# Current issues in the development of fetal growth references and standards

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# Abstract

**Purpose of Review:** This paper discusses the current issues in the development of fetal charts and is informed by a scoping review of studies constructing charts between 2012 and 2018.

**Recent findings:** The scoping review of twenty articles revealed that there is still a lack of consensus on how fetal charts should be constructed and whether an international chart that can be applied across populations is feasible. Many of these charts are in clinical use today and directly affect the identification of at-risk newborns that require treatment and nutritional strategies. However, there is no agreement on important design features such as inclusion and exclusion criteria; sample size and agreement on definitions such as what constitutes a healthy population of pregnant women that can be used for constructing fetal standards.

**Summary:** This paper therefore reiterates some of these current issues and the scoping review showcases the heterogeneity in the studies developing fetal charts between 2012 and 2018. There is no consensus on these pertinent issues and hence if not resolved will lead to continued surge of fetal reference and standard charts which will only exacerbate the current problem of not being able to make direct comparisons of fetal size and growth across populations.

Important points:

* A scoping review of 20 studies between 2012 and 2018 showed several differences in the design considerations and methodologies for the development of fetal charts.
* Important study design features requiring consideration to produce these charts include: the type of approach, sampling methods, sample size and the definition of a healthy population of women.
* The current point arousing much debate around the construction of fetal growth charts is whether ethnicity plays a role in fetal growth and if it should be taken into account during the creation of these charts.

**Key words: fetal growth, references, standards, charts, fetal measurements, fetal biometry**

**Introduction:**

A reference or standard chart depicts a family of curves representing a few selected centiles of the distribution of some physical characteristic of the reference population as a function of age. Such charts allow an individual to be placed in the context of like individuals. Charts of measurements are useful for assessing humans at all stages: fetuses, neonates, children, and adults. Adolphe Quetelet (1796–1874) was the first to investigate the statistical properties of anthropometry and apply the concept of the normal distribution to anthropometry data (1). Francis Galton (1822­ - 1911) introduced the use of percentile scores for comparing measurements with the normal distribution using data on attained height from birth to adulthood (2). A first application of this approach was in growth in height, which is normally distributed from birth to adulthood.

Fetal growth monitoring during pregnancy has been an important practice amongst obstetricians usually done to ascertain the health status of a fetus and relevant interventions may be provided when the health of a fetus is compromised (3, 4). Growth charts are intended to aid clinical judgements. Fetal growth charts are primarily used: to compare the size of a fetus with reference data when gestational age (GA) is known at a specified time (5); to estimate GA from fetal size (e.g., crown-rump length, and fetal head circumference are commonly used for this purpose) (6-8); and to assess a fetus’s rate of growth between two time points (velocity) (9, 10). For example, a fetus classified as being >97th centile according to an estimated fetal weight chart would prompt clinicians to either deliver early or consider a caesarean section to avoid complications that may be associated with delivering a large baby.

The systematic review of 83 published reference charts of fetal biometry across 32 countries were identified in 2012 by Ioannou et. al revealed wide variations between the centile values reported by published studies. There was considerable methodological heterogeneity: the charts were based on different populations and created with different sample selection, methodology, and statistical modelling methods (11). The availability of many charts in use is problematic as it has been shown that the choice of a reference chart in a particular setting could have great impact on the assessment of fetal biometric assessments (3, 12). For example, a study by Salomon et. al. evaluated the impact of using different charts and reported between 2.6% and 23.6% of measurements would be classified as abnormal using three different charts of fetal biometry that are commonly used (12). Due to these differences in the data used in the creation of each fetal growth chart, comparisons between them are difficult.

These differences in fetal growth charts with the need to be able to make direct comparisons were the motivation for the World Health Organisation (WHO) in 1995 to advocate for the creation of a single universal chart that could be used globally to assess fetal and child growth which reflects recent health and feeding recommendations of different populations and settings (13). This recommendation attracted several debates over the varying effects of various factors like environmental and genetic influences on fetal growth (14).

However, regardless of which growth chart is used clinically, there are design and methodological constructs that must be taken into consideration. To understand and summarise current issues in the developments of fetal references and standards, we did a quick review (by no means exhaustive) of studies published between 2012 and 2018 whose aim was to construct fetal references or standards. The review was aimed at understanding and highlighting the current debates and schools of thought regarding the development of fetal growth references and standards. We focussed on studies in the last 5 years as they represent a time period where three large prospective studies purposely designed to construct fetal growth charts for wide use were published i.e., the international fetal charts from the INTERGROWTH-21st Project (5), the WHO fetal growth charts intended for international application (15), and the National Institute of Child Health and Human Development fetal charts that were ethnic specific (16).

**Scoping review**

**Search strategy:**

We searched MEDLINE via PubMed from January 2012 to April 2018 to identify studies that constructed fetal growth charts for fetal growth assessment. The search strategy used was adapted from a previous systematic review by Ioannou *et al* (17) shown in Table 1. In addition, reference lists of all articles included were similarly searched for potential relevant articles. The search included book chapters and documents from organisations such as WHO, and the United States Centers for Disease Control and Prevention (CDC) but excluded abstracts from conference proceedings. The initial search yielded 180 articles. After a review of the titles and abstracts, 27 articles were selected for closer consideration. From the 27 articles, twenty studies produced fetal growth references or standards. A summary of the characteristics of the included studies are shown in Table 2 with details on study designs used and the intended purpose of the study.

**Table 1: Search strategy**

|  |
| --- |
| Search strategy |
| (((fetal OR foetal OR fetus OR foetus) AND growth) OR ((fetal OR foetal OR fetus OR foetus) AND biometr\*)) OR (reference adj (curve\* OR chart\* OR index OR indices OR equation\* OR value\* OR range\* OR equation\* OR centile\* OR percentile\*)) OR (biometr\* adj (curve\* OR chart\* OR index OR indices OR equation\* OR value\* OR range\* OR equation\* OR centile\* OR percentile\*)) OR (size adj (chart\* OR curve\*)) OR (dating adj (curve\* OR chart\*))\*Reference Values/Ultrasonography, Prenatal/(ultrasound\* OR ultrasonogra\* OR sonogra\*) |

**Table 2: Summary of the characteristics of the included studies**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Reference** | **Country** | **Sample size (Number recruited)** | **Design** | **Type of population** | **Type of sampling** | **Recruitment period** | **Chorionicity** | **Fetal Measurements** | **Statistical methodology** | **Type of chart** | **Intended use** |
| Shivkumar et al, 2015 (18) | Canada | 642 | Retrospective | All inclusive | Hospital based | >34 weeks | Twins | Weight | Multilevel linear regression models | Reference | Not clear |
| Daniel-Spiegel et al, 2016 (19) | Israel | 79,328 | Retrospective | All inclusive | Hospital based | 14 – 42 weeks | Singleton | BPD, HC, AC and FL | Quintile regression | Reference | Local |
| Dias et al, 2016 (20) | Sri Lanka | 4256 | Cross-sectional | Healthy pop | Population based | 11 – 13 weeks | Singleton | SFH | Linear and polynomial  regression models | Not clear | Not clear |
| Xu et al, 2015 (21) | Ethnic chinese | 313 | Longitudinal | Healthy pop | Hospital based | 11 – 12 weeks | Singleton | HC, AC, FDL, | mixed-effect linear regression models | Standard | Local |
| Briceño et al, 2013 (3) | Colombia | 792 | Cross-sectional | Healthy pop | Hospital based | 12 – 40 weeks | Singleton | HC, AC, FDL | fractional polynomial  regression models | Reference | Local |
| Liao et al, 2012 (22) | Brazil | 200 | Longitudinal | Healthy pop | Hospital based | <21 weeks | Twins | HC, AC, FDL | Multilevel  regression analysis | Reference | Local |
| Gabbay-Benziv et al, 2017 (23) | USA | 2161 | Retrospective | All inclusive | Hospital based | <20 weeks | Twins | FL, BPD, HC | linear mixed model | Reference | Local |
| Araujo Júnior et al, 2014 (24) | Brazil | 31,476 | Cross-sectional, retrospective | Healthy pop | Hospital based | <14 weeks | Singleton | BPD, HC, AC, FL, EFW | polynomial regression models | Reference | Local |
| Araujo Júnior et al, 2014 (25) | Brazil | 333 | Cross-sectional, retrospective | Not clear | Hospital based | 14 – 38 weeks | Twin | BPD, AC, FL and EFW | polynomial regression models | Reference | Local |
| Kwon et al, 2014 (26) | Korea | 986 | Cross-sectional | Healthy pop | Hospital based | 15 – 19 weeks | Singleton | BPD, HC, AC, FL, TL, HL, UL | fractional polynomial regression models | Reference | Local |
| Buck Louis et al, 2015 (16) | USA | 2,334 | Longitudinal | Healthy pop | Hospital based | 8 – 13 weeks | Singleton | BPD, HC, AC, FL, HL | linear mixed models with cubic splines | Standards | International/ethnic-specific |
| Pay et al, 2017 (27) | Norway | 42018 | Retrospective | All inclusive | Hospital based | All gestational ages | Singleton | SFH | Non-linear regression of symphsio-fundal height | Reference | Local |
| Deter et al, 2016 (28) | USA | 119 | Longitudinal | Not clear | Hospital based | >17 weeks | Singleton | BPD, HC, FDL, AC, ThC, HDL, MAC, FAV, FTV | two-level  mixed-effects modelling | Reference | Local |
| Jiang et al, 2013 (29) | Ethnic chinese | 6832 | Cross-sectional | Healthy pop | Hospital based | >16 weeks | Singleton | BPD, AC, FL | Polynomial regression models | Reference | Local |
| Sotiriadis et al, 2016 (30) | Ethnic greek | 1200 | Longitudinal | All inclusive | Hospital based | >16 weeks | Singleton | BPD, HC, AC, FL, FTV | Polynomial regression | Reference | Local |
| Stirrup et al, 2015 (31) | England | 2025 | Retrospective | All inclusive | Hospital based | >14 weeks | Twin | BPD, HC, AC, FL | Multilevel mixed-effects statistical models | Reference | Local |
| Rizzo et al, 2016 (32) | Italy | 8070 | Retrospective | All inclusive | Hospital based | First trimester | Singleton | BPD, HC, AC, FL | Quantile regression | Reference | Local/gender-specific |
| Moety et al, 2015 (33) | Egypt | 334 | Cross-sectional | Healthy pop | Hospital based | 20 – 41 weeks | Singleton | FTV | Multivariable linear regression model | Reference | Local |
| Papageorghiou et al, 2014 (5) | International | 13108 | Longitudinal | Healthy pop | Population based | 9 – 14 weeks | Singleton | HC, BPD, AC, FL, OFD | second-degree fractional  polynomial modelling | Standards | International |
| Merialdi et al, 2017 (34) | International | 1387 | Longitudinal | Healthy pop | Hospital based | First trimester | Singleton | HC, BPD, AC, FL, HL, FFL | polynomial regression methods, using the generalized  estimating equations method | Standards | International |

Fetal biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL), symphysis-pubis fundal height (SFH), femur diaphysis length (FDL), estimated fetal weight (EFL), Humeral length (HL), ulnar length (UL), mid-thigh circumference (ThC), humerus diaphysis length (HDL), mid-arm circumference (MAC), fractional arm volume (FAV), fractional thigh volume (FTV), occipitofrontal diameter (OFD), triceps and subscapular skinfold thicknesses (TSST), Fetal Foot Length (FFL); NC: Not clear

**Summary findings from the scoping review**

Of the twenty studies, three aimed to develop fetal standards and were all longitudinal studies (the INTERGROWTH-21st project, the NICHD fetal growth studies and the WHO fetal study). Two of the three studies which established standards (the INTERGROWTH 21st project and the WHO fetal study were done in multiple countries while the NICHD fetal growth study was done only in the USA. The other seventeen studies were done in single countries: four in Europe, four in Asia, three in North America, four in South America, one in the Middle East and one in North Africa. The purpose of the study by Dias et al (20) was not clear and the remaining sixteen studies produced fetal reference charts. Four of the sixteen studies, which established references, were also longitudinal studies (16, 17, 22, 24) while the remaining twelve studies were all based on a cross-sectional design.

Five studies established references for twins and 11 studies included healthy populations in their samples. The definition of healthy populations varied greatly amongst these studies making comparisons difficult. For example, Dias et al defined healthy pregnant women as those who did not have a relevant past medical history, were not on long term medications, had no evidence of socio-economic constraints likely to impede fetal growth, no use of tobacco, recreational drugs or alcohol use, no evidence of urinary tract infections or renal disease on urinalysis, had a systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg, no diagnosis or treatment for anaemia during pregnancy and not in an occupation with risk of exposure to chemicals or toxic substances (20). Xu et al included women without pre-pregnancy diabetes, pre-pregnancy hypertension, non-smokers during pregnancy, no alcohol consumption during pregnancy, self-reported pre-pregnancy body mass index (BMI) ≥17 kg/m2 or <27 kg/m2, no pre-eclampsia and/or pregnancy-induced hypertension, and non-diabetics (21). Briceño et al included women without maternal disease that may have affected fetal growth, such as hypertension, preeclampsia, diabetes mellitus, and renal disease (3) which was similar to the inclusion criteria of Araujo Júnior et al (women with absence of maternal diseases and absence of fetal malformations on sonography) (24), Jiang et al (women without maternal diseases, such as hypertension, pre-eclampsia, diabetes mellitus, renal disease; multiple pregnancies; and fetuses without congenital malformations, chromosomal abnormalities, or intrauterine growth retardation) (29) and Kwon et al (women without maternal disease possibly affecting fetal growth such as hypertension, diabetes mellitus, renal disease) (26) while Liao et al included women with uncomplicated pregnancies (22). Moety et al also included only women who did not smoke and those without chronic diseases such as chronic hypertension or diabetes mellitus or give history of recurrent abortions (33).

Similarly, the three large prospective studies that developed fetal standards differed in their definition of what they considered as healthy women. In brief, the NICHD Fetal Growth Studies carried out by Buck Louis et al excluded: women who smoked cigarettes or used illicit drugs in the past 6 or 12 months; who drunk ≥1 daily alcoholic drinks; had previous fetal congenital malformation; a history of non-communicable diseases (asthma requiring weekly medication, autoimmune disorders, cancer, diabetes mellitus, epilepsy or seizures requiring medication, hematologic disorders, hypertension, psychiatric disorders, renal disease, thyroid disease), or history of gravid diseases (gestational diabetes, severe preeclampsia/ eclampsia, or Haemolysis, Elevated Liver enzymes, Low Platelet count Syndrome) (16); while the WHO Fetal growth studies included women with no socioeconomic constraints, normal daily caloric intakes and normal BMI (34). For the Intergrowth 21st study, women were selected from urban areas located at low altitudes (<1600m). These areas were free from contaminants such as pollution, domestic smoke, radiation, and other toxic substances. The definition of a healthy population in this study was: no clinically relevant past medical history: no history of sexually transmitted diseases, no history of a previous pregnancy affected by pre-eclampsia/eclampsia, HELLP syndrome or a related pregnancy-associated condition, no clinically significant atypical red cell alloantibodies, negative urinalysis, systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg and commenced antenatal care before 14 weeks of gestation. Optimal nutritional status defined as first trimester maternal height (≥153 cm), body-mass index (BMI; ≥18·5 and <30 kg/m2), and haemoglobin concentration (≥110 g/L) without receiving supplements or long-term medications (5).

We discuss some of the current issues in the development of fetal growth reference charts and standards in turn as informed by results of the scoping review of studies constructing fetal growth charts since 2012. These issues are by no means exhaustive but we believe represent some of key issues attracting debate in this field.

**How charts are constructed – prescriptive vs descriptive approaches**

One of the key differences in fetal growth charts is whether they are designed to be prescriptive or descriptive. Prescriptive charts describe the process of producing biological norms or a desirable target to be achieved or aspired to at individual and population levels (so as to construct growth *standards*). Prescriptive standards show how growth should occur, independent of time and place (35). Prescriptive fetal standards refer to tools developed after carefully sampling healthy populations which have a low probability of fetal growth restrictions or abnormalities thereby limiting the effects of nutritional and environmental influences on growth patterns (4, 36). The emphasis of these standards is to characterise optimal fetal growth and how fetuses should grow in the absence of factors known to affect fetal growth. For human growth, these are usually based on selected populations considered to be of optimal health (eg., adequate nutritional status and at low risk of abnormal growth) for example the WHO Multi-centre Growth Reference Study (MGRS) (37). Until recently, it was generally accepted that observed differences fetal growth were largely due to biological differences between different regions and ethnicities, resulting in a need for population-specific charts. This concept has been challenged by evidence demonstrating similarities in the genetic make-up of different non-isolated populations worldwide (38, 39), and more specifically by recent comparisons finding similarities in early and late linear fetal growth (40) in diverse populations.

In contrast, descriptive charts arecommonly used to produce a reference chart that describes the anthropometry of a given population at a particular time and place, such as a hospital, region, or country. Descriptive reference charts are usually based on an unselected population with minimal exclusion criteria, for example, known risk factors for optimal health. Although they are used more widely, descriptive charts are only relevant to the source population. Different populations will differ in many aspects, such as rates of smoking during pregnancy, malaria, gestational diabetes, and maternal obesity, which can all affect newborn outcomes. In principle, following the descriptive approach requires separate reference charts for each sub-population of interest.

Many descriptive charts are constructed from fetal measurements as a function of gestational age (GA) of the specified population. An alternative type of descriptive chart is the customised chart. Customised growth charts are constructed following a multivariable analysis that accounts for maternal factors known to affect fetal growth such as age, weight, height, BMI, parity, ethnicity, sex of the fetus etc. or paternal factors such as height (41). An example of such a chart is the gestation-related optimal weight curve (GROW) chart by Gardosi et al., (41, 42). Unlike other fetal growth charts, the development of the customised charts does not need to exclude women based on their demographics as they are intended to be individualised and specific for each pregnant woman. The original computer-generated chart used data from 4179 births from a single hospital between 1989-1990 (42) by first obtaining the 10th, 50th, and 90th centiles at 40 weeks gestation and using a mathematical model to determine expected centiles at earlier gestations. The GROW chart was first constructed based on a UK population (42), and the charts have been used in multiple populations including; Australia (43), the United States of America (44) and New Zealand (45).

There is still debate on whether a unified international standard can be applied universally irrespective of location and ethnicity as demonstrated by three large prospective fetal growth studies published between 2014 and 2017 (5, 15, 16, 40).

We summarise the three studies key characteristics and features:

1) The INTERGROWTH-21st Project was based on a prescriptive approach with an aim to construct a single fetal growth standard for each fetal biometry measurement for international use despite ethnic differences based on overwhelming evidence from the WHO-MGRS that growth amongst healthy populations in diverse geographical settings is similar (36). The INTERGROWTH-21st Project was a longitudinal study conducted across eight different geographical settings; Brazil (Pelotas), China (Beijing), India (Nagpur), Kenya (Nairobi), Oman (Muscat), UK (Oxford), USA (Seattle), and Italy (Turin) in healthy populations demonstrated to have minimal constraints on fetal growth. The participant selection involved defining free-living populations in defined geographic areas with evidence of adequate health outcomes in terms of maternal, perinatal and neonatal morbidity and mortality and then selecting healthy pregnant women with good nutritional statuses and low risk of pregnancy complications from the well-defined populations. The INTERGROWTH-21st project recruited over 4000 women prospectively for the construction of fetal standards. The study was conducted prospectively with recruitment carried out in the first trimester of pregnancy to ensure correct dating of the pregnancy. Follow-up antenatal ultrasound scans were performed every 5 weeks (± 1 week) by trained staff with identical ultrasound machines measuring both skeletal (head circumference, biparietal diameter, femur length and occipito-frontal diameter) and fat-based (abdominal circumference) growth measurement.

2) The WHO Fetal Growth Study is the fetal component of the WHO Multicentre Growth Reference Study, which aimed to establish growth charts for clinical use based on populations recruited from multiple countries - a similar aim to INTERGROWTH-21st Project (15). The WHO fetal study was a longitudinal prospective study of 1439 women recruited from ten countries i.e., Argentina, Brazil, Democratic Republic of the Congo, Denmark, Egypt, France, Germany, India, Norway, and Thailand (15). Similar to the INTERGROWTH-21st Project, the study was done prospectively with recruitment of women in the first trimester between 8 – 13 weeks, who had reliable information on their last menstrual period confirmed by an ultrasound scan of the crown–rump length. The women were then scheduled for follow-up ultrasound scans which were performed monthly. The WHO fetal study also measured both skeletal and fat-based growth measurement of: head circumference, estimated fetal weight, both femur and humerus length, abdominal circumference and biparietal diameter. The WHO fetal study focussed on the estimated fetal weight charts to evaluate variation due to country, maternal characteristics (age, height, weight, BMI, and parity), and sex of the fetus.

3) The National Institute of Child Health and Human Development (NICHD) Fetal Growth Study aimed to produce race/ethnic-specific fetal growth standards (16). This contradicts the prescriptive concept that one standard fits all. The study was, however, restricted to four self-reported ethnic groups of Asian, Hispanic, black, and white women in the USA. The study though prospective in nature, was a hospital-based, with women recruited from 12 centres within the USA, who did not have any constraints on fetal growth or development. In total 2,334 women were recruited onto the study, with analysis performed on 1737 pregnancies.The women were recruited prospectively in the first trimester confirmed by a dating scan and were divided into the aforementioned ethnic groups. The women were then allocated into an ultrasound schedule that was designed to capture weekly fetal growth assessment data without subjecting all the women to weekly ultrasound scanning. As such, each woman attended five follow up appointments. Similarly, both skeletal and fat-based measurements were undertaken; crown rump length, head circumference, biparital diameter, abdominal circumference and both the femur and humerus length until delivery.

**Attained size versus growth - utility of fetal growth charts**

There is a subtle difference between the growth of a fetus and the size of a fetus. In principle, size relates to measurements at a specific time, whereas growth relates to a change in size over time. Whilst fetal growth is evaluated from longitudinal measurements i.e., a series of anthropometric measurements made of each fetus at multiple time points (46-49), fetal size is determined at a single time point (35, 50, 51). However, the term fetal growth is often used to describe both of these measurements and is thus sometimes used inappropriately (50, 52) as fetuses which are determined to have abnormal growth may actually be normal in attained size (53). Longitudinal studies can therefore be used to produce both attained size and growth charts are used in different clinical applications, and have different interpretations.

**Population-based sampling versus hospital-based sampling**

The choice of an appropriate sample and target population is of great importance as comparisons and inferences applicable to the general population can be made. The methodology of some of the studies used to develop fetal growth references sampled pregnant women from selected hospitals as opposed to sampling women directly from the population under investigation. The target population from which the women are selected has implications on whether the aim is to develop a reference or standard charts, generalisability and utility of the charts. For example, a chart based on women who are underweight cannot be applied to the general population. Hospital-based sampling could be problematic especially when there are varying levels of health services available to the population and when healthcare is provided by more than one health system service as is the case with several countries (36). This could also be a potential source of bias in several low-income countries were a substantial number of women do not visit hospitals for pregnancy-related monitoring, prenatal and postnatal care.

**Period of inclusion – pregnancy dating**

The period of inclusion of the women into the study is also an important methodological consideration. For example, during the first trimester of pregnancy, there is less variability in fetal growth. Women recruited during this period using the first day of the last menstrual period could have this information confirmed using ultrasonographical evidence by measurement of the crown-rump length as it has been shown to be most reliable between between 9+0 to 13+6 weeks gestation, but not beyond this range (54) and considered an essential part of routine antenatal care. Recruitment after the first trimester, leads to difficulties in ascertaining of accurate dating for estimating the expected date of delivery. A reliable estimate of gestational age is key as it underpins clinical care and allows the expected delivery date to be estimated accurately, and also necessary for developing reference charts. Newborn outcomes such as preterm birth, small-for-GA, large-for-GA, and appropriate-for-GA are all dependent on having an accurate estimate of GA.

**Study design – longitudinal, cross-sectional, and mixed designs**

There are many design challenges for studies that aim to construct growth charts from fetal measurements. Fetal growth charts are designed to either monitor the fetal growth throughout the pregnancy to allow for medical intervention if required, or to determine the size of a fetus at a specified gestational age. Whether a study is longitudinal or cross-sectional in design is dependent on the question that it is trying to answer. Study design is of fundamental importance for any research study as it determines the appropriateness of the study to address the research question, and helps inform the appropriate analysis of the data obtained. Most studies are based on a cross-sectional design (11) that includes only one examination per fetus whereas a longitudinal design includes measurements at more than one time (49). It is common to construct size charts from longitudinal data by simply treating them as cross-sectional, as was done for example in the WHO Multi-centre Growth Reference Study **(**MGRS) (37). The simplest case is a pure cross-sectional design, for example, Chitty et al., took one measurement per fetus at a random time (55). A longitudinal design based on non-replicated data at each time point, ought to address correlated measurements from the same individual.

In contrast, a mixed design incorporates both longitudinal and cross-sectional measurements i.e., some participants are studied longitudinally and others cross-sectionally therefore for any given participant, the number of measurements included may be one or greater. A mixed design can be useful for studying growth intensively in periods of rapid growth using a longitudinal design and less intensively in periods of slow growth using a cross-sectional design. This may be an efficient, cost-effective approach especially for multicentre studies. An example is the WHO-Multi-centre Growth Reference Study (MGRS), which combined a longitudinal study design from birth to 24 months with a cross-sectional study of children aged 18 to 71 months (56). A mixed design is also likely to arise when using routine data collected from individuals requiring close monitoring who are seen more than once.

**Statistical considerations**

Appropriate statistical methodology is key to the construction of fetal growth references and standards. A desirable feature of fetal charts is that centiles change smoothly with GA, and that the selected statistical methodology for fitting centiles provide a good fit to the raw data (47, 48, 57). Some of the key statistical considerations include: (a) an assessment of whether the normality assumption is reasonable, as is usually the case for fetal data conditional on GA; (b) accounting for the increasing variability with gestation that is typical in fetal growth data; and (c) a goodness-of-fit assessment with graphical evaluation of the superimposed centiles should be conducted to compare the predictive model to the raw data.

**Sample size**

There is very limited literature on what to consider when determining the sample size of fetal growth studies (58-61). A systematic review of the methodology used in published ultrasound studies for developing size or pregnancy dating charts found that only 6 of 83 published ultrasound growth or size charts included their sample size calculations in the description of their methodology (62, 63).

Sample size calculations can be based on either parametric or non-parametric methods. Non-parametric methods can be implemented using simulation and bootstrap techniques as has been demonstrated by Harris et. al. (64), Linnet (65) and Jennen-Steinmetz (60). Regression-based methods for sample size can also be evaluated by either non-parametric or parametric approaches, depending on the distribution of the covariate (66, 67). For example, methods based on regression-based limits are commonly used in clinical chemistry studies involving normal reference ranges (68). These methods can be adopted and applied in fetal and neonatal growth studies (69). Formulae for estimating sample size for regression-based reference ranges were first proposed by Royston (59) and later extended by Bellera and Hanley (58). In 2011, Hanley and Moodie (70) proposed a unified approach for sample size, precision and power calculations that considers various study designs. Later in 2016, Hanley (61) discusses sample size considerations for the case of simple and multiple linear regressions. Regression analysis can be used to obtain reference limits that account for factors such as age, gender, and parity with corresponding confidence intervals (CIs) (71-74).

Precision and power are the key factors in the determination of sample size for constructing reference charts in addition to study design (longitudinal, cross-sectional, or mixed), number of repeated measurements per individual, existence of replicate measurements, and practicality (cost, time and manpower) (75). The precision of estimated centiles is inherently variable. Extreme centiles exhibit large imprecision because there are few observations at the extreme ends of the distribution, while the median has the greatest precision. For normally distributed unreplicated data, the standard error of the pth centile is obtained from the standard formula for the variance of a centile:

SEp = SD(1 + ) / n], (76)

where SE is the standard error, SD is the standard deviation of the measurement (which will increase with GA), zp is the value of the standard normal distribution corresponding to the pth centile, and n is the sample size. For example, for the 2.5th or 97.5th centiles, zp = ±1.96, giving SE = 0.08 SD with a sample size of 500 and 0.03 SD for a sample of 4,000. More extreme centiles will require a larger sample size to estimate than less extreme ones for the same precision. It is also advisable and common practice to inflate the calculated sample size by the expected percentage of attrition for the specific setting.

In general, longitudinal studies are more efficient and have greater power than cross-sectional studies. Royston (1995) defined this efficiency as the design factor, D, which is the number of fetuses in a cross-sectional study that would give the same precision as one fetus in a longitudinal study. He used a simulation study of ultrasound-based biparietal diameter and compared the variance of a centile in longitudinal and equivalent cross-sectional designs. He calculated the design factor (effect) to be ~2.3 (77). A longitudinal study thus requires approximately half to a third the sample size of a cross-sectional study to estimate a given centile with the same precision depending on the number of measurements per fetus. In the case of sub-groups or multi-centre studies, a sufficient power may be required in order to explore ethnic-specific (i.e. site-specific) charts.

**Handling data from multiple sites**

Most studies aiming to construct fetal growth references are done in a single centre. The need for a large sample size and greater generalisability leads naturally to a multicentre design, which brings additional challenges. As multicentre studies are rare in human growth studies, the combinability problem is not common. However, the MGRS, INTERGROWTH-21st Project, NICHD, and WHO fetal study were multicentre studies and so faced this problem. Statistical significance is not appropriate for judging combinability, as even unimportant differences can be statistically significant especially in very large samples. Assessing how appropriate it is to pool data from multiple sites is challenging, as a judgment of the similarities in the fetal growth size patterns across the populations must be made. The combinability of studies in a meta-analysis is usually judged qualitatively using the similarity of the studies, such as the similarity of the participants, interventions, and outcome variables. This is akin to the standardised and careful section process employed by the studies which strives to ensure similarity of women selected from different sites. Judgments on similarity of data from different sites depends on quantifying the differences and variability inherent in the data for which there is no standard statistical approach for evaluating what is an acceptable level of agreement.

Some considerations on how to make judgments on similarity of data include: defining a priori a threshold of acceptable differences based on clinical knowledge for judging whether the differences between the centile curves from each site are acceptable before conducting the analysis, conducting a sensitivity analysis of the inclusion/exclusion of specific data to the overall fitted centiles, quantifying the amount of variability that can be attributed to site differences and defining a priori what differences are considered acceptable based on clinical impact or meaningfulness.

For example, the INTERGROWTH-21st Project used the same criterion as the WHO-MGRS where the impact of the consistency and magnitude of differences in each site compared to all sites was judged according to Cohen (78) with differences of 0.5 SD considered to be medium (an ideological criterion rather than a statistical criterion). This criterion is also widely used in the assessment and evaluation of changes in health-related quality of life measures and patient reported outcomes (79). Therefore a difference of 0.5 SD or greater (defined a priori) between the centile curves from a site and the combined data from all of the sites at any GA would indicate that the data from that site were too different to be pooled (57, 80). In addition, a sensitivity analyses involving an assessment of the impact of excluding each site’s data one at a time on the overall fitted centiles derived from all the pooled data is useful in making judgments on whether any single sites data is incompatible with the rest of the data from other sites or countries. It is recommended that multicentre studies should quantify and evaluate the differences between their sites using pre-specified criteria, as was done in the INTERGROWTH-21st Project (40).

**Concluding remarks**

In this paper we have discussed some of the current developments and debates in the construction of fetal references and standards. We have highlighted some issues regarding how fetal growth reference charts are constructed (prescriptive and descriptive approaches), study design and methodological considerations for constructing reference centile charts. Important design features such as inclusion and exclusion criteria, sample size determination, gestational age (GA) estimation, and handling of data from multiple sites for multi-centre studies are seldom well addressed, considered or reported.

As many of these charts are in clinical use today and directly affect the identification of at-risk newborns that require treatment and nutritional strategies, the establishment of fetal biometric charts for use require careful methodological considerations. The observations by David Barker in the late 1980’s, on the association between early growth parameters such as birthweight and the risk of disease in later life (81, 82) leading to the famous “Barker’s hypothesis” reiterates how crucial and important the first 1000 days of life is. They confirmed the already-overwhelming evidence that fetal growth disorders are risk factors for adverse perinatal outcomes and can predispose infants to adult chronic diseases (83-87). These findings on early fetal programming and associated risk of disease in adulthood stimulated lots of interest among researchers culminating into the formation of an international society for developmental origins of health and disease (DOHaD). This is particularly important as there is still ongoing debate on whether a single growth standard chart can be used internationally (4). Those who argue against this suggest significant differences between racial/ethnic constructs sufficient enough for the production of racial/ethnic-specific charts for use in fetal growth monitoring implying a significant influence of a genetic component in fetal growth patterns existing across ethnicities. Proponents for a single growth standard for international use argue that differences observed in fetal growth patterns arise mainly due to socioeconomic factors like nutritional status and environmental exposures (36).

As demonstrated in Table 2, there have been numerous charts developed for both local (e.g., Liao et al. 2012 (22)), international (e.g., Papageorghiou et al. (5) and Kiserud et al. (15)), as well as customised use (88). The current discussion around fetal growth charts is whether ethnicity plays a role in fetal growth and therefore whether it should be taken into consideration in the creation of the charts. A comparison of the INTERGROWTH-21st Project, NICHD, and the WHO-sponsored fetal charts found that there were minimal differences between the three charts in terms of head circumference across gestational ages (36). The INTERGROWTH-21st Project constructed charts from eight diverse populations following similar methodology, recruitment, standardisation and demonstrated that there was great similarity in fetal growth among healthy women who were well nourished and lived in good environments. The NICHD study hypothesised that there are differences in fetal growth by ethnicity and therefore aimed to construct ethnic specific charts. Gardosi et al have always argued for the need of customised charts that account for a woman’s characteristics that are known to affect growth such as height, weight, BMI and have constructed charts that include these variables.

In summary, we have highlighted some of the current issues related to the development of fetal references and standards. The systematic review of fetal charts published in the last five years shows that these issues still recur with different opinions on how these charts should (or shouldn’t) be constructed. There is no consensus on these pertinent issues and hence if not resolved will lead to continued surge of fetal reference and standard charts which will only exacerbate the current problem of not being able to make direct comparisons of fetal size and growth across populations.

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