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Title

Femur-Sparing Pattern of Abnormal Fetal Growth in Pregnant Women from New York City After Maternal Zika Virus Infection

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Condensation

Femur-sparing pattern of fetal growth restriction following maternal Zika virus infection with smaller head and abdominal circumference in relation to femur length.

Short Version of the Title

Femur-sparing pattern of fetal growth restriction after Zika virus infection

Implications and Contributions

A. Why was this study conducted?

To determine if Zika virus infection during pregnancy is associated with a femur-sparing pattern of fetal growth restriction, similar to observations in a nonhuman primate model of decelerating growth of the fetal head and abdomen with respect to femur length.

B. What are the key findings?

An unusual femur-sparing pattern of fetal growth restriction was detected in the majority of fetuses with congenital ZIKV exposure using Intergrowth-21st Project fetal body ratios comparing head or abdominal circumference to femur length.

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

Fetal body ratios may provide a new screening tool to detect Zika virus-associated fetal injury in pregnancies without overt microcephaly.

Abstract:

Background: Zika virus (ZIKV) is a mosquito-transmitted flavivirus, which can induce fetal brain injury and growth restriction following maternal infection during pregnancy. Prenatal diagnosis of ZIKV-associated fetal injury in the absence of microcephaly is challenging due to an incomplete understanding of how maternal ZIKV infection affects fetal growth and the use of different sonographic reference standards around the world. We hypothesized that skeletal growth is unaffected by ZIKV infection and that the femur length can represent an internal standard to detect growth deceleration of the fetal head and/or abdomen by ultrasound.

Objective: To determine if maternal ZIKV infection is associated with a femur-sparing pattern of intrauterine growth restriction (IUGR) through analysis of fetal biometric measures and/or body ratios using the INTERGROWTH-21st Project (IG-21) and World Health Organization Fetal Growth Chart (WHO-FGC) sonographic references.

Study Design: Pregnant women diagnosed with a possible recent ZIKV infection at Columbia University Medical Center after traveling to an endemic area were retrospectively identified and included if a fetal ultrasound was performed. Data was collected regarding ZIKV testing, fetal biometry, pregnancy and neonatal outcomes. The IG-21 and WHO-FGC sonographic standards were applied to obtain Z-scores and/or percentiles for fetal head, abdominal circumference (HC, AC) and femur length (FL) specific for each gestational week. A novel IG-21 standard was also developed to generate Z-scores for fetal body ratios with respect to femur length (HC:FL, AC:FL). Data was then grouped within clinically relevant gestational age strata (<24 weeks, 24-27 6/7, 28-33 6/7, >34 weeks) to analyze time-dependent effects of ZIKV infection on

fetal size. Statistical analysis was performed using Wilcoxon signed-rank test on paired data, comparing either AC or HC to FL.

Results: A total of 56 pregnant women were included in the study with laboratory evidence of a confirmed or possible recent ZIKV infection. Based on the CDC definition for microcephaly after congenital ZIKV exposure, microcephaly was diagnosed in 5% (3/56) by both the IG-21 and WHO-FGC standards (HC Z-score ≤ -2 or ≤ 2.3%). Using IG-21, IUGR was diagnosed in 18% of pregnancies (10/56; AC Z-score ≤-1.3, <10%). Analysis of fetal size using the last ultrasound scan for all subjects revealed a significantly abnormal skewing of fetal biometrics with a smaller AC versus FL by either IG-21 or WHO-FGC ($p<0.001$ for both). A difference in distribution of fetal AC compared to FL was first apparent in the 24-27 6/7 week strata (IG-21, $p=0.002$; WHO-FGC, $p=0.001$). A significantly smaller HC compared to FL was also observed by IG-21 as early as the 28-33 6/7 week strata (IG-21, $p=0.007$). Overall, a femur-sparing pattern of growth restriction was detected in 52% of pregnancies with either an HC:FL or AC:FL fetal body ratio less than the 10^{th} percentile (IG-21 Z-score ≤-1.3).

Conclusions: An unusual femur-sparing pattern of fetal growth restriction was detected in the majority of fetuses with congenital ZIKV exposure. Fetal body ratios may represent a more sensitive ultrasound biomarker to detect viral injury in nonmicrocephalic fetuses that could impart long-term risk for complications of congenital ZIKV infection.

Keywords

Biomarker, biometry, biparietal diameter, congenital Zika virus syndrome, femur length, fetal growth restriction, fetus, fetal infection, head circumference, Intergrowth-21, intrauterine growth restriction, IUGR, microcephaly, pregnancy, teratogenesis, ultrasound, virus, Zika

Glossary of Terms

AC, abdominal circumference

BPD, biparietal diameter

CDC, Centers of Disease Control

FL, femoral length

HC, head circumference

IG-21, 2014 International Fetal and Newborn Growth Consortium for the 21st Century

IUGR, intrauterine fetal growth restriction

NICHD, Eunice Kennedy Shriver National Institute of Child Health and Development

PRNT, plaque reduction neutralization test

RT-PCR, real-time polymerase chain reaction testing

WHO, World Health Organization

WHO-FGC, World Health Organization Fetal Growth Chart

ZIKV, Zika virus

Introduction

Zika virus (ZIKV) is a mosquito-transmitted flavivirus, recently linked to microcephaly following a maternal infection during pregnancy.[1] Vertical transmission of ZIKV has been associated with fetal microcephaly and development of the Congenital ZIKV Syndrome, a condition encompassing a spectrum of fetal neurologic injury including cortical malformations, ventriculomegaly, ocular injury and arthrogryposis.[2, 3, 4] A maternal ZIKV infection has been associated with a rate of birth defects between 5-8%, but may be as high as 13% when infection occurs in the first trimester.[5, 6] Recently, reports of children with a normal head circumference (HC) at birth that were later found to have abnormal brain imaging, ocular injury and postnatal development of microcephaly, has led to the concept that microcephaly does not capture the broader spectrum of ZIKV-associated brain injury.[3, 7, 8, 9, 10] Identification of fetuses with a normal head size that are at risk for long-term adverse outcomes remains limited due to the incomplete knowledge of how a less overt spectrum of ZIKV-associated fetal injury may be detected prenatally. This limitation is further compounded by weaknesses related to diagnostic testing including: 1) inadequate availability of ZIKV testing in regions at risk, 2) lower sensitivity of real-time polymerase chain reaction testing (RT-PCR) due to the transient nature of ZIKV viremia, and 3) lower positive predictive value of serologic testing due to cross-reactivity between ZIKV and related flaviviruses.

In a nonhuman primate model, ZIKV-associated fetal brain injury was associated with an unusual femur-sparing profile of intrauterine growth restriction (IUGR) notable for a growth arrest in ultrasound biometric measures of the fetal head (biparietal diameter, BPD) and abdomen (abdominal circumference, AC) with continued growth of the femur

(femur length, FL).[11, 12] This profile of IUGR has been noted as “femur-sparing”[13], but has not been characterized in a clinical study nor is it part of the mainstream categories for IUGR; typically, IUGR has been defined as asymmetric (conserved head growth with lagging growth of the abdomen) or symmetric (equal growth restriction of the head, abdomen and femur).[14]

There is a paucity of data to link aberrant fetal growth in the context of a maternal ZIKV infection to long-term adverse outcomes in the neonate, but IUGR may represent a sensitive indicator of viral injury to the placenta or fetus itself. Whether fetuses exposed to Zika virus with abnormal growth patterns, without microcephaly, may be more susceptible to eye injury or late-onset microcephaly is unknown and represents an important knowledge gap.[15] Although IUGR has been reported in pregnant women with a possible ZIKV infection, the profile of IUGR has not been described.[10, 16] Our objective was to determine if maternal ZIKV infection was associated with a femur-sparing profile of growth restriction, similar to observations in a nonhuman primate model of congenital ZIKV infection.[11, 12] Such an observation may be a first step in identifying nonmicrocephalic fetuses at risk for long-term morbidity.

Materials and Methods

Study Population and Ethics Statement

All pregnant women presenting to Columbia University Medical Center from January 1, 2016 through February 1, 2017 from an area with known ZIKV local transmission were offered screening per Centers of Disease Control (CDC) recommendations. The

Columbia University Institutional Review Board approved the study (IRB-AAAQ9686) as a retrospective chart review and informed consent was not required. Cases were excluded if no ultrasound for fetal size or anatomy was completed prior to delivery. The gestational age and due date were estimated according to methods recommended by the American College of Obstetricians and Gynecologists.[17] Following ZIKV diagnosis, a pregnancy ultrasound was performed, and repeated every 3-4 weeks, for the duration of the pregnancy. Timing of ZIKV exposure was estimated based on maternal travel history, but could have occurred later in pregnancy due to sexual exposure from an infected partner; therefore, we included 4 subjects with immediate pre-conception exposure (Table S4). Neonatal outcomes were assessed through measurement of a postnatal HC and head ultrasound scan in the first week of life. A more comprehensive assessment of outcomes was not possible due to limitations on our institutional human subject's approval and the challenge of data procurement from multiple private pediatric clinics in New York City; therefore, results for some recommended neonatal screening tests were not obtained.

ZIKV Diagnosis

Based on uncertainties in the diagnostic testing for ZIKV infection, we followed CDC convention to describe women as having a "possible" ZIKV infection based on: 1) ZIKV infection detected by ribonucleic acid (RNA) testing on maternal, placental or fetal specimen, or 2) diagnosis of ZIKV infection or unspecified flavivirus infection, timing of infection cannot be determined (i.e., positive/equivocal ZIKV IgM and ZIKV plaque reduction neutralization test (PRNT) titer ≥ 10, regardless of dengue virus PRNT value; or negative ZIKV IgM, and positive or equivocal dengue virus IgM, and ZIKV PRNT titer

≥ 10, regardless of dengue virus PRNT titer).[18, 19] We also followed CDC guidance for the interpretation of laboratory testing of the infant for evidence of congenital ZIKV infection.[18] Any positive nucleic acid test from a serum, urine or cerebrospinal fluid sample was considered a confirmed congenital ZIKV infection. Any non-negative IgM result (e.g. positive, equivocal) from infant serum with a negative nucleic acid test was considered a probable congenital ZIKV infection.

Ultrasound Methodology

The INTERGROWTH-21st (IG-21) sonographic standard was used to derive Z-scores for HC, AC and FL, as well as ratios for HC:FL and AC:FL.[20, 21, 22] Ultrasound scans were originally performed using Hadlock methodology, which measures BPD in a cross-section view from outer-to-inner skull edges. As IG-21 measures the BPD from outer-to-outer skull edges, BPD measurements in this study were not directly translatable to the IG-21 sonographic standard. We chose instead to focus the analysis on HC, AC and FL measurements from which we could directly calculate Z-scores. As the sonographic standard or reference used to interpret fetal size is expected to influence detection of IUGR in pregnancies with maternal ZIKV infection, we also corroborated the findings by applying references from the WHO sponsored Fetal Growth Chart study (WHO-FGC).[20, 23]

Online calculators were used to obtain Z-scores for IG-21[22] and published charts allowed estimation of percentiles for WHO-FGC.[20, 23] Notably, the WHO recommends that diagnosis of ZIKV-associated microcephaly use the IG-21 standard when the gestational age is accurately known and WHO-FGC when gestational age is not reliably known.[24] Studies of pregnancy outcomes from Brazilian women with ZIKV

infection have also used the IG-21 standard to determine distribution of fetal biometric measures.[9, 25, 26]

We did not evaluate our data based on sonographic standards developed in the U.S. for two reasons. First, the 1983 Hadlock standard (N=392) was based on a relatively small cohort of Caucasian women and has anecdotally been associated with a common diagnosis of "short femur".[27, 28, 29, 30] Second, application of racial/ethnic specific standards based on the NICHD Fetal Growth Study (N=2,334)[31] would only have allowed for assignment of biometric measures within ranges of centiles (i.e.. <3rd, 3rd – 5th, 5th-10th), but not a more precise and quantitative analysis necessary to test our hypothesis. Our data on subject ethnicity was also incomplete. We ultimately chose to compare our data to the IG-21 and WHO-FGC standards as they were large population-based studies from multiple countries that included an ethnically diverse cohort. Notably, we could also use the IG-21 standard to specifically test our hypothesis of a femur-sparing profile of fetal growth restriction using fetal body ratios.

Definitions for Microcephaly and IUGR

Variations in the definition for prenatal diagnosis of microcephaly with possible ZIKV infection exist among guidelines and standards.[10, 32, 33] The International Society for Ultrasound in Obstetrics & Gynecology recommends heightened surveillance with specialist referral and neurosonography for fetuses with a HC smaller than 2 standard deviations below the mean (Z-score ≤ -2 SD).[34] The WHO definition for fetal microcephaly, in the context of ZIKV infection, is a HC ≤ -2 SD below the mean.[33] After birth, the CDC definition for microcephaly is a HC less than the 3rd centile for gestational age in the setting of congenital ZIKV exposure (≤ -2 SD).[35] Based on this

guidance, we defined microcephaly in our study as a fetal HC Z score ≤ -2 (2.3%, IG-21) or less than the 3rd centile (WHO-FGC).

There is no gold standard to define IUGR and it has been variably defined by deviation of fetal size from a normal distribution at either the 10th, 5th or 3rd centile.[36, 37] The estimated fetal weight (EFW) and AC are consistently identified as important parameters in making the diagnosis and a typical threshold is less than the 10th centile; however, this definition will include many constitutionally small fetuses and miss growth restricted fetuses that are larger than the 10th centile.[38] In this study, we present results using both a conservative (AC <3%, ~Z score ≤ -2) and traditional (AC <10%, ~Z score ≤ -1.3) definition for IUGR to allow comparison of results with AC:FL, a fetal body ratio for AC normalized to FL. Due to the difference in BPD measurements between Hadlock and IG-21, BPD could not be used to calculate EFW; therefore, EFW was not used as a measure of IUGR in this study.

Estimating Population Distribution of Fetal Body Ratios

Fetal body ratios normalized to FL were hypothesized to represent a more sensitive method to detect aberrant growth patterns in fetuses with congenital ZIKV exposure. This approach has the advantage of directly addressing our hypothesis by comparing the size of fetal structures (i.e. head, abdomen) to FL for each fetus, but may not detect constitutionally small fetuses and fetuses with symmetric IUGR. The WHO-FGC has published ratios for FL:HC, but values often overlapped several strata making it difficult to categorize some cases into discrete strata.[20] Therefore, we focused attention on the IG-21 standard from which we could calculate Z-scores for HC:FL and AC:FL.

Published thresholds for IG-21 body ratios did not exist; therefore, we developed these formulas, including mean and standard deviations from the original data (means and standard deviations by gestational week shown in Tables S1, S2, S3). Statistical methods used to construct the fetal biometry ratios were selected using a previously published strategy.[21, 39] In brief, fractional polynomial regression was used, and the resulting functional form further modelled in a multi-level framework to account for the longitudinal design of the study. Goodness-of-fit was evaluated with visual inspection of overall model fit using quantile-quantile plots of the residuals, plots of residual versus fitted values and the distribution of fitted Z-scores across gestational age. All models and goodness-of-fit assessments were fitted with STATA, version 11.2, software (StataCorp LP, College Station, Texas, USA).

Statistical Analysis

Raw measurements for all biometric measures were recorded in millimeters (mm). We analyzed the data in clinically relevant gestational age strata for two reasons: 1) identifying a gestational age threshold at which ZIKV-associated abnormal fetal growth is typically observed has clinical relevance and 2) the effects of ZIKV infection on fetal growth are likely time-dependent with more significant effects occurring in later pregnancy. Gestational age strata were chosen to correspond to transitions classically associated with neonatal viability (18-24 weeks) and morbidity (late second trimester: 24-28 weeks, early third trimester: 28-34 weeks, and near term ≥ 34 weeks). The latest ultrasound per subject was analyzed in each gestational age strata. Wilcoxon signed rank test was used to compare distribution of paired Z-scores for HC to FL or AC to FL.

Statistical significance was reported for p values <0.05. Analysis was completed using STATA version 11.2, software (StataCorp LP, College Station, Texas, USA).

Results

ZIKV Diagnosis and Timing of Exposure

Study participants were pregnant women diagnosed with ZIKV infection after travel to countries with local transmission, who received obstetrical care from Columbia University Medical Center (New York City, NY, USA) between January 1, 2016 and February 1, 2017. A total of 66 pregnant women were retrospectively identified with a recent ZIKV infection and 56 were included based on availability of ultrasound data within the Columbia University health care system. The cohort was of mixed race/ethnicity: 12 Hispanic/White, 7 Hispanic/Black, 2 Hispanic/Pacific Islander, 3 White, and 32 other (unknown/more than one race). Thirteen women (13/56, 23%) recalled symptoms consistent with ZIKV infection including a rash, conjunctivitis, fever and myalgias (Table S4). ZIKV infection was diagnosed based on laboratory evidence for a confirmed ZIKV infection (N=21) or unspecified flavivirus infection (N=35; Table S4) according to the U.S. Zika Pregnancy Registry criteria.[5, 40] By travel history, ZIKV exposure was estimated to have occurred immediately preconception (N=4) or in the first (N=16) or second trimester (N=11). An additional 25 women were more uncertain of exposure timing due to prolonged stays in endemic areas and presented to care in the late second or third trimester (mean 30.8 ± 4.5 weeks).

Pregnancy and Birth Outcomes

Prenatal ultrasound was performed between 14 and 40 weeks gestation with each subject typically having 3 ultrasound scans [range 1-7; ≥ 3 scans, N=29 (52%); 2 scans, N= 15 (27%); 1 ultrasound, N=12 (21%)]. During pregnancy, microcephaly was diagnosed in 5% (3/56) of fetuses by both the IG-21 (HC Z-score ≤ -2) and WHO-FGC (≤ 3rd centile; Table S5). Apart from isolated choroid plexus cysts, no other intracranial abnormalities were detected on prenatal ultrasound. IUGR was diagnosed in 18% of pregnancies by a traditional definition (10/56; AC Z-score ≤-1.3, <10th centile) and 9% by a conservative definition (5/56; AC Z-score ≤-2 or ≤ 2.3 centile, Table 1) using IG-21 standards. The mean Z-score for birthweight for the entire cohort was 0.2 ± 1.0.

Pregnancy outcomes were available in 52 of 56 cases (Table S6). In three pregnancies (3/52; 6%), a pregnancy termination was performed in the second trimester after a diagnosis of microcephaly. One stillbirth occurred at 30 weeks gestation (1/52; 2%) in a microcephalic fetus with symmetric severe growth restriction. Of the remaining 48 pregnancies, term birth occurred in 92% (44/48) and preterm birth in 8% (4/48). A postnatal head ultrasound was performed in 39 cases and identified a grade 1 intraventricular hemorrhage (1/39, 3%) or choroid plexus cyst (4/39, 10%), but no other structural findings associated with the congenital ZIKV syndrome (Table S6). Neonatal HC was measured in 47 of the 48 newborns with a mean Z-score of 0.4 using IG-21. At birth, microcephaly was observed in one neonate (HC Z-score ≤ -2) and no neonates had a HC Z-score ≤ -3 (Table S6). Interpretation of the laboratory testing for ZIKV infection of the neonate is limited by the transient nature of the viremia, but results were available for 41 infants; a possible ZIKV infection was diagnosed in 39% of cases (16/41, Table S4) and one infant had a confirmed ZIKV infection (1/41, 2%).

Microcephaly and Femur-Sparing Pattern of IUGR Identified using Single Fetal Biometric Measures and Fetal Body Ratios

Next, we compared paired biometric measures from each subject to determine if maternal ZIKV infection was associated with differential growth of the HC or AC with respect to the FL. Overall, the AC was significantly smaller than FL based on the last ultrasound scan in pregnancy by either IG-21 or WHO-FGC (Tables 2 and S7, $p<0.001$ for both analyses); this difference was also significant in every strata starting with the 24-27 6/7 week category for IG-21 and most strata for WHO-FGC. The HC was also significantly smaller than FL in the overall analysis by IG-21 ($p<0.001$) and in every strata beginning with 28-33 6/7 weeks; this difference was not significant by WHO-FGC.

Another method to identify ZIKV-associated differential growth of the fetal head or abdomen with respect to the femur would involve an analysis of fetal body ratios (e.g. HC:FL or AC:FL). To this end, we developed IG-21 fetal body ratios based on previously published data from 4,607 normal pregnancies in 18 different countries.[21] These fetal body ratios were used to generate Z-scores in our cohort to compare differences in size of the fetal head and/or abdomen versus the femur. In contrast to a 5% rate of microcephaly, a femur-sparing pattern of fetal growth restriction was observed after 34 weeks gestation in 37% (17/46) of pregnancies based on either a small head (HC:FL; 28%, 13/46) or abdomen (AC:FL; 20%, 9/46) in relation to the femur (Z-scores <-1.3; Fig. 1). If we considered ultrasound data from any time during pregnancy, 52% (29/56) of pregnancies had a differentially small head or abdomen in comparison to the femur [Z-scores <-1.3; HC:FL 39% (22/56) and/or AC:FL 30% (17/56); Fig. 1]; this final analysis allowed inclusion of fetuses from the second trimester

pregnancy terminations and the stillbirth and preterm birth cases. If we considered only women with symptomatic ZIKV infection, an abnormal HC:FL ratio was observed in 46% (6/13) and an abnormal AC:FL ratio in 15% (2/13). In pregnancies with an abnormal HC:FL or AC:FL ratio, the ratio became more skewed over time in most pregnancies (Fig. S1 and S2). Overall, the majority of pregnancies in our study with a possible maternal ZIKV infection developed a femur-sparing profile of growth restriction using fetal body ratios developed from the IG-21 sonographic standard.

Comment

Principal Findings of the Study

Our study is the first to demonstrate a femur-sparing pattern of IUGR in late gestation of women with a possible ZIKV infection. This unusual fetal growth profile was found by application of the IG-21 and WHO-FGC standards and differs from prior models of IUGR (Fig. 2). We found a significant skewing of fetal biometrics with a smaller AC versus FL, which was first apparent in the 24-27 6/7 week strata. Fetal body ratios (HC:FL and AC:FL, by IG-21) were consistent with a femur-sparing pattern of fetal growth restriction in the majority of pregnancies with possible maternal ZIKV infection.

Results in the Context of What is Known

Fetuses that were either small for gestational age or growth restricted were reported to occur in 9% of pregnancies with a possible ZIKV infection in Rio de Janeiro, Brazil.[16] Interestingly, the authors characterized 4 cases of microcephaly in their cohort as either “proportionate” (2/4, 50%) or “disproportionate” (2/4, 50%) relative to the size of the infant; a “disproportionate” microcephaly indicated a grossly differential growth of the

head with respect to other body parts in at least half of their index cases. IUGR has also been described as a hallmark feature of several murine models of ZIKV infection in pregnancy and is associated with spontaneous abortion and stillbirth in these models.[41, 42, 43, 44] Although a femur-sparing pattern of growth restriction has been mentioned in the literature[45], it has not been characterized in the context of maternal complications of pregnancy or exposure to any teratogenic virus. Interestingly, few studies have characterized the IUGR phenotype in pregnancies with viral infections with the exception of a symmetric profile of IUGR associated with congenital cytomegalovirus infection.[13]

Skewed Distribution of Fetal Biometry in Pregnancies with Possible Maternal ZIKV Infection

Beginning in the late second trimester, maternal ZIKV infection was associated with a significantly smaller AC, by both IG-21 and WHO-FGC, and HC by IG-21 compared to FL. Analysis of IG-21 fetal body ratios with respect to FL revealed a femur-sparing profile of growth restriction in the majority of pregnancies with a possible ZIKV infection. The stable or negative trajectory of the AC:FL or HC:FL over time and the high proportion of women with symptoms (nearly half) with an abnormal HC:FL ratio is concerning for ZIKV-associated fetal injury. Identification of a femur-sparing profile of fetal growth restriction using IG-21 fetal body ratios could aid pediatricians in prioritizing neonates for imaging in low-resource settings. It is important to note that this profile of injury may not be obvious using other sonographic standards, primarily due to differences in FL distribution. For example, the Hadlock sonographic standard is anecdotally associated with the finding of “short femurs” and may not yield the same

growth restriction profile.[28, 29, 30] A discordance between the rate of fetuses with a small AC and rate of small for gestational age neonates may be a consequence of this particular type of growth restriction that preserves skeletal growth, which may compensate for birth weight. Whether abnormal growth of the fetus in relation to the femur correlates with long-term adverse outcomes for the developing child is unknown, but identification of an abnormal fetal body ratio (AC:FL or HC:FL) may be superior to measurement of fetal BPD or HC alone as a marker for ZIKV-associated fetal injury.

Clinical and Research Implications

The pathogenesis of perinatal infections resulting in fetal injury is complex and involves both indirect and direct effects. ZIKV infections could have a direct effect on fetal growth through targeted injury of the brain and liver, but also an indirect effect through trophoblast injury and a reduction in oxygen carrying capacity.[46] If viral tropism for cells in the fetal brain and liver is greater than tropism for the skeleton, this could produce differential viral effects on fetal growth that might result in the femur-sparing profile of fetal growth restriction that we observed in our study. As the size of the fetal abdomen directly correlates with liver size [47], ZIKV injury of the fetal liver may depress growth of the abdomen. ZIKV RNA has been detected in the liver in humans and animal models.[11, 48, 49] Liver injury is also a well-known outcome for many viruses related to ZIKV (e.g. Hepatitis C, dengue virus).[50, 51] Future studies of the effect of ZIKV on the fetal liver may in part explain the pathogenesis of fetal growth restriction with this infection.

We would like to emphasize that our results do not suggest that a femur-sparing profile of growth restriction is the only possible phenotype or outcome of perinatal ZIKV

infection. A normal growth profile may occur if the pregnant woman clears the virus before vertical transmission can occur. A fetal growth profile consistent with symmetric IUGR may occur with early and severe placental infections, which could compromise placental function; this effect would be similar to observations of placental infarctions and compromised placental oxygen transport in a nonhuman primate model following experimental ZIKV infection.[46] Additional research may further elucidate the relationship between IUGR and ZIKV infection, and characterize extreme cases of fetal injury, phenotype of IUGR and impact of timing of infection. Finding a more sensitive biomarker of viral injury, such as a sonographic profile of fetal growth, may help guide the pediatricians' evaluation and triage cases for postnatal follow up where resources are limited.

Strengths and Weaknesses

The strengths of this study are in the detailed fetal growth assessment from a relatively large sample of pregnancies with possible maternal ZIKV infection and the novel identification of a variant in fetal growth restriction associated with viral infection. A further strength is in the evaluation and comparison of biometric measures using two contemporary, international fetal growth studies. Finally, the novel use of IG-21 fetal body ratios to interpret fetal size in pregnancies with possible ZIKV infection may be useful for clinical care and also relevant to more common forms of IUGR. One limitation of our study is that the diagnosis of ZIKV infection is challenging due to the transient nature of viremia and cross-reactivity with other flaviviruses. Another important study limitation is the small sample size and lack of a specific fetal growth standard for this population; creating a robust standard would be challenging, however, given the ethnic

diversity of the cohort. Future studies with larger cohorts are necessary to validate our findings and determine if adverse neonatal outcomes might be associated with a femur-sparing profile of growth restriction. Although our study definitions of IUGR and microcephaly were in line with current standards, they may capture some constitutionally small infants; as we did not base IUGR on EFW, this may also limit comparability to other studies. However, the surprising distribution of cases with differential growth of the abdomen and head versus the femur is suggestive of an unusual pattern of fetal growth restriction that is not typically seen in pregnancy.

Conclusion

In summary, our results suggest that infants born following a possible maternal ZIKV infection may have abnormal growth patterns of the fetal head and abdomen with respect to the femur. Calculation of IG-21 fetal body ratios (AC:FL or HC:FL) may provide an early indication of aberrant fetal growth before a clinical or sonographic diagnosis of IUGR or microcephaly. Alerting clinicians to deviations in symmetric growth of a nonmicrocephalic fetus with congenital ZIKV exposure may aid in the identification of cases at risk for a greater spectrum of ZIKV-associated morbidity (e.g. eye abnormalities, postnatal microcephaly). These cases could be prioritized for more intensive neonatal follow-up in low resource settings for earlier interventions after delivery. Ultimately, larger cohorts will be important to validate a femur-sparing profile of growth restriction in women with a possible ZIKV infection in pregnancy and investigate whether this profile might predict adverse fetal and neonatal outcomes.

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Data Availability

Fetal biometric measures from de-identified cases will be made available upon request.

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Figure Legends

Figure 1. Fetal Body Ratio Z-Scores from U.S. Women with Possible Maternal ZIKV Exposure Using the IG-21 Sonographic Standard. A negatively skewed distribution of HC:FL and AC:FL is apparent within every gestational age strata. Data is color coordinated to show individual subjects. Depending on the number of ultrasound scans per subject, one subject may contribute ultrasound data to multiple gestational age strata in the table, but only one (the latest) ultrasound per subject was used in each strata. Application of the IG-21 sonographic standard to generate Z-scores is shown for HC:FL (A), and AC:FL (B).

Figure 2. Femur-sparing Profile of IUGR in Comparison to Normal and Other Abnormal Fetal Growth Patterns. Aberrant fetal growth in association with a possible maternal ZIKV infection is characterized by a femur-sparing profile of aberrant fetal growth. This figure illustrates how the femur-sparing profile of IUGR compares to normal fetal growth and more common IUGR growth patterns (symmetric and asymmetric IUGR).

Table 1. Rates of Microcephaly and IUGR by Exposure Time

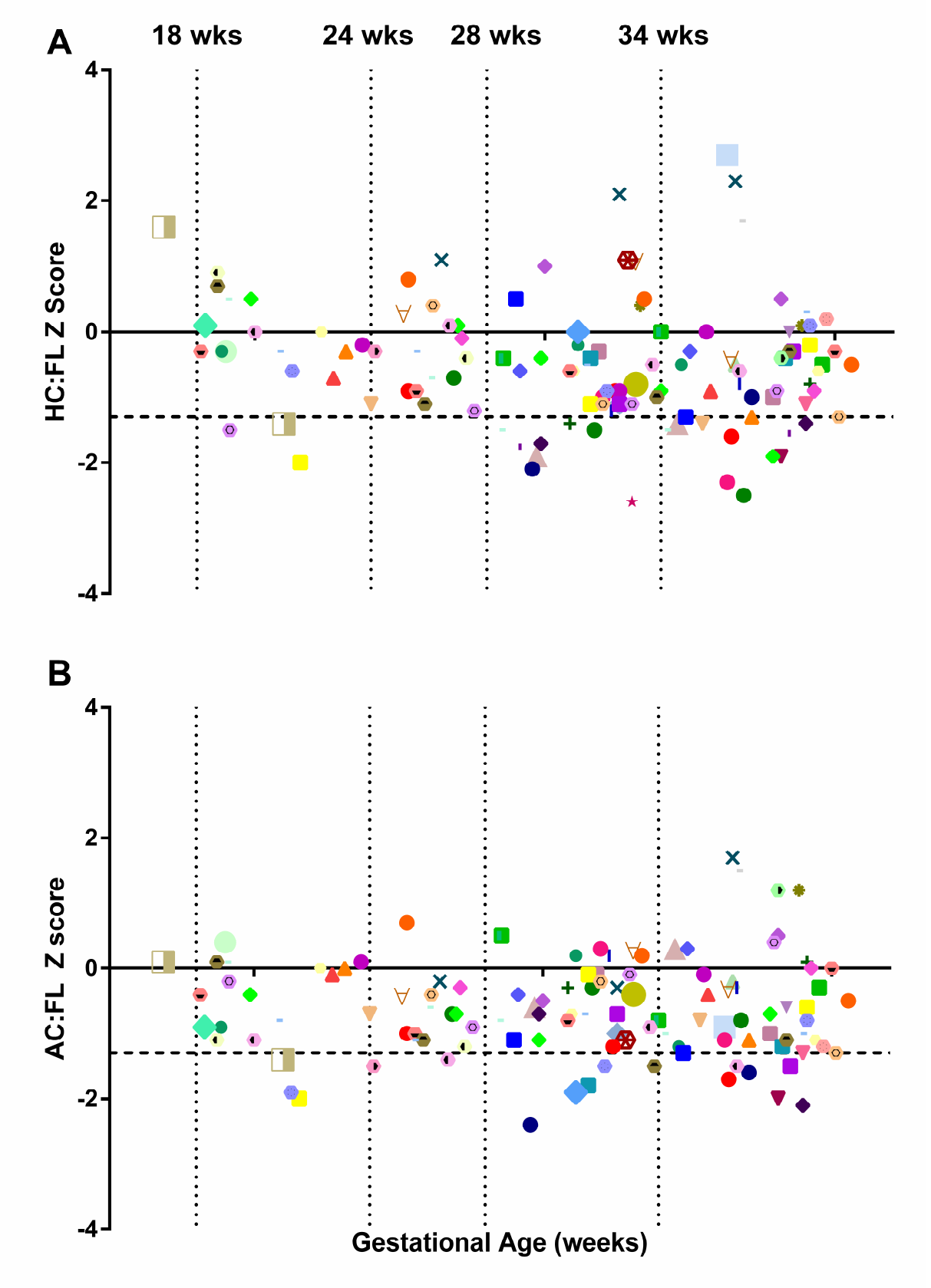
Exposure Time	Gestational Age at Delivery (weeks)	Prenatal Diagnosis of Microcephaly (HC <3%)		Prenatal Diagnosis of IUGR				Birthweight* (g)	Birthweight (% IG-21)
				AC <3%		AC <10%			
		WHO-FGC	IG-21	WHO-FGC	IG-21	WHO-FGC	IG-21		
All (N=56)	37.4 (4.2)	3 (5)	3 (5)	5 (9)	5 (9)	8 (14)	10 (18)	3159 (659)	55 (28.9)
Preconception (N=4)	38 (0)	0	0	0	0	1 (25)	1 (25)	2682 (102)	18.4 (3.3)
First Trimester (N=16)	39.1 (0.8)	1 (6)	1 (6)	3 (19)	2 (13)	3 (19)	5 (31)	3324 (328)	60.2 (22.1)
Second Trimester (N=11)	37.8 (3.2)	1 (9)	1 (9)	1 (9)	1 (9)	1 (9)	1 (9)	3412 (524)	59.0 (36.8)
Unknown Trimester (N=25)	38.2 (2.2)	1 (4)	1 (4)	1 (4)	2 (8)	3 (12)	3 (12)	3111 (676)	54.3 (29.5)

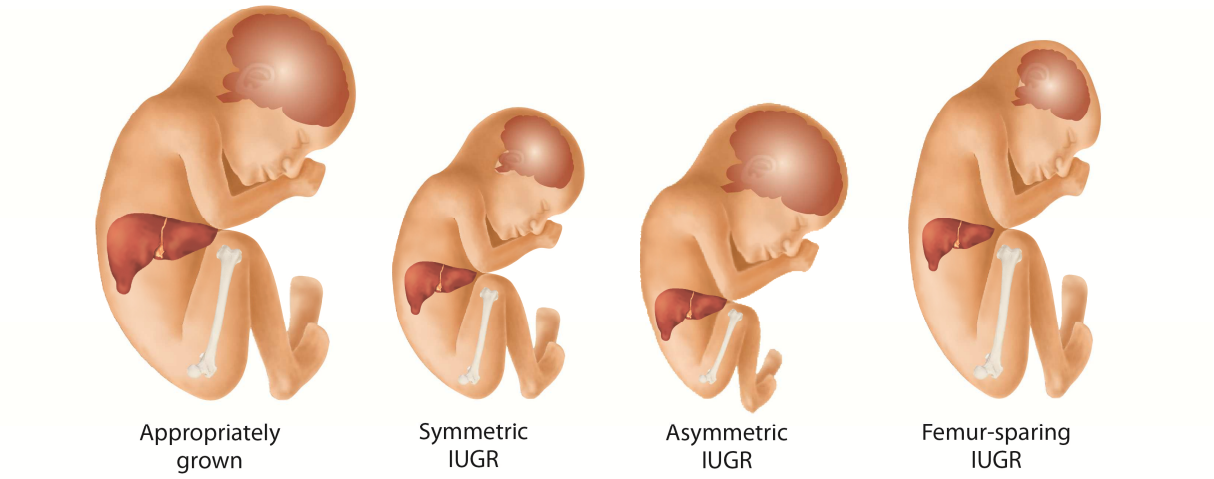
Numbers reflect the mean (standard deviation) or N (%) with Z-score (as indicated) for the entire cohort and also by time of possible ZIKV exposure. Data for prenatal diagnosis of microcephaly and IUGR is based on the last ultrasound obtained per subject. AC, abdominal circumference; FL, femur length; HC, head circumference; IG-21, 2014 International Fetal and Newborn Growth Consortium for the 21st Century; WHO-FGC, World Health Organization Fetal Growth Chart study. *Birthweight data was available for 48 infants (preconception, N=2; first trimester, N=15; second trimester, N=8; unknown trimester, N=23).

Table 2. IG-21 Fetal Z-Scores for Biometric Measures by Gestational Age Strata

Gestational Age Strata	HC	AC	FL	P values	
				FL vs. HC	FL vs. AC
All (N=56)	0.1 (1.2)	0.0 (1.3)	0.7 (1.4)	<0.001	<0.001
>34 weeks (N= 46)	0.4 (0.6)	0.2 (0.8)	0.9 (0.9)	<0.001	<0.001
28 – 33 6/7 weeks (N= 38)	0.1 (1.4)	0.1 (1.4)	0.7 (1.4)	0.007	<0.001
24 – 27 6/7 weeks (N= 17)	0.5 (0.7)	-0.1 (0.7)	0.6 (0.8)	0.8	0.002
18-23 6/7 weeks (N= 19)	0.2 (1.3)	0.0 (1.5)	0.5 (1.2)	0.9	0.7

Values reflect Z-scores within each gestational age strata using the last US scan in each pregnancy or gestational age strata based on the number of subjects. HC, head circumference; AC, abdominal circumference; FL, femur length. P values were calculated using Wilcoxon rank sum to compare paired Z-scores (IG-21) between FL and HC or FL and AC. A p value of <0.05 was considered significant.


A
18 wks
24 wks
28 wks
34 wks
HC:FL Z Score
4
2
0
-2
-4
B
AC:FL Z score
4
2
0
-2
-4
Gestational Age (weeks)


Appropriately grown
Symmetric IUGR
Asymmetric IUGR
Femur-sparing IUGR

Supplemental Materials

Tables

Table S1. Values for BPD:FL Mean and Standard Deviation Derived from IG-21

Gestational Age	Mean -2 SD	Mean -1 SD	Mean BPD:FL	Mean +1 SD	Mean +2 SD
15	1.68	1.83	1.97	2.12	2.26
16	1.57	1.70	1.83	1.97	2.10
17	1.48	1.61	1.73	1.86	1.98
18	1.42	1.54	1.66	1.77	1.89
19	1.38	1.49	1.60	1.71	1.82
20	1.34	1.45	1.55	1.66	1.76
21	1.32	1.42	1.52	1.62	1.72
22	1.30	1.40	1.50	1.59	1.69
23	1.29	1.39	1.48	1.57	1.66
24	1.29	1.38	1.47	1.55	1.64
25	1.28	1.37	1.45	1.54	1.63
26	1.28	1.36	1.45	1.53	1.62
27	1.28	1.36	1.44	1.52	1.60
28	1.27	1.35	1.43	1.51	1.60
29	1.27	1.35	1.43	1.51	1.59
30	1.27	1.35	1.42	1.50	1.58
31	1.27	1.34	1.42	1.49	1.57
32	1.26	1.34	1.41	1.49	1.56
33	1.26	1.33	1.41	1.48	1.55
34	1.25	1.33	1.40	1.47	1.54
35	1.25	1.32	1.39	1.46	1.53
36	1.24	1.31	1.38	1.45	1.52
37	1.23	1.30	1.37	1.44	1.51
38	1.22	1.29	1.36	1.42	1.49
39	1.21	1.27	1.34	1.41	1.48
40	1.19	1.26	1.33	1.39	1.46
41	1.18	1.25	1.31	1.38	1.45
42	1.16	1.23	1.30	1.36	1.43

Values represent the mean, mean ± 1 or mean ±2 standard deviations for BPD:FL ratio for each gestational week as derived from the IG-21 sonographic standard. IG-21 Z-scores for fetal body ratios, biometric measures and neonatal head circumference are publicly accessible on the web.(1)

Table S2. Values for HC:FL Mean and Standard Deviation Derived from IG-21

Gestational Age	Mean -2 SD	Mean -1 SD	Mean HC:FL	Mean +1 SD	Mean +2 SD
15	5.98	6.43	6.87	7.32	7.76
16	5.59	6.00	6.41	6.82	7.23
17	5.30	5.69	6.07	6.45	6.83
18	5.11	5.46	5.82	6.18	6.54
19	4.97	5.30	5.64	5.98	6.32
20	4.87	5.19	5.51	5.83	6.15
21	4.80	5.11	5.41	5.72	6.03
22	4.75	5.04	5.34	5.63	5.93
23	4.72	5.00	5.28	5.57	5.85
24	4.69	4.97	5.24	5.51	5.78
25	4.67	4.94	5.20	5.47	5.73
26	4.66	4.91	5.17	5.43	5.68
27	4.64	4.89	5.14	5.39	5.64
28	4.63	4.87	5.11	5.36	5.60
29	4.61	4.85	5.09	5.33	5.56
30	4.59	4.83	5.06	5.29	5.53
31	4.57	4.80	5.03	5.26	5.49
32	4.55	4.77	5.00	5.22	5.45
33	4.52	4.74	4.96	5.18	5.40
34	4.49	4.71	4.92	5.14	5.36
35	4.45	4.67	4.88	5.10	5.31
36	4.42	4.63	4.84	5.05	5.26
37	4.38	4.59	4.80	5.00	5.21
38	4.33	4.54	4.75	4.95	5.16
39	4.29	4.49	4.70	4.90	5.10
40	4.24	4.44	4.64	4.84	5.05
41	4.18	4.39	4.59	4.79	4.99
42	4.13	4.33	4.53	4.73	4.93

Values represent the mean, mean ± 1 or mean ± 2 standard deviations for HC:FL ratio for each gestational week as derived from the IG-21 sonographic standard. IG-21 Z-scores for fetal body ratios, biometric measures and neonatal head circumference are publicly accessible on the web.(1)

Table S3. Values for AC:FL Mean and Standard Deviation Derived from IG-21

Gestational Age	Mean -2 SD	Mean -1 SD	Mean AC:FL	Mean +1 SD	Mean +2 SD
15	4.92	5.31	5.70	6.10	6.49
16	4.68	5.05	5.42	5.79	6.16
17	4.49	4.84	5.20	5.55	5.90
18	4.35	4.69	5.03	5.37	5.71
19	4.25	4.57	4.90	5.23	5.56
20	4.17	4.49	4.81	5.12	5.44
21	4.12	4.43	4.74	5.04	5.35
22	4.08	4.38	4.68	4.99	5.29
23	4.06	4.35	4.65	4.94	5.24
24	4.05	4.34	4.63	4.92	5.20
25	4.05	4.33	4.61	4.90	5.18
26	4.05	4.33	4.61	4.89	5.17
27	4.06	4.33	4.61	4.88	5.16
28	4.07	4.35	4.62	4.89	5.16
29	4.09	4.36	4.63	4.90	5.16
30	4.11	4.38	4.64	4.91	5.17
31	4.14	4.40	4.66	4.92	5.19
32	4.16	4.42	4.68	4.94	5.20
33	4.19	4.44	4.70	4.96	5.22
34	4.21	4.47	4.73	4.98	5.24
35	4.24	4.50	4.75	5.00	5.26
36	4.27	4.52	4.77	5.03	5.28
37	4.30	4.55	4.80	5.05	5.30
38	4.33	4.58	4.83	5.08	5.33
39	4.36	4.61	4.85	5.10	5.35
40	4.39	4.63	4.88	5.13	5.38
41	4.42	4.66	4.91	5.15	5.40
42	4.45	4.69	4.94	5.18	5.43

Values represent the mean, mean ± 1 or mean ± 2 standard deviations for AC:FL ratio for each gestational week as derived from the IG-21 sonographic standard. IG-21 Z-scores for fetal body ratios, biometric measures and neonatal head circumference are publicly accessible on the web.(1)

Table S4. Laboratory Evidence for Possible Maternal ZIKV Infection

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
First Trimester Exposure										
1	None	**Pos**	Neg	**Pos**	Not Done	Not Done	Not Done	Not Done	Not Done	Not Done
2	Yes-rash	Neg	Not Done	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Neg**
3	None	**Pos**	Neg	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Neg
4	None	Neg	Neg	**Pos**	Neg	**Pos**	Not Done	Neg	Neg	Neg
5	None	**Pos**	Neg	**Pos**	Not Done	Not Done	Not Done	Neg	Neg	Equiv
6	None	Neg	Neg	**Pos**	Equiv	**Pos***	**Pos**	Neg	Neg	Equiv
7	None	Neg	**Pos**	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Equiv
8	None	Neg	Neg	**Pos**	Neg	**Pos**	Not Done	Neg	Neg	Neg
9	None	Not Done	Not Done	**Pos**	**Pos**	**Pos***	Not Done	Not Done	Not Done	Not Done

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
First Trimester Exposure (Cont'd)										
10	Yes-arthralgia	Not Done	Not Done	**Pos**	Neg	**Pos***	Not Done	Not Done	Not Done	Not Done
11	None	Equiv	Neg	**Pos**	Neg	**Pos***	Not Done	Not Done	Not Done	Not Done
12	None	**Pos**	Not Done	Neg	Not Done	Not Done	Not Done	Neg	Neg	Neg
13	Yes - rash, fever	Equiv	Neg	**Pos**	**Pos**	**Pos***	**Pos***	Not Done	Not Done	Not Done
14	Yes - rash, arthralgia	Not Done	Not Done	**Pos**	Equiv	**Pos***	**Pos***	Not Done	Not Done	Not Done
15	Yes-fever, rash, arthralgia	**Pos**	Not Done	Neg	Not Done	Not Done	Not Done	Not Done	Not Done	Not Done
16	Yes-rash	**Pos**	Not Done	**Pos**	Not Done	Not Done	Not Done	Neg	Not Done	Neg
Second Trimester Exposure										
17	No	Not Done	Not Done	**Pos**	**Pos**	**Pos***	Not Done	Neg	Not Done	Neg

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
Second Trimester Exposure (Cont'd)										
18	Yes - rash, fever, headache	Not Done	Not Done	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Neg
19	Yes - rash, headache, conjunctivitis	**Pos**	**Pos**	**Pos**	Not Done	Not Done	Not Done	Neg	Neg	Neg
20	No	Neg	Neg	Equiv	Equiv	**Pos***	Not Done	Neg	Neg	Neg
21	Yes- rash	**Pos**	**Pos**	**Pos**	Not Done	**Pos***	Not Done	Neg	Not Done	Neg
22	Yes – rash	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Not Done	Not Done	Neg
23	No	Not Done	Neg	**Pos**	Equiv	**Pos***	**Pos***	Neg	Neg	Equiv
24	Yes – rash	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Neg	Not Done	Equiv
25	Yes – rash	Neg	Neg	**Pos**	**Pos**	**Pos***	**Pos***	Neg	Not Done	Equiv
26	Yes - fever, myalgias, rash	Neg	Neg	Neg	Neg	**Pos***	Not Done	Not Done	Not Done	Not Done

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
Unknown Trimester Exposure										
27	None	**Pos**	Neg	Neg	Not Done	Not Done	Not Done	Neg	Neg	Neg**
28	None	Neg	Neg	**Pos**	**Pos**	**Pos***	**Pos***	Neg	Not Done	Neg**
29	None	Not Done	Not Done	**Pos**	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done
30	None	Neg	**Pos**	**Pos**	Not Done	Not Done	Not Done	Neg	Neg	Equiv
31	None	Neg	Neg	Equiv	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done
32	None	Neg	Neg	Equiv	**Pos**	**Pos***	**Pos***	Neg	Neg	Equiv
33	None	Neg	Neg	**Pos**	**Pos**	**Pos***	Not Done	Neg	Neg	**Pos**
34	None	Neg	Neg	**Pos**	Neg	**Pos***	Not Done	Neg	Not Done	Equiv
35	None	Neg	Neg	**Pos**	Equiv	**Pos***	Not Done	Not Done	Neg	Equiv
36	None	Neg	Neg	Equiv	Not Done	**Pos***	Not Done	Neg	Neg	Equiv

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
Unknown Trimester Exposure (Cont'd)										
37	None	Neg	Neg	**Pos**	**Pos**	**Pos***	Not Done	Neg	Neg	Equiv
38	None	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Not Done	Neg	Neg
39	None	Neg	Neg	**Pos**	Neg	**Pos***	**Pos***	Neg	Neg	Equiv
40	None	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Equiv
41	None	Neg	**Pos**	**Pos**	Not Done	Not Done	Not Done	Neg	Not Done	Neg
42	None	Neg	**Pos**	**Pos**	Neg	**Pos***	Not Done	Neg	Neg	Neg
43	None	Neg	Neg	**Pos**	Neg	**Pos***	Not Done	Neg	Neg	Neg
44	None	Neg	Neg	**Pos**	**Pos**	**Pos***	Not Done	Not Done	Neg	Equiv
45	None	**Pos**	Neg	Neg	Not Done	Not Done	Not Done	Neg	Neg	Neg
46	None	Neg	Neg	**Pos**	Neg	**Pos***	Not Done	Neg	Neg	Not Done

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
Unknown Trimester Exposure (Cont'd)										
47	None	Neg	Neg	Equiv	Neg	**Pos**	Not Done	Not Done	Not Done	Neg
48	None	Not Done	Not Done	Not Done	Not Done	**Pos***	Not Done	Neg	Not Done	Neg
49	None	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Neg	Neg	Neg
50	None	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done
51	None	Neg	Neg	Equiv	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done
Preconception Exposure										
52	None	Neg	Neg	**Pos**	Not Done	**Pos***	Not Done	Not Done	Neg	Equiv
53	None	Neg	Neg	Equiv	Neg	**Pos***	**Pos***	Neg	Not Done	Neg
54	None	Neg	Neg	Equiv	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done

Subject	Symptom	Serum PCR	Urine PCR	Zika IgM #1	Zika IgM #2	Zika PRNT #1	Zika PRNT #2	Infant Serum PCR	Infant Urine PCR	Infant IgM
Preconception Exposure (Cont'd)										
55	None	Neg	Neg	**Pos**	Neg	**Pos***	**Pos***	Not Done	Not Done	Not Done
56	None	Neg	Neg	Equiv	Not Done	**Pos***	Not Done	Not Done	Not Done	Not Done

Pos, positive; Neg, negative; Equiv, equivocal test result.

*Refers to a positive test result for an "undifferentiated flavivirus."

**Negative ZIKV IgM result, but a West Nile Virus microsphere immunoassay positive. Positive results are known to occur with persons vaccinated or infected with other flaviviruses, like ZIKV.

Table S5. Fetal Biometric Measures Less than the 3rd Centile

Gestational Age Groups	HC (<3%)		AC (<3%)		FL (<3%)	
	WHO-FGC	IG-21	WHO-FGC	IG-21	WHO-FGC	IG-21
All (N=56)	3 (5)	3 (5)	3 (5)	3 (5)	5 (9)	4 (7)
>34 weeks (N= 46)	0	0	1 (2)	2 (4)	2 (4)	1 (2)
28 – 33 6/7 weeks (N= 38)	2 (5)	2 (5)	1 (3)	2 (5)	2 (5)	3 (8)
24 – 27 6/7 weeks (N= 17)	0	0	0	0	0	0
18-23 6/7 weeks (N= 19)	1 (5)	1 (5)	1 (5)	1 (5)	1 (5)	1 (5)

Values reflect N (%) less than the 3rd centile within each gestational age strata using the last US scan in each pregnancy or gestational age strata based on the number of subjects. HC, head circumference; AC, abdominal circumference; FL, femur length.

Table S6. Birth Outcomes for Each Subject

Subject	Gestational Age at Delivery (weeks)	Birthweight (g)	Birthweight Z-score IG-21	HC at birth (cm)	HC Z-score IG-21	Postnatal Imaging	Delivery Outcome
First Trimester Exposure							
1	19	-	-	-	-	-	D+E, Placenta PCR+
2	33	1320	-1.5	27.5	-2.1	Normal	PTD@33 weeks
3	40	3450	0.5	34.5	0.7	Normal	Term
4	36	2640	0.1	34	1.5	Not Done	PTD@36 weeks
5	39	3105	-0.3	35	0.9	Normal	Term
6	37	3135	0.6	32	-0.8	Normal	Term
7	39	3190	0.2	33	-0.4	Normal	Term
8	40	2930	-0.9	33	-0.7	Normal	Term
9	-	-	-	-	-	-	D+E
10	40	3680	0.7	34	-0.3	Normal	Term
11	40	3480	0.5	34	0.2	Not Done	Term
12	40	3165	-0.3	34.5	0.7	Normal	Term

Subject	Gestational Age at Delivery (weeks)	Birthweight (g)	Birthweight Z-score IG-21	HC at birth (cm)	HC Z-score IG-21	Postnatal Imaging	Delivery Outcome
First Trimester Exposure (Cont'd)							
13	39	3440	0.8	-	-	Not Done	Term
14	39	3425	0.7	34	0.5	Normal	Term
15	23	-	-	-	-	-	D+E
16	39	2850	-0.7	33.5	0.1	Normal	Term
Second Trimester Exposure							
17	39	2800	-1.1	33.5	-0.3	Not Done	Term
18	40	3860	1.1	34	-0.3	Normal	Term
19	39	3380	0.3	36	1.7	Normal	Term
20	39	3395	0.4	34.5	0.5	Normal	Term
21	40	3565	0.4	35	0.6	Normal	Term
22	37	2630	-0.7	34.5	1.2	Left CPC	Term
23	38	3315	0.6	34	0.4	Right CPC	Term
24	39	2970	-0.7	34	0.1	Normal	Term

Subject	Gestational Age at Delivery (weeks)	Birthweight (g)	Birthweight Z-score IG-21	HC at birth (cm)	HC Z-score IG-21	Postnatal Imaging	Delivery Outcome
Second Trimester Exposure (Cont'd)							
25	40	3760	0.9	35	0.6	Normal	Term
26	30	725	-3.0	-	-	-	IUFD @30 weeks, Placenta PCR+
Unknown Trimester Exposure							
27	37	2100	-2.0	32.5	-0.4	Normal	IUGR, Hypotonia, Prader-Willi
28	35	2815	0.8	33.5	1.1	Not Done	PTD@35 weeks
29	40	2855	-1.3	34	-0.3	Not Done	Term
30	40	3685	0.7	34.5	0.2	Normal	Term
31	39	2975	-0.4	33.5	0.1	Not Done	Term
32	39	3155	-0.2	34.5	0.5	Normal	Term
33	39	3285	0.4	33.5	0.1	Normal	Term
34	39	3275	0.4	34.5	1.0	Left CPC	Term
35	37	3690	2.1	34	1.2	Not Done	Term

Subject	Gestational Age at Delivery (weeks)	Birthweight (g)	Birthweight Z-score IG-21	HC at birth (cm)	HC Z-score IG-21	Postnatal Imaging (First Week of Life)	Delivery Outcome
Unknown Trimester Exposure (Cont'd)							
36	40	3700	0.8	35	0.6	Grade 1 IVH	Term
37	38	3650	1.6	35	1.7	Normal	Term
38	33	1859	-0.2	29	-1.4	Normal	PTD@33 weeks
39	38	2990	-0.2	34	0.4	Normal	Term
40	37	3130	0.8	34.5	1.6	Normal	Term
41	39	3420	0.4	34.5	0.5	Not Done	Term
42	39	3135	0.0	34	0.5	Normal	Term
43	40	3840	1.1	34	-0.3	Normal	Term
44	39	3845	1.7	35.5	1.8	Normal	Term
45	41	3940	2.0	37	2.0	Not Done	Term
46	39	4290	2.3	36	1.7	Normal	Term
47	40	3060	-0.8	34.5	0.2	Normal	Term
48	38	2985	0.03	34	0.8	Normal	Term
49	38	3790	1.6	34	0.4	Normal	Term

Subject	Gestational Age at Delivery (weeks)	Birthweight (g)	Birthweight Z-score IG-21	HC at birth (cm)	HC Z-score IG-21	Postnatal Imaging	Delivery Outcome
Unknown Trimester Exposure (Cont'd)							
50	-	-	-	-	-	-	-
51	40	3305	-0.2	35	0.6	Not Done	Term
Preconception Exposure							
52	38	2610	-1.0	33	0	Normal	Term
53	39	2755	-0.8	33	-0.4	Normal	Term
54	38	-	-	-	-	-	-
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-

-, Information not available.

D+E, second trimester termination of pregnancy with or without prior fetal demise,

PTD, preterm delivery

CPC, choroid plexus cyst

IUFD, intrauterine fetal demise

Postnatal imaging reflects a neonatal head US performed within the first week of life.

Table S7. Distribution and Comparison of Fetal Biometric Measures by WHO-FGC

Gestational Age Groups	HC	AC	FL	P values	
				FL vs. HC	FL vs. AC
All (N=56)	54 (24)	49 (29)	59 (28)	0.6	<0.001
>34 weeks (N= 46)	57 (19)	53(26)	63 (25)	0.4	0.05
28 – 33 6/7 weeks (N= 38)	59 (26)	55 (29)	64 (30)	0.07	0.004
24 – 27 6/7 weeks (N= 17)	67 (23)	39 (25)	63 (28)	0.3	0.001
18-23 6/7 weeks (N= 19)	68 (28)	60 (30)	70 (25)	0.08	1

Values reflect Mean (SD) within each gestational age strata using the last US scan in each pregnancy or gestational age strata based on the number of subjects. HC, head circumference; AC, abdominal circumference; FL, femur length. The p values were calculated using Wilcoxon rank sum to compare percentiles determined by WHO-FGC between HC and FL or AC and FL. A p value of <0.05 was considered significant.

Figure S1. HC:FL Ratio Across Gestational Age in Subjects with a Ratio Z-Score Less than 10th Centile

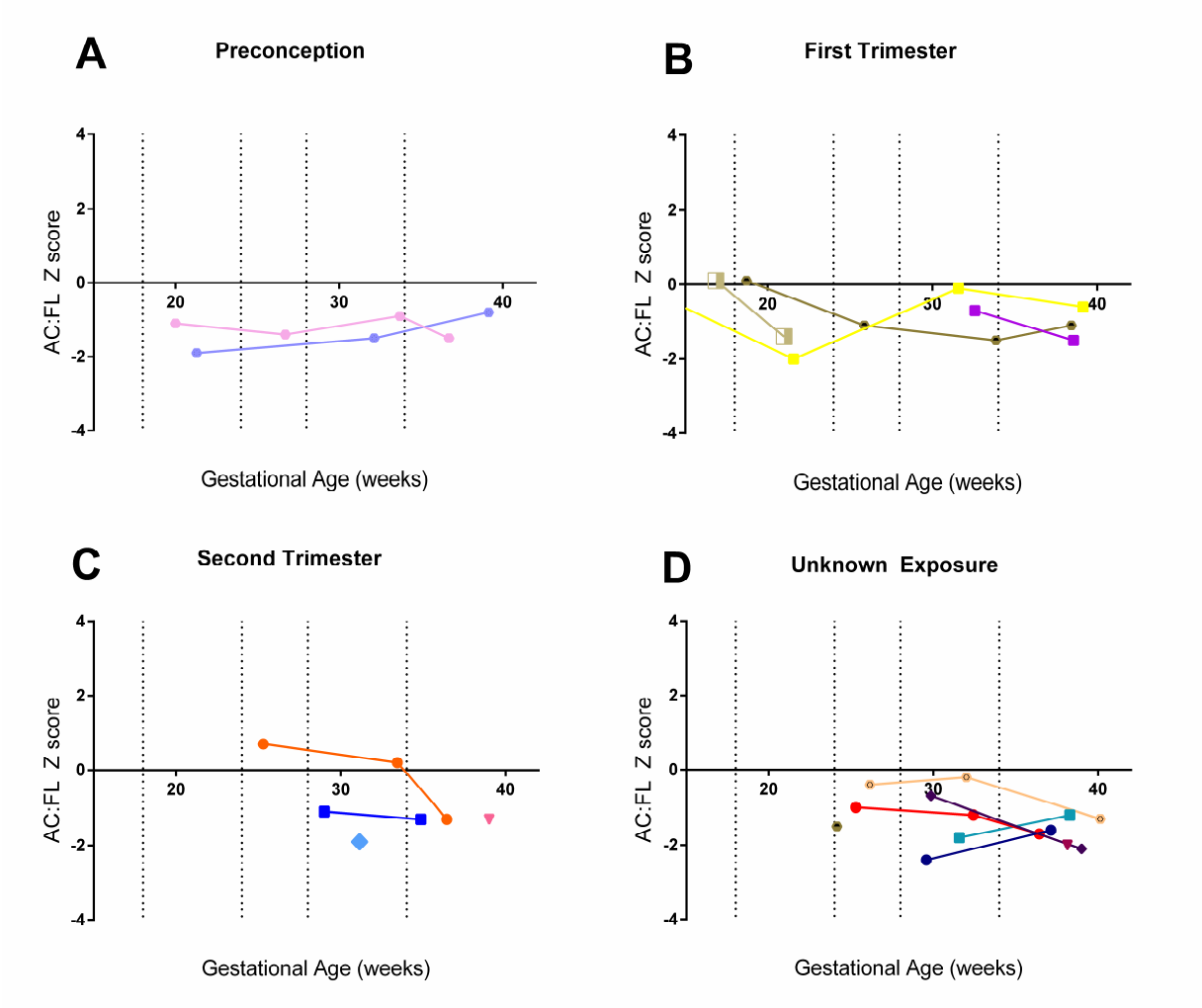
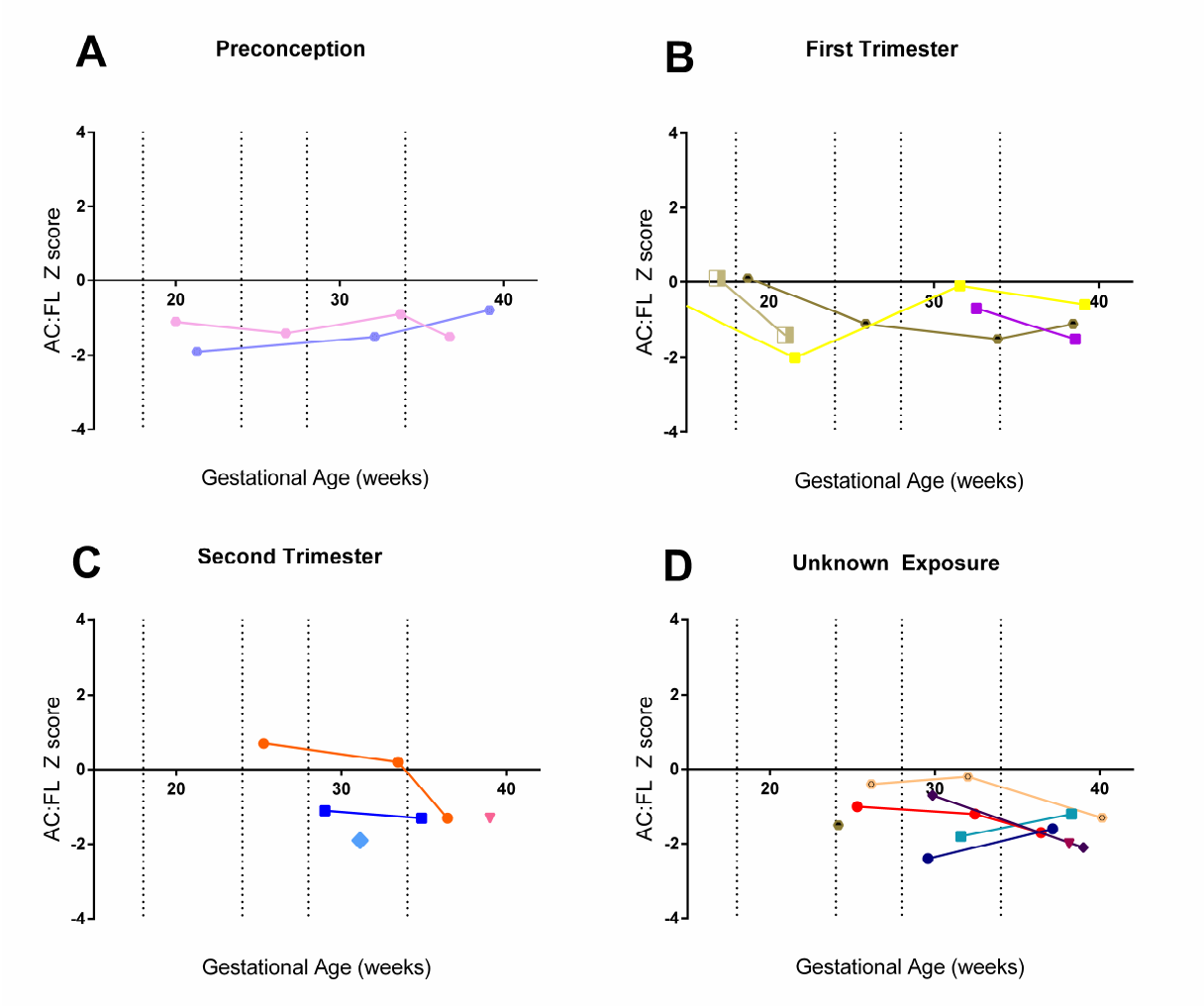


Figure S2. AC:FL Ratio Across Gestational Age in Subjects with a Ratio Z-Score Less than 10th Centile



References

1. INTERGROWTH-21st Applications and Calculators 2017 [Available from: https://intergrowth21.tghn.org/intergrowth-21st-applications/.