

**1 International gestational age-specific centiles for umbilical artery Doppler indices: a**  
**2 longitudinal prospective cohort study of the INTERGROWTH-21<sup>st</sup> Project**

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## Condensation

We have produced international standards for umbilical artery Doppler indices, based on prospective, serial measurements from low-risk pregnancies.

## Short title

Standards for umbilical artery Doppler

## AJOG in a glance

*A. Why was the study conducted?*

Many of the studies that have produced reference charts for umbilical artery Doppler indices have methodological limitations, which explain the large differences in the centiles for these indices.

*B. What are the key findings?*

We have produced international gestational age-specific umbilical artery Doppler indices centiles based on the rigorous methodology of the INTERGROWTH-21<sup>st</sup> Project, using a standardized, population based, prospective, longitudinal approach with long term follow up of infants.

*C. What does this study add to what is already known?*

The use of international gestational age-specific centiles for Doppler indices should improve the management of high-risk pregnancies and standardize research outcomes in observational and interventional studies involving umbilical artery Doppler.

## **Keywords**

Umbilical artery, Doppler, ultrasound, Pulsatility Index, Resistance Index, Systolic/Diastolic Ratio, INTERGROWTH-21<sup>st</sup>, reference ranges, perinatal morbidity, perinatal mortality, antepartum testing, INTERBIO, fetal well-being, multinational study, longitudinal study, fetal growth restriction, placenta

## **ABSTRACT**

### **Background**

Reference values for umbilical artery Doppler indices are used clinically to assess fetal wellbeing. However, many studies that have produced reference charts have important methodological limitations, and these result in significant heterogeneity of reported reference ranges.

### **Objectives**

To produce international gestational age-specific centiles for umbilical artery Doppler indices based on longitudinal data and the same rigorous methodology used in the original Fetal Growth Longitudinal Study (FGLS) of the INTERGROWTH-21<sup>st</sup> Project.

### **Study design**

In Phase II of the INTERGROWTH-21<sup>st</sup> Project (the INTERBIO-21<sup>st</sup> Study), we prospectively continued enrolling pregnant women according to the same protocol from three of the original populations in Pelotas (Brazil), Nairobi (Kenya) and Oxford (UK) that had participated in FGLS. Women with a singleton pregnancy were recruited at <14 weeks' gestation, confirmed by ultrasound measurement of crown-rump length, and then underwent standardized ultrasound every 5±1 weeks until delivery. From 22 weeks of gestation umbilical artery indices (Pulsatility Index, Resistance Index and Systolic/Diastolic Ratio) were measured in a blinded fashion, using identical equipment and a rigorously standardized protocol. Newborn size at

birth was assessed using the international INTERGROWTH-21<sup>st</sup> Standards and infants had detailed assessment of growth, nutrition, morbidity and motor development at 1 and 2 years of age. The appropriateness of pooling data from the three study sites was assessed using variance component analysis and standardized site differences. Umbilical artery indices were modeled as functions of the gestational age using an exponential, normal distribution with second-degree fractional polynomial smoothing; goodness of fit for the overall models was assessed.

## Results

Of the women enrolled at the three sites 1629 were eligible for this study, 431 (27%) met the entry criteria for the construction of normative centiles, similar to the proportion seen in the original fetal growth longitudinal study. They contributed a total of 1243 Doppler measures to the analysis; 74% had three measures or more. The healthy low-risk status of the population was confirmed by the low rates of preterm birth (4.9%), and pre-eclampsia (0.7%). There were no neonatal deaths and satisfactory growth, health and motor development of the infants at 1 and 2 years of age were documented. The only a very small proportion (2.8-6.5%) of the variance of Doppler indices was due to between site differences; in addition standardized site difference estimates were marginally outside this threshold in only one of xx comparisons, and this supported the decision to pool data from the three study sites. All three Doppler indices decreased with advancing gestational age. The 3<sup>rd</sup>, 5<sup>th</sup> 10<sup>th</sup>, 50<sup>th</sup>, 90<sup>th</sup>, 95<sup>th</sup> and 97<sup>th</sup> centiles according to gestational age for each of the three indices are provided, as well as equations to allow calculation of any value as a centile and z-scores. The mean Pulsatility Index (PI) according to gestational age (GA) =  $1.02944 + 77.7456 \cdot GA^{-2} - 0.000004455 \cdot GA^3$ .

## Conclusions

We present here international gestational age-specific normative centiles for umbilical artery Doppler indices produced by studying healthy, low-risk pregnant women living in

125 environments with minimal constraints on fetal growth. The centiles complement the existing  
126 INTERGROWTH-21<sup>st</sup> Standards for assessment of fetal wellbeing.

127

## INTRODUCTION

The umbilical artery waveform, obtained using Doppler ultrasonography, reflects the impedance to blood flow in the fetal compartment of the placenta.<sup>1, 2</sup> The ability to assess the umbilical artery waveform using Doppler was first described in 1977<sup>3</sup>; just a few years later, Trudinger and Cook first showed that in normally grown fetuses the impedance decreased with advancing gestation, whereas the impedance increased in growth restricted fetuses.<sup>4</sup> The clinical value of measuring the umbilical artery Doppler is now well-established in high-risk pregnancy as one of the few interventions that reduce perinatal mortality; but not in low-risk pregnancies.<sup>5, 6</sup>

The approach to umbilical artery Doppler acquisition is standardized.<sup>7</sup> However, our recent systematic review of the studies that have produced umbilical artery Doppler reference charts found considerable methodological heterogeneity and limitations in study design, statistical analysis and reporting.<sup>8</sup> High potential for bias in studies reporting on umbilical artery Doppler was noted with only one study being multicenter; with only one study demonstrating comprehensive quality assurance; and only one study reporting that sonographers were blinded to the measurement recorded during the examination. Reference ranges varied significantly with important clinical implications on what is considered normal or abnormal, even when restricting the analysis to the highest scoring studies.<sup>8</sup> For example, in the three studies with the best methodology, the reported 95<sup>th</sup> centile of the umbilical artery Pulsatility Index (PI),<sup>9-11</sup> ranged between 1.28 and 1.48 at 32 weeks and between 1.03 and 1.40 at 39 weeks' gestation. This is important because, apart from absent or reversed end-diastolic flow, Doppler indices are used to monitor high-risk pregnancies over time and contribute to the decisions regarding early delivery. It is easy to see that the differences in what is "normal" or "abnormal" between these studies can result in differences in classification of fetal well-being.

Our aim was to address the methodological limitations identified in our systematic review,<sup>8</sup> so as to produce international gestational age-specific centiles for umbilical artery Doppler indices for use alongside the INTERGROWTH-21<sup>st</sup> Standards for fetal growth;<sup>12</sup> symphysis-fundal height;<sup>13</sup> gestational weight gain;<sup>14</sup> early and late pregnancy dating;<sup>15</sup> newborn size at birth<sup>16</sup> and body composition,<sup>17</sup> and postnatal growth of preterm infants.<sup>18</sup> To that end, we prospectively collected longitudinal data from pregnant women matching the recruitment criteria of the INTERGROWTH-21<sup>st</sup> standards at both population and individual level, because they met the World Health Organization (WHO) prescriptive criteria for optimal health, nutrition, education and socioeconomic status.<sup>19, 20</sup>

## **MATERIALS AND METHODS**

INTERGROWTH-21<sup>st</sup> is an international, multicenter, population-based project. Phase I of the INTERGROWTH-21<sup>st</sup> Project, conducted between 2009 and 2016, consisted of nine complementary studies designed to describe optimal human growth and development, based conceptually on the WHO prescriptive approach.<sup>21</sup> The study sites were eight urban areas worldwide, with no or low levels of major, known, non-microbiological contamination, that were geographically delimited to ensure the study was population-based.<sup>20</sup>

In the Fetal Growth Longitudinal Study (FGLS), one of the components of the INTERGROWTH-21<sup>st</sup> Project, we enrolled, before 14 weeks' gestation, a large cohort of healthy, well-nourished women with a naturally conceived singleton pregnancy who met rigorous individual inclusion criteria<sup>12</sup>. The specific aim was to monitor their babies prospectively until 2 years of age so as to generate international standards.

Doppler measurements were not included in FGLS for pragmatic reasons in the implementation of such a large multi-country project. However, given the lack of robust data supporting the choice of cut-off points for umbilical artery Doppler indices in clinical practice



while assessing complicated pregnancies, we specifically included Doppler measurements in Phase II of the INTERGROWTH-21<sup>st</sup> Project (the INTERBIO-21<sup>st</sup> Study),<sup>22</sup> with the aim of producing international gestational age-specific centiles to facilitate standardization of the technique and the clinical decision-making in high-risk pregnancies.

Phase II of the INTERGROWTH-21<sup>st</sup> Project (The INTERBIO-21<sup>st</sup> Study)<sup>23</sup> aims to improve the functional classification of the preterm birth and FGR syndromes<sup>24, 25</sup> through a better understanding of how environmental exposures (e.g. HIV, malaria), clinical conditions (e.g. pre-eclampsia) and malnutrition influence patterns of human growth from early pregnancy to childhood. Improvements in phenotypic characterization of these complex syndromes at clinical, molecular and biochemical levels may help in the development of better screening and prevention strategies. The INTERBIO-21<sup>st</sup> Study prospectively collected information on pregnancy and perinatal outcomes, newborn anthropometric measures, and the child's growth and development until 2 years of age using the same protocols, standardized tools and data collection systems as in the construction of international fetal growth and newborn size standards in Phase I of the INTERGROWTH-21<sup>st</sup> Project. In addition, a comprehensive set of biological samples was collected. Details on study sites, population characteristics, study design, methodology and standardization procedures for the collection of longitudinal clinical data and biological samples have been reported elsewhere.<sup>23, 26</sup>

INTERBIO-21<sup>st</sup> participants were enrolled following the protocols<sup>22</sup>, data collections system, and standardization procedures, between 2012 and 2015, from six geographically diverse populations worldwide, including three of the eight study sites that also took part in FGLS. Those sites were the cities of Pelotas, Brazil (Hospital Miguel Piltcher, Hospital São Francisco de Paula, Santa Casa de Misericórdia de Pelotas, and Hospital Escola da Universidade Federal de Pelotas), Oxford, UK (John Radcliffe Hospital), and the Parklands suburb of Nairobi, Kenya (The Aga Khan University Hospital).

The selection criteria at the population level in FGLS were: the areas had to be located at an altitude <1,600m with a low risk of fetal and infant growth and developmental disturbances, as well as an absence or low levels of major, known, non-microbiological contamination. Within each area, all institutions classified locally as “private” or “corporation” hospitals and/or serving the middle to upper socioeconomic population were selected, provided that most institutional deliveries from the target population took place there. Women receiving antenatal care had to plan to deliver in these institutions or in a similar hospital located in the same geographical area.

In the INTERBIO-21<sup>st</sup> Study, we enrolled women from the three original FGLS sites (out of six included in INTERBIO-21<sup>st</sup>), *irrespective of their risk profile for adverse pregnancy/perinatal outcomes*, provided they were at least 18 years old; their pregnancy was conceived naturally; they initiated antenatal care before 14 weeks’ gestation, and their body mass index (BMI) was less than 35 to avoid difficulties scanning the overweight.

Umbilical artery Doppler indices were measured in all INTERBIO-21<sup>st</sup> participants. However, only those women who fulfilled the strict FGLS inclusion criteria of optimal health, nutrition, education, and socioeconomic status, contributed data to the present analysis. The aim was to produce centiles using data acquired from healthy, low-risk women comparable to those who participated in FGLS; we have previously adopted this concept and produced an FGLS-like population.<sup>19</sup>

The INTERGROWTH-21<sup>st</sup> Project was approved by the Oxfordshire Research Ethics Committee ‘C’ (reference: 08/H0606/139), the research ethics committees of the individual participating institutions and the corresponding regional health authorities in which the project was implemented. Participants provided written consent to be involved in the study.

### ***Standard procedures***

We enrolled women between 9<sup>+0</sup> and 13<sup>+6</sup> weeks' gestation as determined by ultrasound measurement of crown-rump length.<sup>15</sup> Following the dating scan, women were scanned every 5±1 weeks until delivery. At each visit, we obtained fetal biometric measures and, from 22<sup>+0</sup> weeks' gestation, three umbilical artery Doppler indices: PI (=systolic velocity-diastolic velocity/mean velocity), Resistance Index (RI= systolic velocity-diastolic velocity/systolic velocity) and Systolic/Diastolic Ratio (S/D Ratio). The end-diastolic flow was recorded as present, absent or reversed. Detailed documentation on measurement acquisition protocols, the unique standardization procedures, data collection forms and electronic data transfer strategies are available at the study website.<sup>26</sup>

The technique for acquiring the Doppler indices was standardized across sites based on the following criteria: [1] sample taken from a free-floating loop of the umbilical cord; [2] fetal quiescence ensured, i.e. absence of significant limb/breathing movements; [3] avoidance of venous signal; [4] magnification of the screen with the zoom box so the umbilical artery occupied no less than 50%; [5] sample gate within the center of the vessel; [6] angle correction employed to ensure angle of insonation of less than 30° and confirmed using color Doppler; [7] sweep speed yielded 4-6 consistent waveforms of similar signal; [8] velocity scale of approximately 75% of the peak systolic velocity, and [9] image clarity secured by adjustment of pulse repetition frequency and color gain correction; [10] the average of three waveforms used in the analysis.

The acquisition was repeated if the image quality did not satisfactorily meet all ten criteria. One image was then selected by the sonographer for all three measurements: PI, RI, and S/D ratio.<sup>27</sup> These were performed via auto-tracing of three or more consecutive similar waveforms, from the beginning of the systolic to the end of the diastolic signal, selecting the “limited trace” or “automatic trace” options on the ultrasound machine.

Twenty-four experienced sonographers participated in the study (six in Brazil, eight in Kenya and ten in the UK); all were locally accredited and underwent uniform standardization. To avoid expected-value bias, the ultrasound machines were modified so that Doppler measures were not visible to the sonographer on the screen; this was also the case for data collection of the fetal biometric measures. Only at the end of a completed scan were the measures revealed. All scans were performed using identical ultrasound machines (Philips HD-9, Philips Ultrasound, and Bothell, WA, USA) with curvilinear abdominal transducers (C5-2, C6-3, V7-3). Ultrasound data were entered locally and submitted electronically to the study database.<sup>28</sup> Our umbilical artery Doppler measurements quality control methods have previously been published, which include the inter-observer variability on a large sample of measurements.<sup>29</sup>

The infants in the INTERBIO-21<sup>st</sup> Study were seen at 1 and 2 years of age for a detailed assessment of growth, nutrition, morbidity and motor development. These data were collected by a certified examiner and by interviewing parents. Achievement of milestones ('sitting without support', 'standing with assistance', 'hand-and-knees-crawling', 'walking with assistance', 'standing alone' and 'walking alone') were considered satisfactory if the time of achievement was within the expected WHO windows (< 99<sup>th</sup> centile child age for each of the expected windows).<sup>30, 31</sup>

### ***Statistical methods***

Sample size and justification for the present study was performed prior to analyzing the prospectively collected Doppler data. Sample sizes are based on a balance between pragmatic, biological and statistical considerations. Statistical considerations focused on the precision and accuracy of a single centile which we have demonstrated a posteriori that was adequate.<sup>12, 32</sup> Our selection of the final study sample was mostly guided by biological and pragmatic considerations: the desire to use the same study sites that contributed to the Fetal Growth

Standards of the INTERGROWTH 21<sup>st</sup> Project<sup>12</sup>, providing continuity across the complete set of standards and the need to follow up infants for evaluation of growth and development to two years. Overall, 431 fetuses with 1243 repeated scans were available for analysis which mean that it is (to our knowledge) the largest to date to capture umbilical artery Doppler measures longitudinally in a cohort of pregnancies followed from the first trimester of pregnancy up to 2 years of age. Furthermore, longitudinal studies of fetal growth require half the sample size of a cross-sectional study to estimate a given centile with the same precision.<sup>33</sup> Hence, our cohort of fetuses, contributing 1243 Doppler measures, has the power equivalent to a sample of 2500 measures in a cross-sectional study.

Following the INTERGROWTH-21st Project policy that has been implemented in all our previous publications, we planned to remove from the analyses values that were either implausible within each study site's distribution or not within 5 standard deviations (SD) of the mean of the overall gestational-age specific values<sup>12, 16</sup>. This latter criterion was used, rather than more conservative definitions, to minimize the risk of excluding extreme yet valid cases within a very healthy cohort – a scenario that is made worse whenever measures or indices are not normally distributed and skewed.

First, the heterogeneity in umbilical artery Doppler indices within sites was evaluated using variance component analysis to calculate the percentage of variance in each index due to between-site and within-site differences. Only data from women with three or more scans were used for this analysis. Separate multilevel mixed-effects models were fitted with random intercepts for the study-site and the woman levels (with women nested within sites) and adjustment for gestational age (treated as a fixed effect), using the restricted maximum likelihood option in the STATA 15 (StataCorp. 2017, StataCorp LLC, College Station, TX) *mixed* module.

Second, similarities between sites were measured using standardized site differences (SSD), defined as the site mean of each Doppler index minus the pooled mean for all sites relative to the SD of all sites together, adjusted by the gestational age at which the scan was performed within three pre-specified windows: 23-28, 29-33 and 34-41 weeks' gestation. In line with previous publications,<sup>22,34</sup> pooling the data from different sites was considered appropriate if differences were less than 0.5 SD of the pooled means for each gestational age and measure.

Different distributions and smoothing techniques were explored for the construction of the curves using the GAMLSS (Generalized Additive Models for Location, Scale and Shape) package in R<sup>35</sup> and the *xrml* module in STATA.<sup>36</sup> Starting with the simplest model assuming a normal distribution, goodness of fit was evaluated using the Akaike Information Criteria,<sup>37</sup> quantile-quantile (q-q) plot of residuals, plots of residuals versus fitted values, and the distribution of fitted Z scores across gestational ages to decide if modelling complexity needed to be increased.

In summary, the exponential normal (EN) distribution<sup>36</sup> with second-degree fractional polynomial smoothing<sup>38</sup> was as good as more complex methods with a higher number of parameters that account for skewness and kurtosis in the distribution of the values.<sup>39</sup> Models fitted in a multilevel framework accounting for repeated measurements showed little impact on the estimated centiles. Goodness of fit for the overall models was assessed by comparing empirical centiles (calculated per completed gestational week) with fitted centiles.

Eleven scans, performed on 10 women before 23 weeks' gestation, were excluded from the analysis to avoid edge effects contributing to undesirable model fit at lower gestational ages. Twenty-two scans performed at 23- and 41-weeks' gestation were included in the modeling to stabilize the curves at the tails of the gestational age range. However, reporting

was restricted to the period between 24- and 40-weeks' gestation, which represents the window of established clinical utility.

## RESULTS

### *Population*

Amongst the 1,716 women enrolled at the three INTERBIO-21<sup>st</sup> Study sites that also participated in FGLS, 87 were excluded because of loss to follow-up, withdrawn consent, termination or pregnancy loss, leaving 1,629 with live singleton births. Of these, 434 (27%) fulfilled the FGLS individual criteria, which is similar to the proportion seen in the original fetal growth longitudinal study.<sup>12</sup> Three women had babies with a postnatal diagnosis of a congenital abnormality and were also excluded, resulting in data for analysis from 431 women who had 1243 ultrasound scans. (Figure 1) The contribution of each site to the total study population was 88 women from Brazil (20.4%), 219 from Kenya (50.8%) and 124 from the UK (28.8%).

From 24 to 40 weeks' gestation, there were between 20 and 119 individual scans per gestational week. The median number of umbilical artery Doppler scans per woman was three (range 1-5), with 319 women (74.0%) having three or more measurements. As planned, based on the INTERGROWTH-21<sup>st</sup> policy, we excluded six measures because they were not within 5 SD of the mean of the overall gestational-age specific values. Removing this small number of outliers had no effect on the centiles.

The maternal, pregnancy and newborn characteristics of the women who contributed data to the present analysis (Table 1) were strikingly similar to the baseline characteristics of the original FGLS population whose data were used to produce the international INTERGROWTH-21<sup>st</sup> Fetal Growth Standards<sup>12</sup>.

Assessment of the infants at one (n=329; 76%) and two years of age (n=319; 74%) confirmed their adequate health and nutritional status (Table 2), and that their developmental milestones were reached at a similar age to the infants in the original FGLS, all within the WHO recommended range for these gross motor milestones.<sup>30, 31</sup> (Figure 2).

### *Doppler indices*

For the three Doppler indices, the percentage of the total variance due to the difference between study sites was 2.8% to 6.5%, while the percentage of total variance explained by differences between individuals within a site ranged from 20.9% to 25.4%. In other words, the within-site percentage variance was 4-6 times higher than the between site percentage of the total variance (Table 1S). From the 27 comparisons made across gestational age, only one SSD estimate was marginally outside this threshold: RI SSD for Brazil in the 23-28 weeks' gestational age window = -0.52, (Figure 3). These two findings strongly supported the decision to pool the data from the three study sites to produce the international gestational age-specific normative centiles.

The gestational-age specific 3<sup>rd</sup>, 50<sup>th</sup>, and 97<sup>th</sup> fitted centiles for each of the Doppler indices are shown in Figure 4, along with the observed centiles for each completed week. The comparison between smoothed and empirical centiles suggests that the models have reasonable fit to the data. Gestational age-specific standard values for use in clinical practice for the 3<sup>rd</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 50<sup>th</sup>, 90<sup>th</sup>, 95<sup>th</sup> and 97<sup>th</sup> centiles of umbilical artery PI, RI, and S/D ratio are presented in Tables 3 to 5. The corresponding regression equations for the model parameters are presented in Table 6, along with the equations to calculate Z-scores and centiles.

## **DISCUSSION**

### **Principal Findings**



We have presented here a set of normative values for the interpretation of Doppler measures in the clinical care of high-risk pregnancies. These are based on serial ultrasound measures, obtained prospectively from low-risk, singleton pregnancies in healthy women from three geographically delimited, diverse populations. They match, in their study population and methodology, the comprehensive set of tools previously published for the standardized assessment of fetal, pregnancy, newborn, infant and child growth and developmental parameters. In the present analysis we have overcome the methodological limitations of previous studies by meeting 22 out of the 24 criteria used to evaluate their quality in our systematic review.<sup>8</sup> Crucially, the data were collected from three diverse populations in the context of a large scale project to standardize fetal, neonatal and infant monitoring tools, whereas, with one exception,<sup>11</sup> all past studies were performed in a single hospital with limited relation to other pregnancy parameters or newborn and infant follow up. Remarkably, the proportion of low-risk pregnancies (around 30%), the low adverse outcome rates including preterm birth, and results of long term follow up were similar to the previously observed samples of the INTERGROWTH 21<sup>st</sup> Project demonstrating the interoperability of these basic biological makers when health, nutrition and socio-economic conditions are adequate.

## **Results**

We have confirmed that Doppler indices fall with advancing gestational age as the physiological adaptation of the umbilical-placental bed leads to a decrease in vascular flow resistance.<sup>40</sup> A failure in this physiological process results in increased vascular resistance, evidenced by a fall in diastolic flow. In combination with the increasing demands of the growing fetus on the placenta, there is an increase in PI, RI and S/D ratios.

Uniquely in the literature, we were interested to document that the studied fetuses were clinically healthy at birth and up to 2 years to support the concept that they were eligible for the construction of normative values. We explore this question by assessing the health, growth

and development of the infants up until 2 years of age, as has been the policy with all our standards<sup>18</sup>. We strongly believe that the failure to follow up infants enrolled in perinatal studies in general and in ultrasound studies specifically, particularly those focused on fetal wellbeing, has been a major shortcoming of our specialty. The finding of satisfactory growth, health and neuro-developmental outcomes at 2 years of age, evaluated by researchers masked of the hypotheses tested in the present study, that we have prospectively documented, should provide clinicians with confidence regarding: a) the appropriateness of selecting our study population for determining normative values, and b) the use of the presented centiles in clinical practice during fetal wellbeing assessment, validated against outcomes of long term relevance.

### ***Clinical Implications***

In summary we propose two take-home messages: first, from a biological perspective, we have shown that the feto-placental circulation functions, expressed by these Doppler indexes, are similar across different populations when optimal health, uncomplicated pregnancies, nutritional and environmental conditions are met. As previously reported for early and late fetal, newborn, preterm postnatal growth, infant and child skeletal growth, maternal weight gain, symphysis fundal growth, cerebellum and Sylvian fissure maturation, neurodevelopment and related behaviors and by WHO for term infants and children, the proportional magnitude of the variance in the Doppler indices *between* fetal cohorts from these different study sites is very small (around 5% of the total variance) as compared with the large proportion of the total variance explained between fetuses *within* a study site. This evidence confirms the similarities in fundamental biological human characteristics across regions, ethnic groups and ancestries.

Second, the current reference charts for clinical interpretation of umbilical Doppler indices demonstrate large differences in the 95<sup>th</sup> centile values, which may be having an adverse effect on perinatal outcomes. It is certainly very difficult to generate high-quality, evidence-

based guidelines for the management of the compromised fetus and coordinate referral systems when an important component of the clinical armamentarium offers normal PI value in one chart that is above two standard deviations on another.<sup>41</sup> These inconsistencies should concern clinicians and parents alike. The lack of standardization, which pervades obstetric practice, is probably not found in any other field of medicine that involves such important decision-making. A strong commitment is required in our profession to avoid retaining these patterns of care.

### ***Research Implications***

In the current literature there are large differences in umbilical artery cut-offs. This has several implications for research: individual studies where an abnormal umbilical artery Doppler index is used as an enrolment criterion may be difficult to combine depending on what reference is used; while in multicenter studies, charts used in different institutions may lead to heterogeneous participant selection. The same is true of course where a Doppler index is used as a diagnostic criterion (umbilical PI >95<sup>th</sup> centile figures as a criterion for both early and late growth restriction), or to guide an intervention, such as delivery; a recent study has shown that differences in umbilical artery PI cut-off values would result in differential management in a cohort of small-for gestational age fetuses from 20-40%.<sup>41</sup>

This need to standardize practice is not only relevant to clinical management but also research into fetal growth restriction as we have proposed before.<sup>42, 43</sup> We now need to start defining not just what to measure, but how to measure it; in time this will allow harmonization of care, research and aid better data synthesis of evidence in future.

### ***Strengths and Limitations***

Our work has several unique features and strengths. First, the ultrasound data were obtained with the same degree of scientific rigor, standardization and quality assurance as in

the fetal growth during pregnancy standards we have published<sup>44-46</sup> including using identical ultrasound equipment at each site and a single validated acquisition protocol. Uniquely, we have masked all Doppler values to the sonographers, reducing “expected values” bias often recognized in this field.<sup>46-48</sup>

Second, our achieved sample size compared favorably with the published literature:<sup>8</sup> our study involved 431 fetuses with 1243 repeated scans which mean it is, to our knowledge, the largest to date to capture umbilical artery Doppler measures longitudinally in a cohort of pregnancies followed from the first trimester of pregnancy up to 2 years of age. Furthermore, longitudinal studies of fetal growth require half the sample size of a cross-sectional study to estimate a given centile with the same precision.<sup>33</sup> Hence, our cohort of fetuses, contributing 1243 Doppler measures, has the power equivalent to a sample of 2500 measures in a cross-sectional study. This is reflected in the high level of precision we achieved in the estimation of the centiles i.e. the width of the 95% confidence intervals, when compared with the range of expected values at that gestational age. For example, at the clinically relevant gestational age of 34<sup>+0</sup> weeks, when decisions are made partially based on Doppler values, for the 50<sup>th</sup> centile the width of the 95% CI was 0.02, 0.01 and 0.07 for PI, RI, and S/D ratio, respectively. The values for the 95<sup>th</sup> and 97<sup>th</sup> centiles were 0.04, 0.01 and 0.16, and 0.04, 0.02 and 0.22, respectively.

We accept that the work has limitations. There were two out of the 24 criteria that we identified in our systematic review as required for the construction of ultrasound normative charts, with which we did not comply: the first is that each Doppler measure was only taken once despite our recommendation that ultrasound measures for the construction of standards should be taken in triplicate and the average used in the analyses<sup>8</sup>, as we have done for all previous standards.<sup>12-18</sup> We took only single Doppler measures because, although Doppler ultrasound is considered safe, we felt it was important, in the absence of any obstetric

indication, to minimize fetal insonation in a research study, based on the As Low As Reasonably Achievable (ALARA) principle.<sup>49</sup>

The second limitation was that we did not perform an inter- and intraobserver evaluation. Nevertheless, we have undertaken strict quality assessment using a scoring system that was used in this study and this has been shown to be more reproducible than subjective assessment<sup>29</sup>, therefore all possible measures to improve reproducibility have been addressed.

We did not examine other Doppler parameters that are suggested in maternal-fetal medicine and concentrate on those that are widely used. This was done mainly because this was an already complex prospective study and it is recognized that the addition of more measures and examinations to healthy subjects, burdens participants and reduces follow up compliance; it also increases observers' measure error. It has been our policy to concentrate on the most used perinatal practices as priority for standardization of clinical practice across medical specialties. We hope, that our work should encourage other researchers to adopt a similar approach to other parameters such as the cerebro-placental flow ratio. There is promising evidence that such evaluation could help to predict adverse perinatal and/or neurodevelopmental outcomes in growth-restricted fetuses.<sup>50</sup>

Comparisons with presently used charts are a challenge because of the methodological limitations<sup>8</sup>, including lack of standardization of equipment and measurement methods, pregnancy outcomes, the unreliability of gestational age estimates, observer bias and limited information of the underlying population served by mostly high-risk hospitals. Perhaps, as a result of these limitations, some of the observed patterns are not plausible, for example large up and downs in the values according to gestational age.<sup>11, 51</sup> In addition, INTERGROWTH-21<sup>st</sup> centiles are not intended for comparison with all single hospital charts produced because that will be a never ending process considering the number of institutions around the world

producing such local charts. The task is to create normative values from prescriptive populations that are compatible with adequate fetal growth, pregnancy and neonatal outcomes and that are associated with adequate child growth, health and development.

## **Conclusions**

In conclusion, to overcome the limitations of previous ultrasound studies and standardize clinical practice, we adopted a prescriptive approach to the production of international gestational age-specific centiles for umbilical artery Doppler indices. The work has contributed a helpful clinical tool for the assessment of fetal wellbeing and placental function in high-risk pregnancies, which complements the existing INTERGROWTH-21<sup>st</sup> tools for monitoring growth and development from early pregnancy to 2 years of age.<sup>12-18</sup> The healthy outcomes we report at 2 years of age in the infants whose intrauterine growth and Doppler indices we so rigorously evaluated should give clinicians and parents confidence in the benefits of using the centiles in clinical practice to manage high-risk pregnancies.

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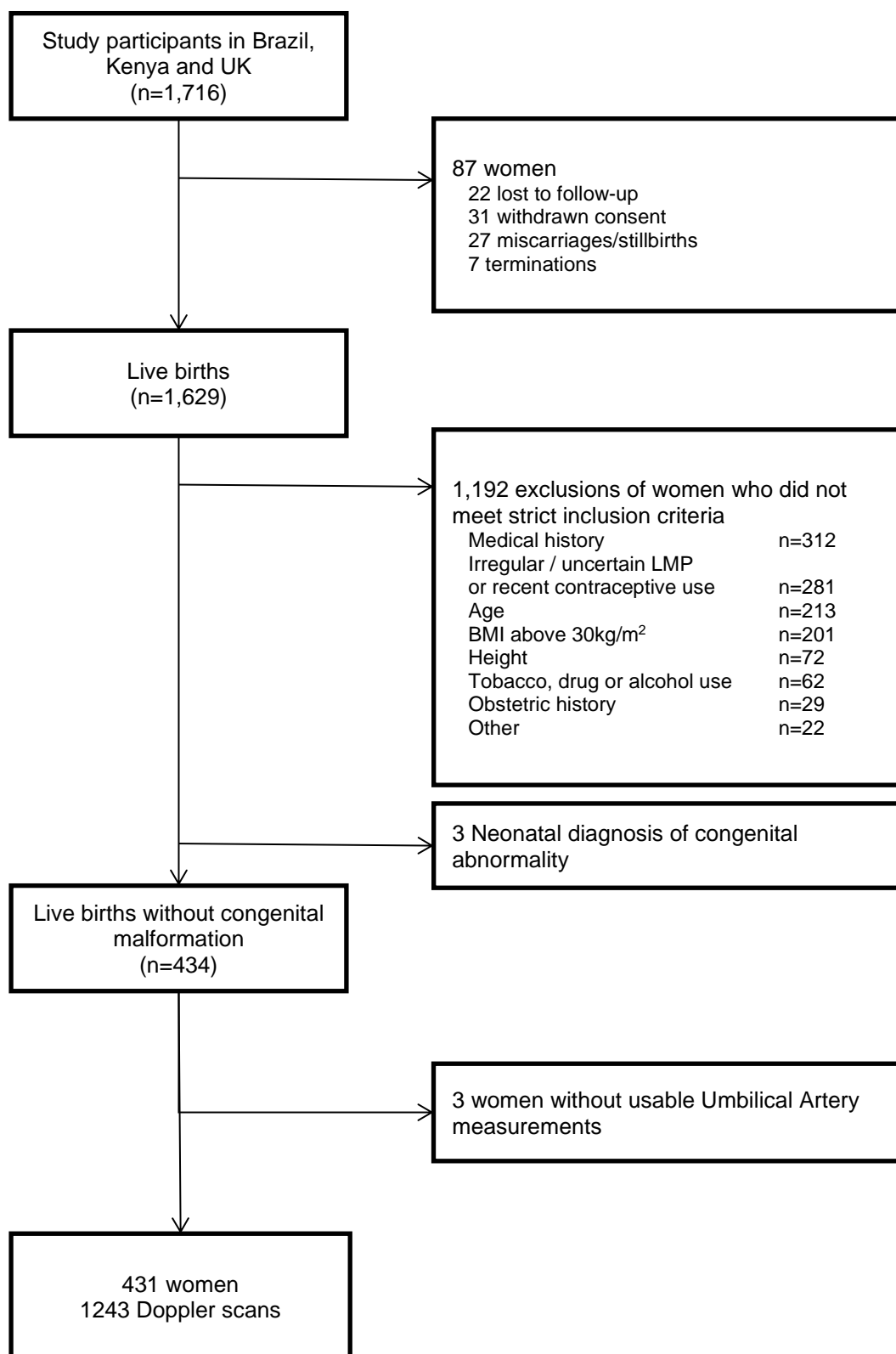
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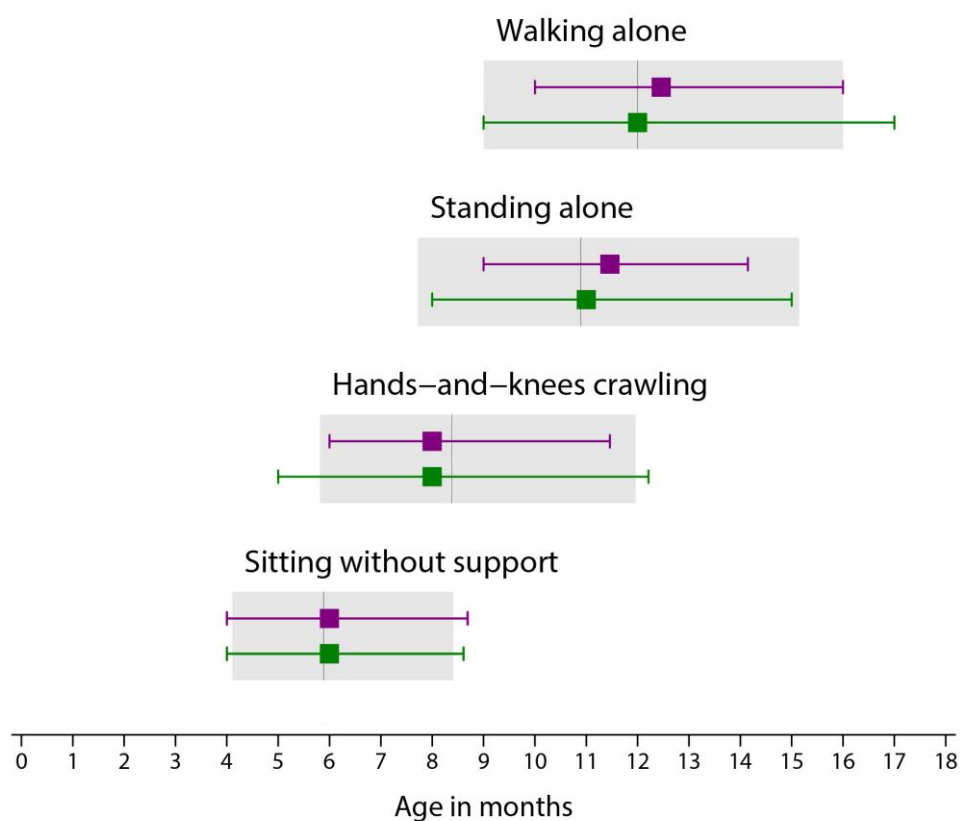
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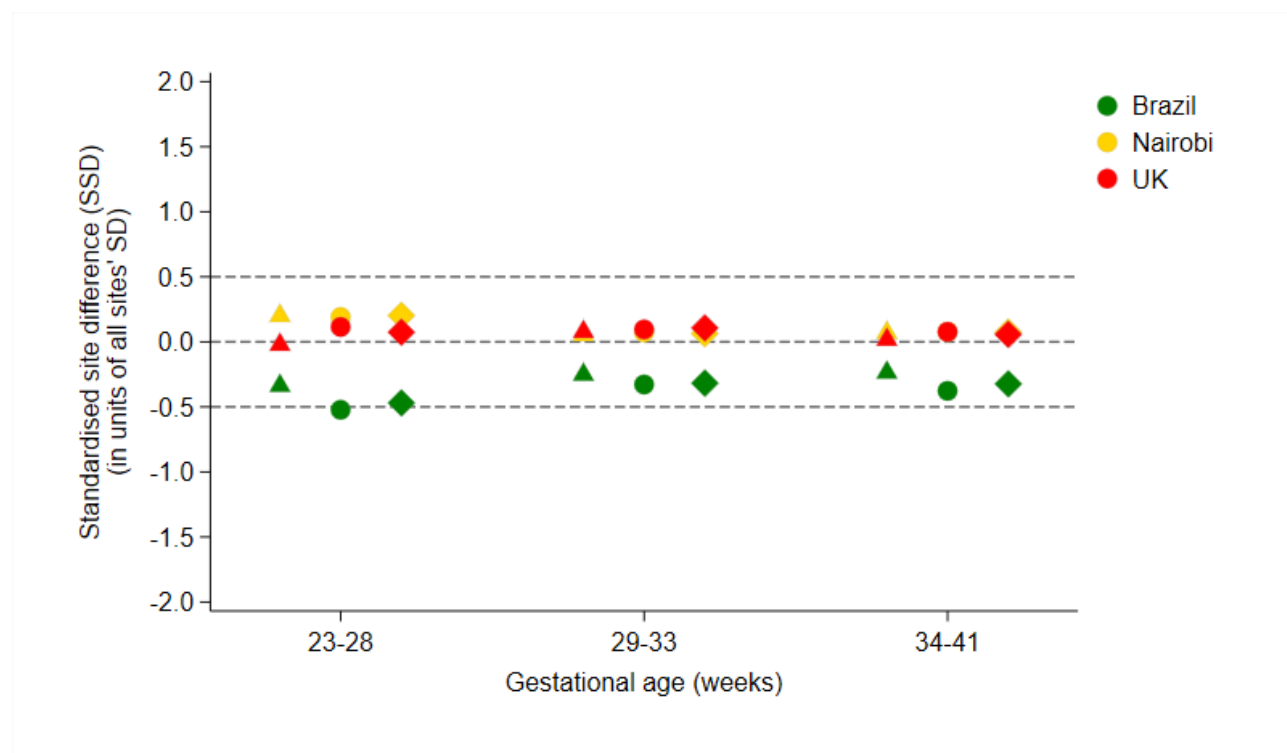
**Figure 1.** Flow chart of participants in the study



**Figure 2.** Median age of achievement (3<sup>rd</sup> and 97<sup>th</sup> centiles) of four gross motor development milestones for infants that were included in the INTERGROWTH-21<sup>st</sup> Fetal Growth Standards (purple) and those included in the present analysis (green). For comparison, the median, 3<sup>rd</sup> and 97<sup>th</sup> centiles of the WHO windows of achievement for the same milestones are presented as grey bars.

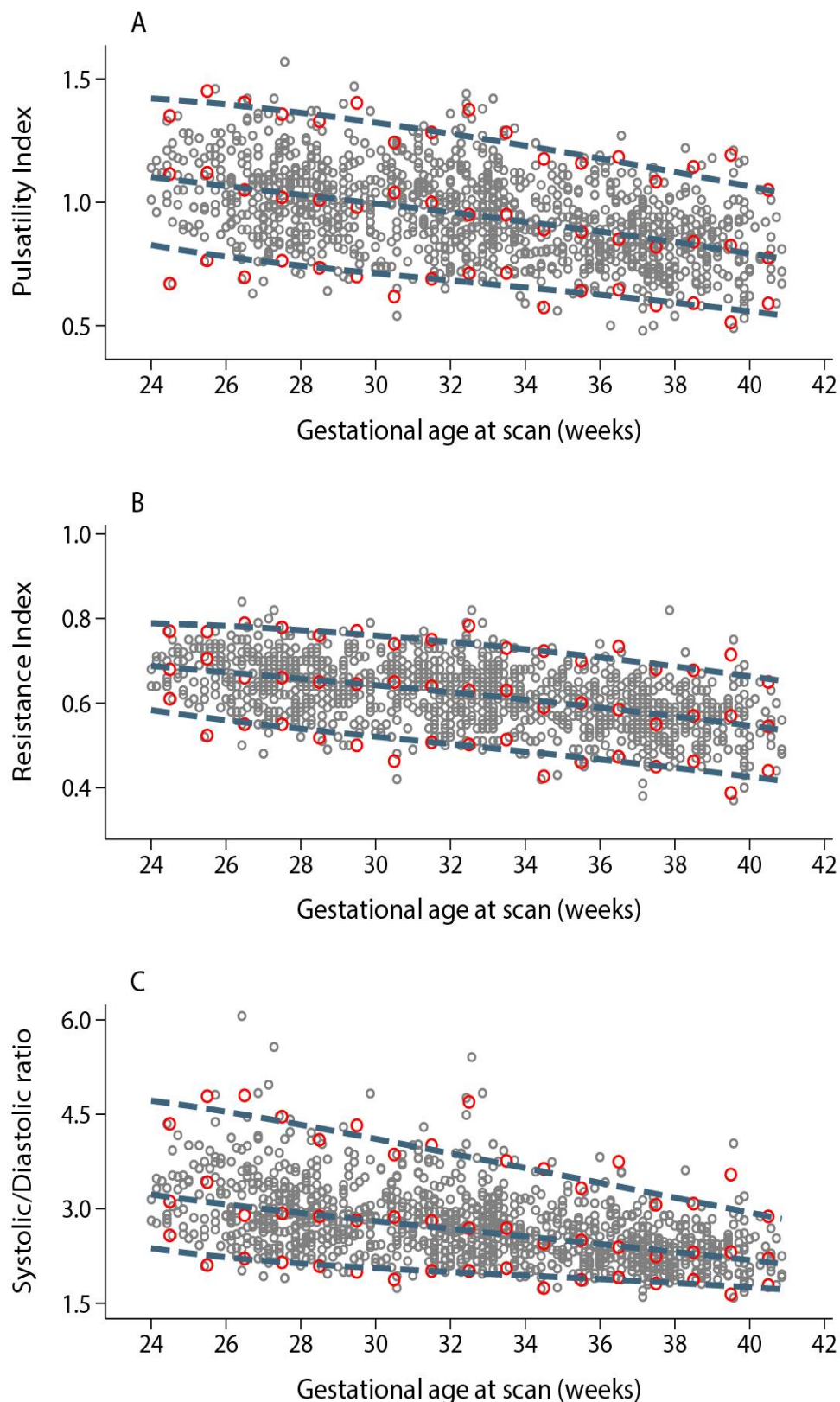


**Figure 3.** Standardized site differences (SSD) for three umbilical artery Doppler indices for Pulsatility Index (triangles), Resistance Index (circles) and for the Systolic/Diastolic ratio (diamonds). SSDs were calculated as the site mean of each index minus the pooled mean divided by the standard deviation of all sites together, adjusted at the mean gestational age for the specified window.





**Figure 4.** Smoothed 3<sup>rd</sup>, 50<sup>th</sup> and 97<sup>th</sup> centile curves of umbilical artery Doppler indices. Fitted centiles according to gestational age in weeks are presented as blue dashed lines for A) Pulsatility Index, B) Resistance Index and C) Systolic/Diastolic ratio. Red circles show empirical 3<sup>rd</sup>, 50<sup>th</sup> and 97<sup>th</sup> centiles for each completed week of gestation; grey circles show individual observations.



690 **Table 1.** Maternal, pregnancy, and newborn characteristics of the study population

	FGLS <i>n</i> = 4321	Present study <i>n</i> = 431
Maternal age, years	28.4 (3.9)	28.9 (3.7)
Maternal height, cm	162.2 (5.8)	163.9 (5.7)
Maternal weight, kg	61.3 (9.1)	64.3 (8.7)
Paternal height, cm	174.4 (7.3)	176.8 (6.8)
Body Mass Index, kg/m <sup>2</sup>	23.3 (3.0)	23.9 (2.9)
Gestational age at first visit, weeks	11.8 (1.4)	12.0 (1.1)
Years of formal education, years	15.0 (2.8)	15.4 (2.7)
Hemoglobin level at <15 weeks, g/L	125 (11)	128.8 (9.3)
Married or cohabiting	4204 (97%)	400 (93%)
Nulliparous	2955 (68%)	259 (60%)
Pre-eclampsia	31 (<1%)	3 (<1%)
Pyelonephritis	16 (<1%)	3 (<1%)
Any sexually transmitted infection	3 (<1%)	17 (4%)
Spontaneous initiation of labor	2868 (66%)	266 (62%)
Preterm Premature Rupture of Membranes (PPROM; <37 weeks)	80 (2%)	9 (2%)
Caesarean section	1541 (36%)	159 (37%)
Neonatal Intensive Care Unit (NICU) admission >1 day	240 (6%)	24 (6%)
Preterm (<37 weeks' gestation)	195 (5%)	21 (5%)
Preterm and spontaneous onset of labor	126 (3%)	10 (2%)
Term Low Birth Weight (LBW; <2500 g; ≥37 weeks' gestation)	128 (3%)	11 (3%)
Neonatal mortality	7 (<1%)	0 (0%)
Male sex	2149 (50%)	232 (54%)
Exclusive breastfeeding at discharge	3786 (88%)	399 (93%)
Mother admitted to intensive care unit	17 (<1%)	3 (<1%)
Newborn weight (≥37 weeks' gestation), kg	3.3 (0.4)	3.3 (0.5)
Newborn length (≥37 weeks' gestation), cm	49.4 (1.9)	49.4 (1.9)
Newborn head circumference (≥37 weeks' gestation), cm	33.9 (1.3)	34.5 (1.2)

691 Data are mean (Standard Deviation) or number (percent).

692 FGLS, Fetal Growth Longitudinal Study of the INTERGROWTH 21<sup>st</sup> Project

693 **Table 2.** Morbidity in the second year of life for the 319 infants in the analysis

Morbidity in the second year of life	Infants in the analysis ( <i>n</i> = 319) <sup>1</sup>
Hospitalized at least once	27 (8.5)
Any prescription made by a health-care practitioner	275 (86.2)
Antibiotics ( $\geq 3$ regimens)	55 (17.2)
Iron/folic acid/vitamin B12/other vitamins ( $\geq 3$ regimens)	72 (22.6)
Up-to-date with local vaccination policies	313 (98.1)
Otitis media/Pneumonia/Bronchiolitis	42 (13.2)
Parasitosis/Diarrhea/Vomiting	15 (4.7)
Exanthema/skin disease	88 (27.6)
Urinary tract infection/pyelonephritis	2 (0.6)
Fever $\geq 3$ days ( $\geq 3$ episodes)	36 (11.3)
Other infections requiring antibiotics	5 (1.6)
Asthma	11 (3.4)
Gastro-esophageal reflux	6 (1.9)
Cow's milk protein allergy	6 (1.9)
Food allergies	9 (2.8)
Injury trauma	22 (6.9)
Surgery	8 (2.5)

694 Data are number (%). Missing data below 1% for all variables.

695 <sup>1</sup>For five infants, information on morbidities in the first year of life was used.

696 Table 3. Umbilical artery Pulsatility Index (PI) centile values according to gestational age

Gestational age (weeks + days)	Centile						
	3 <sup>rd</sup>	5 <sup>th</sup>	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	97 <sup>th</sup>
24+0	0.83	0.86	0.91	1.10	1.31	1.38	1.42
25+0	0.80	0.84	0.89	1.08	1.30	1.37	1.41
26+0	0.78	0.81	0.87	1.07	1.29	1.35	1.40
27+0	0.76	0.79	0.85	1.05	1.27	1.34	1.38
28+0	0.74	0.78	0.83	1.03	1.25	1.32	1.36
29+0	0.73	0.76	0.81	1.01	1.23	1.30	1.34
30+0	0.71	0.75	0.80	1.00	1.21	1.28	1.32
31+0	0.70	0.73	0.78	0.98	1.19	1.26	1.30
32+0	0.68	0.72	0.77	0.96	1.17	1.24	1.28
33+0	0.67	0.70	0.75	0.94	1.15	1.21	1.25
34+0	0.66	0.69	0.74	0.92	1.13	1.19	1.23
35+0	0.64	0.67	0.72	0.90	1.10	1.16	1.20
36+0	0.63	0.66	0.70	0.88	1.08	1.14	1.18
37+0	0.61	0.64	0.69	0.86	1.05	1.11	1.15
38+0	0.59	0.62	0.67	0.84	1.03	1.08	1.12
39+0	0.58	0.60	0.65	0.82	1.00	1.06	1.09
40+0	0.56	0.59	0.63	0.79	0.97	1.03	1.06

698 **Table 4.** Umbilical artery Resistance Index (RI) centile values according to gestational age

Gestational age (weeks + days)	Centile						
	3 <sup>rd</sup>	5 <sup>th</sup>	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	97 <sup>th</sup>
24+0	0.58	0.60	0.62	0.69	0.76	0.78	0.79
25+0	0.57	0.58	0.61	0.68	0.75	0.77	0.79
26+0	0.56	0.57	0.60	0.67	0.75	0.77	0.78
27+0	0.55	0.56	0.59	0.67	0.74	0.76	0.78
28+0	0.54	0.55	0.58	0.66	0.74	0.76	0.77
29+0	0.53	0.55	0.57	0.65	0.73	0.75	0.77
30+0	0.52	0.54	0.56	0.64	0.72	0.75	0.76
31+0	0.51	0.53	0.55	0.63	0.72	0.74	0.75
32+0	0.50	0.52	0.54	0.63	0.71	0.73	0.74
33+0	0.49	0.51	0.53	0.62	0.70	0.72	0.74
34+0	0.49	0.50	0.52	0.61	0.69	0.71	0.73
35+0	0.48	0.49	0.52	0.60	0.68	0.70	0.72
36+0	0.47	0.48	0.51	0.59	0.67	0.69	0.71
37+0	0.46	0.47	0.50	0.58	0.66	0.68	0.70
38+0	0.45	0.46	0.49	0.57	0.65	0.67	0.69
39+0	0.44	0.45	0.48	0.56	0.64	0.66	0.68
40+0	0.43	0.44	0.46	0.55	0.63	0.65	0.66

700 **Table 5.** Umbilical artery Systolic/ Diastolic Ratio (S/D Ratio) centile values according to  
 701 gestational age

Gestational age (weeks + days)	Centile						
	3 <sup>rd</sup>	5 <sup>th</sup>	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	97 <sup>th</sup>
24+0	2.38	2.46	2.61	3.23	4.12	4.46	4.72
25+0	2.30	2.39	2.53	3.15	4.03	4.38	4.63
26+0	2.23	2.32	2.46	3.07	3.95	4.29	4.54
27+0	2.18	2.26	2.40	3.00	3.86	4.19	4.44
28+0	2.13	2.22	2.35	2.93	3.77	4.09	4.33
29+0	2.09	2.17	2.31	2.87	3.68	3.99	4.22
30+0	2.06	2.14	2.26	2.81	3.58	3.89	4.11
31+0	2.03	2.10	2.22	2.74	3.49	3.78	4.00
32+0	2.00	2.07	2.19	2.68	3.40	3.67	3.88
33+0	1.97	2.04	2.15	2.62	3.30	3.57	3.76
34+0	1.94	2.01	2.11	2.56	3.21	3.46	3.65
35+0	1.91	1.97	2.08	2.50	3.12	3.35	3.53
36+0	1.88	1.94	2.04	2.44	3.02	3.24	3.41
37+0	1.85	1.91	2.00	2.38	2.93	3.14	3.30
38+0	1.82	1.87	1.96	2.32	2.83	3.03	3.18
39+0	1.79	1.84	1.92	2.25	2.73	2.92	3.06
40+0	1.75	1.80	1.87	2.19	2.64	2.81	2.94

**Table 6.** Equations for parameters and computations of Z-scores and centiles for three umbilical artery Doppler indices according to gestational age (GA) in weeks

Parameter	Equation
Skewness	Pulsatility Index (PI)
	$\lambda(\text{GA}) = -0.0768617$
	Resistance Index (RI)
	$\lambda(\text{GA}) = 0.0172944$
Mean	Systolic/Diastolic Ratio (S/D Ratio)
	$\lambda(\text{GA}) = -0.2752483$
	Pulsatility Index (PI)
	$\mu(\text{GA}) = 1.02944 + 77.7456 \cdot \text{GA}^{-2} - 0.000004455 \cdot \text{GA}^3$
Coefficient of variation	Resistance Index (RI)
	$\mu(\text{GA}) = 0.674914 + 25.3909 \cdot \text{GA}^{-2} - 0.0000022523 \cdot \text{GA}^3$
	Systolic/Diastolic Ratio (S/D Ratio)
	$\mu(\text{GA}) = 2.60358 + 445.991 \cdot \text{GA}^{-2} - 0.0000108754 \cdot \text{GA}^3$
Z-score	Pulsatility Index (PI)
	$\sigma(\text{GA}) = -0.00645693 + 254.885 \cdot \ln(\text{GA}) \cdot \text{GA}^{-2} - 715.949 \cdot \text{GA}^{-2}$
	Resistance Index (RI)
	$\sigma(\text{GA}) = 0.0375921 + 60.7614 \cdot \ln(\text{GA}) \cdot \text{GA}^{-2} - 183.336 \cdot \text{GA}^{-2}$
Centile	Systolic/Diastolic Ratio (S/D Ratio)
	$\sigma(\text{GA}) = -0.503202 + 1268.37 \cdot \ln(\text{GA}) \cdot \text{GA}^{-2} - 3417.37 \cdot \text{GA}^{-2}$
Z-score	$z = \lambda^{-1} \cdot [\exp[(y - \mu) \cdot \lambda \cdot \sigma^{-1}] - 1]$
Centile	$c = (1 - \text{normal}(z)) \cdot 100$

GA, Gestational age in exact weeks. ln, natural logarithm. y, Doppler index value

Example: calculating the PI centile at a certain gestational age

Measurement: PI = 1.00 at gestational age 36+4

Calculations:

$$y = 1.00$$

$$36+4 = 256 \text{ days}$$

$$GA = 256 / 7 = 36.571429 \text{ (exact weeks)}$$

$$\lambda = -0.0768617$$

$$\begin{aligned} \mu &= 1.02944 + 77.7456*(36.571429)^{-2} - 0.000004455*(36.571429)^3 = \\ &1.02944 + 77.7456*0.00074768 - 0.000004455*48913.168 = \\ &1.02944 + 0.05812883 - 0.21790816 = \\ &0.869660 \end{aligned}$$

$$\begin{aligned} \sigma &= -0.00645693 + 254.885*\ln(36.571429)*(36.571429)^{-2} - 715.949*(36.571429)^{-2} = \\ &-0.00645693 + 254.885*3.5992673*0.00074768 - 715.949*0.00074768 = \\ &0.144163 \end{aligned}$$

$$\begin{aligned} Z &= \lambda^{-1} * [\exp[(y-\mu) * \lambda * \sigma^{-1}] - 1] = \\ &(-0.0768617)^{-1} * [\exp[(1.00-0.86966067)*-0.0768617*(0.14416339)^{-1}] - 1] = \\ &-13.010381 * [\exp[0.13033933*-0.0768617*6.9365738] - 1] = \\ &-13.010381 * [\exp[-0.06949131] - 1] = \\ &-13.010381 * [0.93286824 - 1] = \\ &0.87340977 \end{aligned}$$

$$\begin{aligned} c &= (1 - \text{normal}(0.87340977)) * 100 = \\ &80.9 \end{aligned}$$

Conclusion: A PI value of 1.00 measured at 36+4 gestational weeks has a Z-score of 0.87 and is placed at the 80.9<sup>th</sup> centile of the distribution.



**Table 1S.** Variance components analysis\* for three umbilical artery Doppler indices

	<b>Pulsatility Index (PI)</b>	<b>Resistance Index (RI)</b>	<b>Systolic/Diastolic ratio (S/D Ratio)</b>
Number of measures	1021	1020	1022
Variance between study sites	2.8	6.5	5.6
Variance between individuals within a site	25.4	22.9	20.9
Residual variance	71.8	70.6	73.5

\*From multilevel mixed effects models fitted with random intercepts for the study-site and individual levels (with individuals nested within sites) and adjustment for gestational age (treated as fixed effect).

Models included women with three or more scans performed between 23 and 40 weeks' gestation (316 women and 1023 scans were available for these analyses).

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