothers included in our sample was 32 years, and 52.3% of the mothers were primiparous. A total of 33.16% of the mother conceived using some form of artificial reproductive technology. The median baseline body mass index of mothers in our sample fell in the normal range at 23.09 kg/m^2, while approximately 14% of the mothers experienced diabetes of any severity during pregnancy, and 10% experienced pregnancy-induced hypertension. Only about 4% of women included reported any smoking during pregnancy, and about 4% reported consuming alcohol occasionally during pregnancy. The mean birth weight of the twin infants included in our analysis was 2535g. The median gestational age at birth was 37 weeks.

Modeling Fetal Growth

Regardless of whether we included random intercepts and slopes for pregnancy-level clustering or for the correlation of serial measurements on each fetus, the AIC values and residual standard errors remained the same across models. This was true also when we stratified by sex and chorionicity. Therefore, we selected the most parsimonious model, only allowing the intercepts to vary for each pregnancy and fetus. The predicted values from those models for the overall sample, and by sex and chorionicity, are shown in tables 2, 3a, 3b, 4a and 4b. As expected, the change in fetal weight was rapid over the second trimester and early third trimester, with a slowing observed closer to term. Males were heavier than female fetuses at each week of completed gestation, until week 34, when females caught up with males. Monochorionic twins were consistently smaller than dichorionic twins after week 22 of gestation until about 37 weeks.

Sensitivity analyses showed no difference in these estimates, regardless of whether dating of pregnancies was done by LNMP only or by ultrasound only.

Overall, the standard error for each of the models run was rather low, with small differences between the 1^{st} percentile, 10^{th} percentile, 50^{th} percentile, 90^{th} percentile and 99^{th} percentile fetal weight values. This was observed regardless of stratification by sex or chorionicity. However, the distance between the percentiles increased with increasing gestational age.

Discussion

We constructed a reference for fetal size at each gestational age for twins. We used ultrasound measurements to calculate EFW of fetuses using the published Ong formula.[28] We additionally stratified our sample by sex and chorionicity and generated reference values for males, females, monochorionic twins, and dichorionic twins.

The trajectory of fetal growth was found to follow an S-shape over the course of pregnancy, with a period of rapid change in median fetal weight observed in the second trimester and early third trimester, and a reduction in the change of median fetal weight observed from week 31 and onward.

As expected, male fetuses were heavier than females until around week 34. However, after this time, the predicted values from our model were similar for males and females. This is surprising, especially as birth weight of males was significantly higher than females in our sample. In fact, the median predicted fetal weight at week 37 was higher than the median birth weight for both males and females, implying an upward skew in estimated fetal weight close to term. The catch-up observed in EFW of females relative to males may then be explained if the positive bias in EFW close to term is greater in female fetuses than males.

Similarly, the predicted median fetal weight at week 37 in monochorionic and dichorionic twins was higher than their respective median birth weights, with the difference larger in monochorionic twins. Again, this implies that there is a positive bias in EFW close to term, and that the magnitude of this bias is greater in monochorionic twins than in dichorionic twins. This would explain the similarity in predicted fetal weights at week 37 between monochorionic and dichorionic twins. Prior to week 37, however, monochorionic twins were consistently lighter than dichorionic twins over the course of pregnancy, with a marked difference observed between weeks 29 and 36. This could be explained by the rising demands placed on the mother with increasing gestational age. In monochorionic twins that share a placenta, this increased demand may not be met as efficiently as in dichorionic twins, resulting in the weight difference between the two groups.

Comparison with Other Studies in Twin Populations

The median fetal weight values predicted by our model compared well with those predicted by Min et al.,[30] and by Yarkoni et al.[29] The predicted fetal weights from our model were lower than those reported by Min et al. up to around week 27 and, after this time, they were higher than Min et al.'s estimates until term. The predicted fetal weights from this study were lower than those predicted by Yarkoni et al. up to week 30, after which they were higher than Yarkoni et al.'s estimates. Overall, however, the predicted fetal weights from our model were very close to those from the model by Yarkoni et al.

Comparison with Studies in Singletons

We compared the results from our study with those from Gallivan et al.,[40] who also constructed a fetal weight reference for singletons based on ultrasound measurements. While the median fetal weights from our study were consistently lower than those from the study by Gallivan et al., a more pronounced deviation was observed by week 32, with weight differences further increasing until term. Specifically, the difference was 85 g at week 32 and 422 g by week 37. These differences were slightly lower than those reported from the study by Min et al.[30] After comparing their weight reference against a number of singleton birth weight references, these authors reported a 347g difference by week 34 and a 450 g difference by week 36. The discordance in these results may arise from the fact that they compared a twin *in utero* weight reference to a number of singleton birth weight references, while we compared a twin *in utero* reference with a singleton *in utero* reference. Birth weight closer to term is thought to be higher than ultrasound estimates,[41] which may account for the discrepancy between the two studies.

Strengths and Limitations

This is one of few studies to construct fetal weight references in a twin population. To the best of our knowledge, it is the only one that has accounted for clustering by pregnancy and for the correlation between serial ultrasound measurements on the same fetus. While, theoretically, this is the most appropriate way to model fetal growth in twins with serial ultrasound measurements, in reality, it may not have affected our results much. In fact, when examining the intraclass correlation coefficient (=0), clustering did not explain the variability in fetal weight, indicating that the twin clusters could be ignored. Moreover, when examining the AIC values, a model that adjusted for the two levels of clustering performed as well as one that did not adjust for any clustering, and treated twins, and serial measurements on each fetus as independent. The use of restricted cubic splines to model fetal growth, however, markedly improved the fit of the models.

Our sample size was substantial, as we had access to data from a tertiary-care hospital over a 10-year period and since we included more than one ultrasound measurement per fetus. While the fact that our sample is drawn from a tertiary care center may limit the generalizability of our results, we do not consider this to be very likely, given that twin pregnancies are considered high-risk, and often referred to tertiary-care centers.

Our inability to match the birth record and ultrasound record of each fetus hindered our analysis to some degree by forcing us to exclude birth weight as another potential data point for modeling. Including birth weight as a third data point for each fetus may have improved the fit of the model.

A potential consequence of using multiple ultrasounds per fetus is that we may have included ultrasounds that were not routine. This is generally a limitation of studies that are retrospective in nature, as it is not possible to differentiate if a fetus had multiple ultrasounds due to fetal distress, abnormal growth, or was simply highly monitored. This may not be a major issue in a twin population, which is, in general, closely monitored. However, it does limit our ability to differentiate between routine and indicated ultrasounds. In order to alleviate this somewhat, we only included the first ultrasound conducted in each trimester, as this had the highest probability of being routine. Although this does not rule out the possibility of an ultrasound being conducted for medical indication, it somewhat goes towards ensuring that only routine ultrasounds were included in analysis.

Finally, a general concern with ultrasound is the error associated with the estimation of fetal weight using biometric measurements. However, we had validated the Ong formula within a subset of this same population prior to this study, and we thus have a high degree of confidence in our estimates.

In general, the difference between the 1^{st}, 10^{th}, 50^{th}, 90^{th} and 99th percentiles was quite low at each gestational age, although there was an increase in the distance between them observed with increasing gestational age. This may be explained by the tendency for fetal weight values predicted by a regression model to be more closely distributed than the original distribution. Moreover, adjusting for clustering may have accounted for much of the variance, contributing to the narrow distribution of predicted fetal weights at each gestational age. However, this limits the clinical applicability of this reference due to the error inherent in ultrasound-based EFWs in general. If the difference between the 1^{st}, 10^{th}, 50^{th}, 90^{th}, and 99^{th} percentiles is low, the use of the references in predicting clinically relevant SGA becomes limited. Moreover, if the 1^{st}, or 10^{th} percentiles are artificially inflated due to the narrow distribution of predicted fetal weights, there may be over-estimation of the prevalence of SGA when using this ultrasound-based fetal weight reference for the purposes of comparison.

Conclusion

This fetal weight reference adds to literature in the field of fetal growth monitoring in twins. This could prove to be a useful tool for monitoring of both cross-sectional EFW and longitudinal growth, when serial ultrasounds are performed. In a high-risk population such as twins, this could provide clinically important information on fetal growth. However, it is necessary to assess the ability of these fetal weight references to identify an SGA fetus, and associated clinically relevant outcomes accurately before recommending the widespread use of this standard. In this regard, the small differences between the 1st, 10th, 50th, 90th, and 99th fetal weight percentile values may be a significant limitation.

Tables and Figures for Manuscript 1

Figure 1: Observed and Predicted Fetal Weights for Entire Study Sample

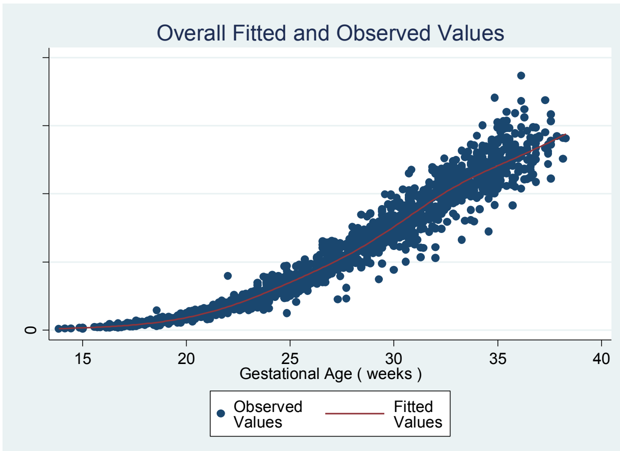

Figure 2: Predicted Median Fetal Weights for Males and Females

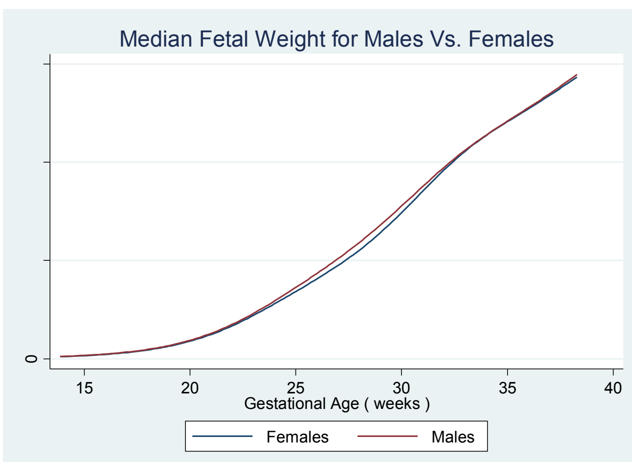

Figure 3: Predicted Median Fetal Weights for Monochorionic and Dichorionic Twins

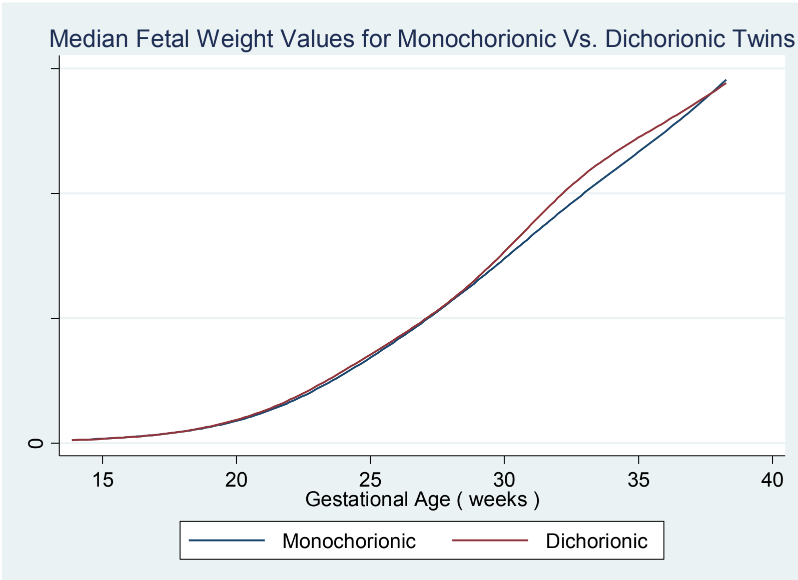

Table 1: Maternal and Fetal Characteristics of Study Population (Women delivering at the Royal Victoria Hospital, 1996-2006)

Mean Maternal Age (years)	32.8 (95% CI: 32.5, 33.1)
Primiparous	52.3%
Any smoking during pregnancy	4.5%
Any alcohol intake during pregnancy	4.6%
Pregnancy conceived through ART	33.2%
Median BMI (kg/m^2)	23.1 (IQR:20.9, 26.4)
Occurrence of any diabetes during pregnancy	14.1%
Occurrence of any Hypertensive Disorders during Pregnancy	10.5%
Mean number of ultrasounds received	5.8 (95% CI: 5.7, 5.9)
Mean Birth weight (g)	2534.9 (95% CI: 2504.8, 2565.2)
Median Gestational Age (days)	259 (IQR: 248, 265)
Male Fetuses	48.3%
Monochorionic Twins	17.8%
First-Born Twins	48.8%

*kg: Kilograms; m: Metre; g: grams; CI: Confidence Interval; IQR: Inter-quartile Range; BMI: Body Mass Index

Table 2: Predicted Fetal Weights (grams) for Overall Sample (Weeks 22-37)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	335	338	342	346	349
23	440	444	450	455	460
24	560	565	572	578	583
25	688	694	701	709	715
26	821	828	837	847	854
27	959	969	981	993	1003
28	1113	1124	1139	1154	1166
29	1291	1303	1318	1334	1347
30	488	1501	1517	1533	1547
31	1692	1707	1726	1745	1761
32	1889	1908	1931	1954	1973
33	2070	2090	2114	2139	2159
34	2229	2248	2272	2297	2317
35	2362	2385	2412	2440	2463
36	2477	2508	2546	2586	2618
37	2587	2631	2686	2742	2789

Table 3a: Predicted Fetal Weights (grams) for Males (Weeks 22-37)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	340	345	350	356	360
23	448	454	461	469	675
24	572	579	588	597	604
25	706	714	725	735	744
26	845	855	868	882	892
27	990	1003	1020	1037	1051
28	1147	1163	1183	1204	1221
29	1324	1341	1362	1384	1402
30	1515	1533	1555	1577	1596
31	1706	1727	1753	1780	1802
32	1890	1915	1947	1978	2005
33	2062	2089	2122	2155	2183
34	2218	2244	2276	2309	2336
35	2349	2379	2417	2456	2487
36	2455	2499	2554	2610	2657
37	2555	2618	2698	2780	2848

Table 3b: Predicted Fetal Weights (grams) for Females (Weeks 22-37)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	326	330	336	341	346
23	428	434	441	449	455
24	544	551	559	568	575
25	666	674	684	694	702
26	791	801	813	825	835
27	922	935	950	966	980
28	1069	1085	1104	1123	1139
29	1246	1263	1283	1304	1321
30	1448	1465	1487	1509	1527
31	1659	1679	1705	1731	1752
32	1864	1889	1921	1952	1979
33	2051	2078	2112	2146	2175
34	2213	2240	2273	2307	2335

35	2345	2375	2413	2451	2482
36	2451	2492	2544	2597	2642
37	2548	2607	2681	2757	2821

Table 4a: Predicted Fetal Weights (grams) for Monochorionic Twins (Weeks 22-37)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	312	320	328	338	345
23	411	421	432	444	454
24	527	538	553	567	579
25	655	668	684	701	716
26	788	805	826	847	865
27	926	948	975	1003	1027
28	1074	1100	1133	1167	1195
29	1239	1266	1301	1337	1366
30	1411	1440	1477	1514	1546
31	1578	1612	1656	1701	1738
32	1741	1782	1834	1888	1932
33	1906	1950	2005	2061	2109
34	2070	2113	2168	2224	2271
35	2211	2263	2329	2396	2453
36	2322	2397	2493	2593	2677
37	2420	2529	2668	2815	2941

Table 4b: Predicted Fetal Weights (grams) for Dichorionic Twins (Weeks 22-37)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	339	342	347	352	355
23	446	450	456	463	468
24	566	572	579	587	593
25	693	700	708	717	724
26	824	832	843	853	862
27	960	971	985	999	1010
28	1113	1126	1143	1159	1173
29	1294	1308	1325	1343	1358
30	1498	1513	1531	1550	1565
31	1710	1728	1749	1771	1789
32	1916	1937	1963	1990	2011
33	2101	2124	2152	2180	2203

34	2259	2281	2309	2337	2360
35	2388	2413	2444	2476	2502
36	2494	2528	2571	2615	2651
37	2594	2642	2702	2764	2816

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5.3 Preface to Manuscript 2

The second manuscript addresses the secondary objective of this thesis, and details the results from the comparison of five formulae to estimate fetal weight in a subset of the twin population from the RVH. The estimated fetal weight from the last ultrasound, calculated by each of the five formulae, was compared with birth weight in those infants that were born within seven days of receiving their last ultrasound in order to calculate accuracy and precision of each of the formulae. The results from this part of the study were used to decide which formula would be used to estimate fetal weight for the purposes of constructing the ultrasound-based fetal weight reference in twins, the primary objective of this thesis.

5.4 Manuscript 2: A Comparison of Five Commonly Used Formulae to Estimate Fetal Weight in Twins

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ABSTRACT

Background: The estimation of fetal weight during pregnancy is an important component of the monitoring of fetal growth. This is especially true in twin pregnancies, which are known to experience a higher incidence of fetal growth abnormalities, and adverse perinatal outcomes than singleton pregnancies. However, of all the methods developed to estimate fetal weight, only one has been developed specifically in a twin population.

Objective: To compare the accuracy and precision of five commonly used formulae to estimate fetal weight relative to birth weight in a twin population.

Methods: A population of live-born non-anomalous non-reduced twins were included in this comparative study based in a tertiary care hospital. Only those pregnancies with ultrasounds conducted within seven days of delivery, and with complete ultrasound measurements of head circumference, abdominal circumference, biparietal diameter, and femur length were included in the study. The mean percentage difference between estimated fetal weight (EFW) and birth weight was used as a measure of accuracy, while the percentage of EFWs falling within 5% and 10% of birth weight was used as a measure of precision. The accuracy and precision of each formula was assessed by fetal sex, birth order, chorionicity, birth weight, gestational age, and presence of maternal disease or obesity.

Results: The correlation between EFW and birth weight was high for all five formulae examined. However, the Ong formula, which has been developed in twins, exhibited the overall highest accuracy (2.41%; 95% CI:1.03, 3.79), and highest accuracy regardless of maternal or fetal characteristics. While the Combs formula performed with the highest precision (74.4% of EFWs within 10% of birth weight), the Ong formula performed comparably. Fetal sex, and birth order influenced the accuracy of estimated fetal weight formulae, while chorionicity, presence of maternal hypertension, diabetes, or obesity showed no significant influence when accuracy was compared using the Student’s t-test.

Conclusion: The Ong formula, which only incorporates abdominal circumference and femur length performs better in twins than the four other formulae, which have been developed in singletons. This is encouraging given that it is difficult to obtain head measurements in twins due to uterine over-crowding and fetal presentation in twin pregnancies. However, overall, EFW calculated by all five formulae show a high correlation with birth weight.

Introduction

Twin pregnancies are known to be of higher risk for adverse perinatal outcomes, preterm delivery and intrauterine growth restriction (IUGR) compared to singletons.[1-8] Moreover, twins are known to demonstrate fetal growth trajectories that are distinct from those of singletons, with twin median fetal weights falling significantly below those of singletons around week 30 of gestation.[1] Thus, the estimation of fetal weight is an important component of the clinical management of twin pregnancies.

Since the routine use of ultrasound in pregnancy began, a number of research groups have used *in utero* biometric measurements, such as head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) in different combinations to estimate fetal weight.[9-12] However, while a number of formulae have been developed in singletons, to our knowledge, there is only one formula, the Ong formula, that has been developed specifically in a twin population.[13]

Research groups have examined the performance of formulae developed in singletons in twin populations, with variable results. The Warsof formula[12] has been found to perform comparably in singletons and twins,[14] as have the Hadlock formulae (incorporating HC, AC and FL or AC and FL or AC and BPD),[15] and the Shepard formula.[10, 16] In twins, just as in singletons, the Hadlock and Shepard formulae have been observed to perform poorly at very low birth weights.[10, 16]

Other factors that have been suggested to affect the accuracy of estimated fetal weight (EFW) are inter- and intra-observer variability in obtaining measurements.[17-19] Operator experience has also been suggested to affect the accuracy of EFW measurements,[20] while there is conflicting evidence on the effect of maternal body mass index (BMI),[21-22] chorionicity,[23-24] and birth order.[24-25] Fetal sex has been suggested to have no or minimal influence on the accuracy of EFW.[21, 24, 26] The presence of IUGR in a twin increases the error associated with estimating its fetal weight.[24]

Within this context, and as part of a larger project involving the construction of an ultrasound-based fetal weight reference for twins, we assessed the accuracy and precision of five commonly used formulae to estimate fetal weight in twins using ultrasounds conducted within seven days of delivery, and comparing EFWs with birth weights.

Methods

This was a retrospective cohort study based in a tertiary care centre, the Royal Victoria Hospital (RVH) in Montreal, QC. Data were derived from the McGill Obstetrical and Neonatal Database (MOND), which records antenatal and postnatal information on all women delivering at the RVH and their infants, and hospital ultrasound records, which contain information from all ultrasounds performed at the hospital.

Merging of the databases

We merged the MOND and ultrasound records in order to compare EFW by ultrasound with birth weight. In twins, at the time of the earliest ultrasound, a letter assignment ("A" or "B") is made for each fetus. Each twin then retains this letter as an identifier in the ultrasound database, and at any subsequent ultrasounds. At the time of birth, however, a letter is assigned and recorded separately in the MOND based on the twin's birth order: first-born twins are labeled "A" and second-born twins are labeled "B". As the ultrasound letter assignment is made early in pregnancy and the two databases are independent, it is impossible to ascertain whether the letter assigned at birth corresponds with the letter assigned at the time of ultrasound.

Therefore, in order to ensure an exact match between the ultrasound and birth record of each fetus in a twin pair, we only included discordant-sex twin pairs, in which a match could be made based on the sex of the fetus, and those twin pairs that had a match between a variable (the last EFW) that appeared in both the MOND and the ultrasound database. The last estimated fetal weight is derived from the ultrasound records and recorded in the MOND. However, in cases of inaccurate entry or in cases where the last

EFW was the same in both twins, it was not possible to use this variable as a unique identifier for each twin.

Pregnancies in which at least one fetus had a congenital anomaly or died, underwent spontaneous or iatrogenic fetal reduction, or had missing information on HC, BPD, AC or FL were excluded from our analysis. Moreover, our sample was restricted to those women who delivered within seven days of ultrasound, in order to allow a comparison of EFW with birth weight.

We assessed two Hadlock formulae, the Shepard formula, the Ong formula and the Combs formula for their accuracy and precision in this study sample. Table 1 provides details of the formulae assessed in this study.

Statistical Analysis

We examined the correlation coefficient between EFW calculated by each formula and birth weight, when ultrasounds were conducted within seven days of delivery.

In order to measure accuracy, the mean percentage difference, the difference between EFW and birth weight as a percentage of birth weight, was calculated for each formula. The mean percentage difference then allowed us to assess whether a formula, on average, over- or under-estimated birth weight. Moreover, we estimated the percentage of observations within 10% of birth weight as the primary measure of precision, although the number of observations within 5% of birth weight was also considered. Measures of accuracy and precision were compared when the interval between ultrasound and delivery was within seven days, and within two days.

We used the Student's t-test to compare the accuracy of each formula between groups (eg. Male vs. Female; Monochorionic Vs. Dichorionic, etc.)

Covariates

We conducted analyses to assess accuracy of the formulae by stratifying on fetal sex, birth order, chorionicity, 500g birth weight categories (<2500g, 2500-2999g, 3000-3499g, 3500-3999g), two-week strata of gestational age (<30 weeks, 30-31 weeks, 32-33 weeks, 34-35 weeks, 36-37 weeks, ≥38 weeks) and, in the cases of maternal obesity, hypertension or diabetes.

Maternal obesity was defined as a pre-pregnancy BMI of 30 kg/m^2, calculated from pre-pregnancy weight and height, which was either self-reported or charted at the first antenatal visit. Those mothers missing height or weight estimates were excluded from analysis. Gestational hypertension was defined as pregnancy-induced hypertension, varying in severity from mild pre-eclampsia to the HELLP syndrome. Diabetes was defined as any pre-existing or gestational diabetes developed during pregnancy, regardless of severity.

Results

Our study sample consisted of 242 twins who had one ultrasound each within seven days of delivery. The mean birth weight of this group was 2363 g, which was significantly lower than the mean birth weight of the hospital-based twin population (2535g) from which this smaller group was drawn. Of the sample, 54.5% were born weighing ≤ 2500 g compared to 45.2% of the original twin population, and 67.8% were born preterm (≤ 37 weeks) compared with 52.6% of the original twin population.

In this sample, 49.2% of the fetuses were male and 42.9% of the sample were first-born twins. Of all women, 92 (38.1%) were missing information on either height or pre-pregnancy weight. Therefore, we were unable to calculate BMI for them. Of the remaining 150 women who had complete information on height and pre-pregnancy weight, 9.3% were obese with a BMI ≥ 30 kg/m^2 compared with 11.8% of mothers from the original hospital-based twin population. Pregnancy-induced hypertension was present in 7.4% of the women compared with only 10.9% of the original twin population, and 14.5% had either pre-existing or gestational diabetes, compared with 14.3% of the original twin population.

The correlation coefficients of EFW for each formula with birth weight were comparable, with the Hadlock 1 formula, showing the highest correlation (0.9409).The Combs formula had a correlation of 0.9347, while the Hadlock 2 formula had a correlation of 0.9267, the Ong formula had a correlation of 0.9206, and the Shepard formula had a correlation of 0.9185. The accuracy and precision of each formula, and by maternal and fetal covariates are listed in Table 2.

The Ong Formula

As seen in Table 2, the Ong formula showed the lowest overall mean percentage difference (2.41%, 95% CI: 1.03, 3.79), and the lowest percentage difference in males, females, first-born twins, monochorionic twins, dichorionic twins, infants weighing less than 2500g at birth, infants born to diabetic as well as non-diabetic mothers, in infants born to mothers without pregnancy-induced hypertension, and in mothers born to obese mothers as well as those with BMI $<30kg/m^2$. Moreover, the Ong formula showed the highest precision in infants born between 2500g and 2999g, infants born to diabetic mothers, and those born after 38 weeks.

The Shepard Formula

When the interval-to-delivery was within two days, the Shepard formula had the lowest mean percentage difference, but was not significantly better than the Ong formula, which performed next best. Like the Ong formula, it showed the lowest mean percentage difference from birth weight in females; and the most number of observations falling within 10% of birth weight. Moreover, it showed the highest accuracy in second-born twins, although not significantly different from the Ong formula; in infants born between 3000g and 3499g; and in those infants born between 36 and 37 weeks. Similar to the Ong and Combs formula, the Shepard formula showed the highest precision in second-born twins, and additionally, was the most precise formula in infants born below 2500g, and infants born to mothers with a normal BMI.

The Combs Formula

Overall, the Combs formula showed the highest precision when intervals-to-delivery were within two days and seven days. The Combs formula also showed the highest accuracy in infants born between 2500g and 2999g, those born weighing more than 3500g, those born between 34 and 35 weeks, and in infants of mothers with pregnancy-induced hypertension. Moreover, the Combs formula had the highest precision in infants born to obese mothers, non-diabetic mothers, infants born before 30 weeks and after 36 weeks, and in infants born weighing between 3000g and 3499g.

Hadlock1 Formula

The Hadlock1 formula under-estimated fetal weight regardless of maternal or fetal covariate examined. Despite the fact that the accuracy of this formula was low, the standard deviation of the mean percentage difference was consistently the lowest across strata, implying that the Hadlock1 formula performs well with respect to precision. Moreover, it showed the highest accuracy and precision in those infants born between 30 and 31 weeks, and the highest precision in those born between 32 and 33 weeks, when the number of observations falling within 5% and 10% of birth weight were used to measure precision.

Hadlock2 Formula

The Hadlock2 formula showed the highest accuracy in estimating the fetal weight of those infants born before 30 weeks of gestation, between 32 and 33 weeks, and when gestational age was greater than or equal to 38 weeks.

Accuracy by Covariates

When the study sample was stratified by fetal sex, the Hadlock1 and 2 formulae performed significantly worse in male compared to female fetuses, while the Ong and Combs formulae performed worse in females compared to males. When birth order was considered, the Hadlock1 formula performed significantly worse in first-born compared to second-born twins, while the Hadlock2, Ong and Combs formulae performed significantly worse in second-born twins compared with first-born twins. The accuracy of all the formulae were similar regardless of chorionicity, whether mothers were diabetic or not, whether mothers had pregnancy-induced hypertension or not, and whether mothers were obese or not.

Discussion

All five formulae tested in this sample showed a high correlation between the EFW and birth weight. Moreover, they all showed fairly high accuracy, with only five instances in which the absolute value of the mean percentage error was larger than 10%. Three of these were for the Hadlock1 formula when birth weight was between 3000g and 3999g or gestational age was greater than or equal to 38 weeks. The fourth case was for the Ong formula when birth weight was greater than 3500g, and the fifth was for the Combs formula when gestational age was between 30 and 31 weeks.

Overall, and regardless of the maternal and fetal covariates being examined, the Ong formula had low mean percentage errors relative to the other formulae. Even in those cases, where another formula performed the best with respect to accuracy, the Ong formula was the next best performing formula. The Ong formula was ranked below two or more formulae only when gestational ages were below 32 weeks. Although, as mentioned above, the Ong formula showed a high mean percentage difference in those infants born weighing more than 3500g, this may be of less concern in this population due to their general tendency towards lower birth weights.

The Combs formula tended to have the most number of observations within 10% of birth weight, while the Ong formula performed comparably with respect to precision. Between 21% and 80% of observations fell within 10% of birth weight, depending on the stratum of the covariate being analyzed. The low value of 21% was in those fetuses, who were born before 30 weeks of gestation. Similarly, the Ong formula performed poorly in the infants born before 32 weeks, with only 50% of observations falling within 10% of birth weight and, among those born after 38 weeks, with only 56% of observations falling within 10% of birth weight. As is the case with birth weight, in light of the fact that most twins are born at or before 38 weeks, this may be less of a concern in this population. However, on the other hand, extremely preterm infants and those who are born very late in gestation may be the very groups in which accurate estimation of fetal weight may be important.

The tendency of the Hadlock1 formula to under-estimate fetal weight was consistent across the entire sample. Moreover, the observed high mean percentage difference of the Hadlock1 formula was in contradiction with results from singletons.[10, 27] A previous study by Hadlock et al. found that the Hadlock1 formula over-estimated fetal weight in those infants born <2500g, which was also in contradiction with the results from our study.[10]

Surprisingly, we found that fetal sex influenced the accuracy of the Hadlock formulae, the Ong and Combs formulae. Previous literature has found that fetal sex does not influence the accuracy of EFW. [21, 26] Birth order was also found to influence the accuracy of formulae with the Hadlock1 formula performing worse in first-born twins, and the Hadlock2, Ong and Combs formulae performing worse in second-born twins. Although the presence of maternal disease, such as pregnancy-induced hypertension is associated with higher risk of IUGR, and IUGR is known to affect the accuracy of EFW, the presence of pregnancy-induced hypertension itself did not influence the accuracy of the formulae. Also, we found that maternal BMI, chorionicity, and diabetic status of the mother did not influence the accuracy of the formulae.

The comparisons conducted here were exploratory, since we were restricted by a small sample size. Ideally, in a larger cohort, a more detailed exploration of the influence of maternal and fetal covariates, as well as the influence of covariates such as interval between ultrasound and delivery, would be conducted using regression analysis in order to assess the magnitude of their effect on accuracy.

Factors that may have influenced the results of our study could include our choice of population. Due to database limitations in the identification of fetuses, we were forced to use a much smaller sample than originally intended. The formulae for EFW were compared in a sample that selected for discordant-sex twin pairs and for those with different last recorded EFWs (i.e., those twin pairs that were discordant in fetal weight); it is thus possible that our results are not generalizable beyond this population.

Moreover, the fact that women delivered so close to receiving their last ultrasound could introduce bias in our study, if the reason for receiving ultrasounds so late was a high-risk pregnancy (e.g. maternal disease) or suspicion of IUGR, or if findings from the ultrasound prompted iatrogenic intervention. This would have resulted in the inclusion of fetuses that were less healthy than those that were not monitored so close to delivery. However, this is a limitation of all retrospective studies that attempt to compare EFW with birth weight. The alternative would be to carry out a prospective study, or to use an EFW from earlier in pregnancy and adjust for average estimated daily or weekly growth till delivery, although this approach is associated with bias as well.[26]

Conclusion

Although all the five formulae tested performed fairly well in this population, in this preliminary analysis, the Ong formula, which incorporates AC and FL, appears to have the highest accuracy regardless of maternal and fetal covariates. This finding is encouraging as it obviates the need for head measurements, which are difficult to obtain late in pregnancy when the fetal head is engaged, or more pertinently to this study, in twins, where uterine over-crowding leads to difficulties in obtaining head measurements.[28]

Tables and Figures for Manuscript 2

Table 1: Formulae assessed in study

Authors	Formula
Hadlock 1	Log (Fetal Weight)=1.5662-(0.0108 x HC)+ (0.0468 x AC)+(0.171 x FL)+(0.00034 x HC^2)-(0.00386 x AC x FL)
Hadlock 2	Log (Fetal Weight)=1.304+(0.05281 x AC)+(0.1938 x FL)-(0.004 x AC x FL)
Shepard	Log (Fetal Weight)=(0.166 x BPD)+(0.046 x AC)-(0.002546 x AC X BPD)-1.7492
Ong	Log (Fetal Weight)=(0.0259 x AC)+(0.6720 x FL)-(0.0475 x FL^2)-2.7606
Combs	Fetal Weight=(0.23718 x AC^2 x FL)+(0.03312 x HC^3)

*HC: Head Circumference; AC: Abdominal Circumference; FL: Femur Length. All Biometric Measurements are in cm.

Table 2: Accuracy and Precision for Each Formula by Maternal and Fetal Covariates

Covariate	Sample	Formula with highest accuracy	Accuracy (95% CI)	Formula with highest precision	% of observations within 10% of birth weight
Interval-to-Delivery	≤7 days) (n=242)	Ong	2.41% (1.03, 3.79)	Combs	74.38%
	≤2 days) (n=101)	Shepard	1.26% (-0.69, 3.2)	Combs	80.2%
Fetal Sex	Male (n=119)	Ong	0.84% (-1.09, 2.77)	Shepard	75.63%
	Female (n=123)	Shepard	3.51%(1.32, 5.71)	Combs	74.79%
Birth Order	First	Ong	-0.07% (-2.06,	Combs	79.81%

	Order (n=104)		1.91)		
	Second Order (n=138)	Shepard	3.02% (1.06, 4.97)	Shepard, Ong, Combs	70.28%
Chorionicity	Monochorionic (n=20)	Ong	2.79%(-3.82, 9.41)	Shepard	75%
	Dichorionic (n=212)	Ong	2.63%(1.19, 4.06)	Combs	74.5%

Table 2: Accuracy and Precision for Each Formula by Maternal and Fetal Covariates (Contd.)

Covariate	Sample	Formula with highest accuracy	Accuracy (95% CI)	Formula with highest precision	% of observations within 10% of birth weight
Birth Weight	<2500 g (n=128)	Ong	3.89% (1.78, 6.01)	Shepard	70.31%
	2500g-2999g (n=85)	Combs	1.96% (0.26, 3.65)	Combs	81.18%
	3000g-3499g (n=26)	Shepard	0.19% (-3.9, 4.27)	Hadlock2	80.77%
	3500g-3999g (n=3)	Combs	-4.64% (-22.26, 12.97)	NA	
Gestational Age	<30 weeks (n=14)	Hadlock2	0.41% (-6.85, 7.67)	Combs	71.43%
	30-31.86 (n=6)	Hadlock1	-3.56% (-9.95, 2.84)	Hadlock2	83.33%
	32-33.86 (n=21)	Hadlock2	0.6% (-3.89, 5.1)	Ong	76.19%
	34-35.86 (n=49)	Combs	2.61% (1.17, 6.39)	Ong	75.51%
	36-37.86 (n=127)	Shepard	3.1% (1.49, 4.71)	Combs	81.1%
	≥38 weeks (n=25)	Hadlock2	0.99% (-3.23, 5.22)	Combs	76%
Maternal Diabetes	Non-diabetic (n=207)	Ong	2.98% (1.55, 4.42)	Combs	72.95%
	Diabetes (n=35)	Ong	-1% (-5.44, 3.44)	Combs	82.86%
Pregnancy-Induced Hypertension	No pregnancy-induced hypertension (n=200)	Ong	2.08% (0.5, 3.66)	Combs	77%
	Pregnancy-Induced Hypertension	Combs	3.58% (-1.07, 8.24)	Ong	69.05%

Maternal Obesity (BMI≥30 kg/m^2)	Maternal obesity (n=14)	Ong	1.93%(-0.05, 3.91)	Combs	92.86%
	Normal BMI (n=136)	Ong	2.52% (-4.32, 9.37)	Shepard	71.32%

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Chapter 6: Discussion

6.1 Interpretation of Findings

To date, to our knowledge there has not been any work comparing formulae to estimate fetal weight in twins born at the RVH. Despite the limitations encountered when merging the two databases, and the resulting restricted sample, there was convincing evidence that, in the examined sample, the Ong formula performed consistently better than the other formulae. Moreover, it is encouraging that all the formulae tested had mean percentage errors below 15%, and most had mean percentage errors below 10%. The ultrasound machines at the RVH currently use the Hadlock formula, which incorporates HC, AC, FL and BPD.[215] The five formulae tested, all of which use fewer biometric measurements than the Hadlock formula in the estimation of fetal weight, performed well. Since, in twins it is difficult to accurately measure the head due to uterine over-crowding, it is useful to note that these formulae perform with high accuracy and precision.

The fetal weight references that have been constructed from this twin population could be useful for the clinical management of pregnancies. Although the cross-sectional assessment of weight-for-gestational-age will not be sufficient to determine whether a fetus attains its own growth potential, serial ultrasounds and the use of references for comparison could be useful in monitoring longitudinal growth. For instance, if an infant is physiologically small, it may rank in the 10th percentile of fetal weight distribution in the second trimester. If the fetus then remains at the 10th percentile in the third trimester, this is more likely to imply normal growth than the situation where a fetus shows a decline in its percentile ranking, which would suggest IUGR. Therefore, although cross-sectional in nature, the references generated in this study could be of clinical use for monitoring fetal growth over the course of pregnancy.

6.2 Strengths

Although there has been extensive research conducted in singletons and a number of birth weight references created for use in twin populations, this study is one of the few that has constructs a fetal growth reference using *in utero* estimations of fetal weight.

Moreover, the high accuracy and precision of the Ong formula, which has been specifically developed in twins and only uses AC and FL to estimate fetal weight, is an advantage in a population in which head measurements are notoriously difficult to obtain due to over-crowding.[186] This is also potentially useful in estimating fetal weight when mothers are in labour, and the fetal head is engaged.[176]

Since data were available for over ten years from a tertiary care center, the sample size for this study was substantial. The inclusion of more than one ultrasound observation per infant further added to the sample size. Although Altman and Chitty recommend against the use of more than one ultrasound observation per infant, due to the correlation of serial measurements taken on the same fetus,[241] this was accounted for by using hierarchical (mixed) models. Furthermore, this study appropriately accounts for the correlation between twins born to the same mother.

Unlike the previous studies in twins that model fetal growth, the response variable was log-transformed, in order to ensure that the assumptions of homoscedasticity of residual errors and normal distribution of the response variable were not violated.[223] In fact, when the fit of the models with and without log-transformation of the fetal weight variable in preliminary analyses were compared, there was a large difference in fit as measured by AIC, with the model including log fetal weight showing far superior fit than the model including the non-transformed fetal weight variable.

Most studies use an arbitrary threshold as being indicative of small-for-gestational age.[12] Fetal size distributions and deviations are presumably better described along a continuum and not at a threshold that is statistically defined. Therefore, the 1st, 10th, 50th, 90th and 99th percentile fetal weight values were included for reference.

6.3 Limitations

Percentile Values

The small distances between the 1st, 10th, 50th, 90th, and 99th fetal weight percentile values are of concern. When compared with the study by Min et al.,[228] for instance, which had a larger sample size than this study (1,831 pregnancies and more than one ultrasound observation per fetus), the 10th and 90th percentile values from this study are much closer to the median fetal weight value for each gestational age.

Predicted values from a regression model, in general, will have a narrower distribution than the original study sample distribution. Moreover, accounting for the two levels of clustering may have captured much of the variance around the parameter estimate in the present study. This was further evidenced by the low residual standard error observed in the overall sample. However, the fact that the 1st and 50th or the 50th and 99th percentile values were relatively close somewhat limits the clinical applicability of these references, especially in the light of error associated with ultrasound estimations of fetal weight, and may lead to the over-estimation of the prevalence of SGA when used for comparison.

While, theoretically, the most appropriate way to model fetal growth in twins with serial ultrasound measurements would be to account for the two levels of clustering, in reality, it may not have affected our results much. In fact, when examining the intraclass correlation coefficient (=0), clustering did not explain the variability in fetal weight, indicating that the twin clusters could be ignored. Moreover, when examining the AIC values, a model that adjusted for the two levels of clustering performed as well as one that did not adjust for any clustering, and treated twins, and serial measurements on them as independent.

Assessment of size rather than growth

As early as 1989, Altman described the conflation of fetal size and growth in literature focusing on IUGR in fetuses.[241] A single measure of fetal size is not an adequate marker of fetal growth, even when compared to the distribution of fetal weights for that gestational age. A single cross-sectional comparison of fetal size cannot distinguish between those fetuses that may be small but healthy and growing normally, and those that appear to be AGA at the time of measurement, but either experienced a deviation in growth prior to this point, or will in the future. In order to appropriately assess fetal growth, two or more longitudinal measurements are required on the same fetus, although this approach may still miss growth restriction that may have set in at an earlier point in pregnancy. There is, however, some evidence that suggests that the identification of an SGA fetus is at least as good in predicting adverse perinatal outcome as the identification of deviations from a fetus' own growth trajectory.[9, 242] These studies have, however, been conducted using birth weight references and thus need to be corroborated using ultrasound-based references.

The Influence of Zygosity

In this study, fetal weight references were stratified on chorionicity. However, as information on zygosity is not available in the MOND, it was not possible to take into account any potential impact of zygosity on the degree of correlation between fetal weights of twins born to the same mother, or difference in fetal size.

Number of Ultrasounds Conducted

A general limitation of all studies that use serial ultrasound measurements to construct size references is the clinical indication associated with ultrasound. When a mother has more than one ultrasound, it is possible that this is due to fetal distress or suspected IUGR. Inclusion of these fetuses in the construction of size references could bias the results towards smaller predicted EFW.[241] This is less of an issue with this study since twins are closely monitored as a rule. However, the fact that some of these ultrasounds were performed as a result of suspicion of fetal distress or growth restriction cannot be ruled out.

Study Sample

Altman and Chitty recommend using as unselected a population as possible in the creation of fetal size references, and not selecting a population based on information that is not available at the time of ultrasound.[241] The exclusion of fetuses with congenital or structural abnormalities or cases of fetal death, even if discovered later, are the only exceptions they allow. With this in mind, the study sample was restricted to live-born twins without congenital and chromosomal anomalies. However, pregnancies were excluded if iatrogenic fetal reduction occurred since the timing of reduction was unknown, and since it was not possible to ensure that the ultrasound record of a reduced fetus would mistakenly be matched with the birth record of a surviving one.

The exclusion of these fetuses from analysis could have resulted in a selection bias, since this ultimately resulted in the exclusion of a sub-sample of live-born twins. For instance, if surviving twins in the case of a multifetal pregnancy reduction are smaller than spontaneous twins, for which there is some evidence,[120-123] excluding these pregnancies would inflate the predicted fetal weight values from this study relative to a more general population. The exclusion of fetuses with congenital anomalies or those who died *in utero*, and their co-twins may have additionally resulted in a shifting of predicted fetal weights for each reported percentile to the right. Moreover, we excluded those ultrasound observations that were missing information on biometric measurements. This may have also resulted in a bias if, for instance, the reason that biometric measures were not recorded at the time of ultrasound was that the ultrasound was conducted due to an indication for fetal distress, or monitoring due to maternal disease. This would have, again, resulted in the exclusion of fetuses that may have shifted the fetal weight references to the left.

The inclusion criteria specified in this study resulted in a general fetal weight reference, and did not describe fetal weight changes that could be described as “ideal” in a healthy population ascertained retrospectively after birth. There is value in the description of what could be termed “ideal” growth for the purposes of comparison, defined as either fetal growth in low-risk pregnancies or associated with the lowest risk of adverse perinatal outcome. This could be an avenue of future exploration.

Random Error Associated with Ultrasound

The random error associated with calculating fetal weight by ultrasound was a limiting factor in this analysis as in any ultrasound-based study, mitigated somewhat by the validation of the Ong formula in our population. If the error was 10% or greater, then the cut-off of the 10^{th} percentile as representative of SGA would lose its value. This would be exacerbated in this study due to the close values of the predicted percentile fetal weight values.

Database Limitations

Since the MOND does not record race or ethnicity data of the mother or infant, it was not possible to explore the influence of these factors on the fetal weight distribution at each gestational age.

6.4 Future Research

This research project was meant to create a fetal weight reference for a general hospital-based population of twins. Therefore, fetal weight references were only presented specific to sex and chorionicity. However, race, parity, and the use of ART are all known to influence fetal weight.[145] Future research could examine the influence of the above covariates on fetal weight, although, for race, additional information from census records or based on the mother’s names recorded on birth certificates may be required.

The ability of this fetal weight reference to identify an SGA fetus and its association with clinical outcomes relative to other fetal weight or birth weight references is important, and could be explored in future studies. Such an assessment could be either cross-sectional or longitudinal in nature. For instance, the value of the identification of an SGA fetus from a cross-sectional comparison with different references at a particular time point during pregnancy could be compared.

Alternatively, as outlined above, the weight of a fetus at a subsequent ultrasound or at birth, conditional on its weight and its percentile in the weight distribution at a previous ultrasound could be explored as a means of identifying a growth-restricted fetus. The ability of the current method to identify an SGA fetus could be compared with other conventional means of identifying clinically relevant SGA status, for instance, a cross-sectional comparison with a birth weight reference. Hutcheon et al. have previously used this approach in a singleton population.[9] While a significant difference was not found between this method, and the use of a birth weight reference, this may have been due to the small number of ultrasounds available in singletons.[9] In twins who are more highly monitored, this method may have more value.

Conclusion

Despite the limitations faced in merging two independent databases in a twin population, the use of the Ong formula was validated to estimate fetal weight in this particular sub-sample from a tertiary-care hospital. Moreover, a fetal weight reference was constructed for this population, stratifying by sex and chorionicity and adjusting for the clustering inherent in twin pregnancies and the use of longitudinal measurements. However, it may not have been necessary, in this study sample, to adjust for clustering by pregnancy or for the serial correlation between ultrasound measurements on the same fetus. Finally, more research is needed to explore the limitations outlined in the current study and to validate these references in other populations and larger samples.

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APPENDIX 1: Procedure for merging the MOND and Ultrasound Records

Preparation of Datasets Prior to Merge

Preparation of the McGill Obstetric and Neonatal Database (MOND)

There are two versions of the MOND dataset, one for the period up to 2000, and the other for the period between 2001 and 2006. Using coding manuals for both periods, variables were re-coded and renamed in order to ensure that both versions were consistent. Then, the two versions were appended to include birth records for all infants born between 1996 and 2006. Data between April 1, 1997 and March 31, 1998, and between April 1, 2000 and March 31, 2001 was missing due to administrative reasons (Ms Danielle Vallerand, personal communication)

Preparation of Ultrasound Records

Since the recording of information in the ultrasound database has remained consistent over time, it was ready to be merged with the MOND database. Pertinent variable were renamed to be consistent with naming of variables in the MOND.

Merging of Ultrasound Records and MOND for Construction of the Fetal Weight Reference (Primary Objective)

The mother's MRN, and the birth order of the twin are unique identifiers of the twin in the MOND dataset. Conventionally, the twin that presents first at the time of delivery is labeled the "A" twin and the one that presents second is labeled the "B" twin. This would correspond with the first order and second order twins respectively. This would potentially have been a straightforward method upon which to exactly link the MOND and ultrasound record for each baby, since the ultrasound records also identify each twin based on the mother's MRN and an assigned twin letter which is related to presentation at the time of the earliest ultrasound

However, in the ultrasound records, the twin letter that is assigned at the earliest ultrasound follows each fetus as a unique identifier, regardless of its position as viewed on subsequent ultrasounds. Therefore, a twin that is labeled "A" initially may become the non-leading twin over time and be labeled the "B" twin when it presents second at birth. Since this twin letter assignment is made at the earliest ultrasound visit, the likelihood of a change in position is quite high.

The merge between the MOND and ultrasound records was therefore done using the mother's MRN and infant's birth order as unique identifiers. This resulted in a merged database in which it was not possible to ascertain whether the ultrasound record of a particular fetus, in fact, matched its own birth record in the MOND. However, since the only covariates that were used in analysis were either pregnancy-specific and derived from the MOND (eg. Chorionicity), or were assigned at the time of each ultrasound in the ultrasound database (eg. Sex), an exact match between the ultrasound and MOND records for each baby was unnecessary for the purposes of this analysis.

Merging of Ultrasound Records and MOND for the Validation of Formulae to Estimate Fetal Weight (Secondary Objective)

For the section of the project in which we validated the accuracy of estimated fetal weight, an exact match was required between the ultrasound and MOND records of each infant. Since it was not possible to do this for the entire sample, a smaller dataset was created of those infants for which an exact match was possible based on the following criteria:

Last estimated fetal weight (EFW)

A last EFW derived from the ultrasound records is also entered in the MOND dataset. Therefore, a match is presumably possible based on the mother's MRN and last EFW as a unique identifier of each infant. However, although the last EFW recorded in the MOND is derived from the ultrasound records, it is not necessarily derived from the last conducted ultrasound. Therefore, an algorithm was used that checked the last EFW from the MOND against the fetal weight estimate from each of the ultrasound visits for each fetus. The probability that an infant's last EFW from the MOND incorrectly matched one of its co-twin's fetal weight estimates was low. However, in cases where the last EFW recorded in the MOND was the same for both twins, or where there were data entry errors in recording the last EFW, this strategy was untenable.

Discordant sex

Additionally, sex was used to match the MOND record of each twin with its ultrasound record in twin pairs of discordant sex. However, there were fetuses for which there were entry errors in fetal sex at the time of ultrasound. When these errors were easily identifiable, they were manually corrected and a match could then be verified.

For this part of the project, infants, for whom we were unable to match the last EFW with a fetal weight estimate from one of the ultrasounds, and who were either not part of a discordant sex twin pair, or had either no entry of sex in the ultrasound database or errors that could not be corrected, were excluded.

APPENDIX 2: Results from Sensitivity Analysis

Table 1: Predicted Fetal Weight (grams) (Estimation of Gestational Age by LNMP)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	320	324	330	336	341
23	418	425	432	440	446
24	534	542	551	561	569
25	664	672	683	694	704
26	801	812	826	840	851
27	946	961	978	996	1011
28	1103	1120	1142	1164	1182
29	1277	1296	1319	1343	1363
30	1466	1485	1510	1534	1555
31	1658	1680	1707	1735	1758
32	1844	1870	1903	1936	1964
33	2021	2050	2087	2123	2154
34	2188	2217	2253	2290	2320
35	2335	2367	2407	2448	2481
36	2460	2503	2557	2613	2659
37	2574	2636	2714	2794	2861

Table 2: Predicted Fetal Weight (grams) (Estimation of Gestational Age by Ultrasound)

Gestational Age (weeks)	1st Percentile	10th Percentile	Median Fetal Weight	90th Percentile	99th Percentile
22	270	275	281	287	292
23	343	349	357	365	372
24	444	452	461	471	479
25	583	592	604	616	625
26	758	771	788	804	818
27	963	982	1006	1030	1050
28	1180	1204	1234	1265	1291
29	1378	1404	1436	1469	1496
30	1543	1569	1601	1633	1660
31	1673	1701	1737	1774	1804
32	1789	1822	1865	1908	1944
33	1928	1964	2009	2054	2092
34	2100	2136	2180	2225	2262
35	2285	2326	2377	2429	2473
36	2465	2524	2598	2675	2739
37	2643	2730	2840	2955	3052

Figure 1: Comparison of Median Predicted Fetal Weight (grams) By the Three Methods of Estimating Gestational Age