Effects of *Vivax* Malaria Acquired Before 20 Weeks of Pregnancy on Subsequent Changes in Fetal Growth

Amantino C. Machado Filho, Elenice P. da Costa, Emely P. da Costa, Iracema S. Reis, Emanuela A. C. Fernandes,
Bernardo V. Paim, and Flor E. Martinez-Espinosa*

Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Amazonas, Brazil;
Instituto Leônidas e Maria Deane, FIOCRUZ, Amazônia, Brazil

Abstract. The resistance index (RI), pulsatility index (PI), fetal biometry, fetal heart rate (FHR), placental thickness, and hemoglobin levels were compared in 30 *Plasmodium vivax*-infected women between 14 and 20 weeks of pregnancy and a control group. Evaluations were performed at the moment of the malaria diagnosis and 26 weeks of pregnancy. The malaria group had lower levels of hemoglobin and greater placental thickness in both assessments, higher FHR in the first evaluation, and lower values on fetal biometry in the second assessment. There were no differences when comparing RI and PI on umbilical arteries between the two groups. Birth weight and height were lower in newborns in the malaria group than the control group. The results suggest that *P. vivax* infections at an earlier gestational age do not affect umbilical arteries blood flow but do affect fetal biometry in the second trimester of pregnancy and at birth.

INTRODUCTION

It is well-recognized that the morbidity and mortality attributed to malaria differ between the two most prevalent species, *Plasmodium falciparum* and *P. vivax*.¹ Several studies suggest that the different susceptibilities between pregnant and non-pregnant women arise from the infecting parasite's cytoadherence to chondroitin sulfate A, which is present at the syncytiotrophoblast.² It has been shown that *P. falciparum*-infected erythrocytes migrate from peripheral circulation to internal organs, a phenomenon called sequestration, which plays a key role in the pathogenic event.¹ The mechanisms underlying severe disease in *P. vivax* malaria remains poorly defined.³ Nowadays, infection by *P. vivax* has been reported as a cause of severe malaria in endemic regions.⁴ The increasing number of cases of malaria infection by *P. vivax* with severe clinical manifestations similar to those manifestations observed *P. falciparum* suggests that adhesion mechanisms are shared by both parasites and that the strength of interaction with blood vessels is similar but less in *P. vivax*.⁴ Recent studies have also shown that *P. vivax*-infected erythrocytes cytoadhere in the intervillous space, although to a lesser degree compared with *P. falciparum*.^{4–6} Another study found *P. vivax*-infected erythrocytes in the intervillous space but no hemozoin in macrophages or increased intervillous inflammatory cells.⁷ *P. vivax* infection may exert its adverse effects on the fetus through maternal anemia or induction of a potent inflammatory response with abundant cytokine production, which will interfere with uteroplacental hemodynamics.^{8,9} It has been shown that cytokine production, such as production of tumor necrosis factor (TNF), is more intense during *P. vivax* infection compared with *P. falciparum* with similar parasite loads.¹⁰ A reversible acute placental insufficiency develops during *P. falciparum* infections. In such cases, placenta acts like a filter by retaining infected erythrocytes, thus leading to placental deterioration characterized by chorionic

villous degeneration, fibrin and malarial pigment deposition, basement membrane swelling, and accumulation of macrophages on intervillous space.¹¹ The infection by *P. vivax* in the placenta, is associated with less pronounced changes, being the major finding the increase hemozoin.¹²

In malaria-endemic regions, Doppler velocimetry studies have related malaria during pregnancy with maternal–fetal blood flow changes and also associated the occurrence of this disease during pregnancy to fetal growth restrictions.^{13,14} Placental malaria contributes to maternal morbidity and low birth weight in endemic countries.¹⁵ Studies on the impact of malaria in pregnancy generally point to a higher frequency of low birth weight. However, in infections occurring at early gestational age, fetal head circumference may be a more appropriate marker of fetal growth restriction. Although fetal head circumference and weight have similar growth velocity curves, they take place at different occasions during pregnancy.¹⁶

In the context of malaria transmission in the Western Brazilian Amazon, the city of Manaus faces a situation of unstable transmission, with an annual parasite incidence of 29 cases per 1,000 inhabitants.¹⁷ In this region, *P. vivax* infection accounts for more than 80% of the malaria cases, and the association between malaria and pregnancy occurs in nearly 1% of the pregnancies and may result in a wide range of complications, including miscarriage, pre-mature delivery, or low-birth weight infants.¹⁸ In this population, one in four pregnancies suffers some kind of threat during the acute event of malaria.¹⁷ To evaluate the effects of *P. vivax* infection on fetal growth and umbilical arteries by Doppler velocimetry, a case control study was carried out in pregnant women from Manaus.

MATERIALS AND METHODS

Study population. We conducted a case-control study at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) encompassing a group of pregnant women diagnosed with *P. vivax* infection between 14 and 20 weeks of gestational age. Evaluations were performed at the moment of the malaria diagnosis and 26 weeks of pregnancy. A control group of non-infected pregnant women from the same area was used for comparison.

*Address correspondence to Flor E. Martinez-Espinosa, Posgraduate Program on Tropical Medicine, Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Avenida Pedro Teixeira, No. 25, Dom Pedro, CEP 69.040-000, Manaus, Amazonas, Brazil. E-mail: florespinosa@gmail.com

Sample size was estimated using Epi Info 7.0 (Centers of Disease Control and Prevention). α -Error was fixed on 5%, and β -error was fixed on 20%, assuming an expected frequency of 35% altered Doppler velocimetry indices in the malaria group¹¹ and 3.5% altered Doppler velocimetry indices in the control group.¹⁹ A sample size of 25 patients was, therefore, achieved. This value was increased to a rate of 35% because of presumable losses, and a final number of 34 patients in each group was established. Patients' recruitment was consecutively held among pregnant women who spontaneously sought care at the FMT-HVD and had a positive thick blood smear for *P. vivax* from June of 2011 to July of 2012. As a control group, 34 pregnant women were enrolled who sought care at the FMT-HVD for pre-natal blood testing purposes and did not have malaria. Malaria group inclusion criteria were *P. vivax* infection diagnosed at the FMT-HVD; gestational age ranging from 14 to 20 weeks as determined by obstetric ultrasound evaluation; single gestation; and absence of any known illnesses before the pregnancy, such as diabetes mellitus and cardiovascular diseases. Patients who did not attend additional evaluations, patients who presented infections, such as syphilis, rubella, or human immunodeficiency virus (HIV), and patients whose fetuses presented cardiac arrhythmias were excluded. Control group patients were recruited using the same inclusion and exclusion criteria, except for a negative malaria microscopic test.

Clinical, laboratory, and ultrasound procedures. A single venous blood sample of 10 mL was obtained from each patient in a vacuum blood tube and evaluated for hematocrit and hemoglobin levels on an automated counter. Maternal parasite load was estimated using a semiquantitative method as recommended by the Brazilian Ministry of Health: ≤ 2 (40–60 parasites per 100 higher magnification microscopic fields), 1+ (1 parasite per microscopic field), 2+ (2–20 parasites per microscopic field), 3+ (21–200 parasites per microscopic field), and 4+ (> 200 parasites per microscopic field). An acute malaria episode was defined as the length of time ranging from early symptoms to the first negative thick blood smear. Maternal anemia was defined as hemoglobin values lower than 11 g/dL or hematocrit lower than 33% detected at any gestational age. Low birth weight (LBW) was defined as a newborn weighing < 2,500 g. Umbilical arteries (UAs) Doppler velocimetry flow was evaluated for resistance index (RI) as described by Pourcelot²⁰ and pulsatility index (PI) as described by Gosling and King²¹ as well as qualitative evaluation for the presence/absence of zero diastole flow (ZDF) or reverse diastole flow (RDF) in that vessel.

An Xario Ultrasound (Toshiba Medical System Corporation, Barueri, São Paulo, Brasil) convex transducer with frequency ranging from 3.5 to 5.0 MHz equipped with color Doppler was used for ultrasound scanning. Filter settings were fixed between 50 and 100 Hz, the size of Doppler sample window was adjusted between 1 and 2 mm, and the angle of insonation was always lower than 60°. Placental thickness was determined at a perpendicular plan to placental axis at the point of insertion of the umbilical cord. The upper limit of normality was obtained adding 10 mm to gestational age in weeks. To perform Doppler velocimetry analysis of UAs, we used color Doppler scan to localize the umbilical cord, and then, we used pulsatile Doppler analysis next to placental insertion. At least five similar consecutive waveforms were required for the sonogram be considered adequate. A wave-

form corresponding to a complete cardiac cycle was automatically determined, and the indices were calculated by the ultrasound machine. Each index was measured three times, and the mean was used for statistical purposes. All ultrasound and Doppler scanning were performed by a single observer. Patients were evaluated two times for hematocrit, hemoglobin, placental thickness, fetal biometry, fetal heart rate, and UAs Doppler velocimetry flow at the time of laboratorial diagnosis of malaria and the 26th week of pregnancy (all showing a negative blood smear) period when the fetal growth rate is considered to be higher.²² Fetal biometry encompassed assessment of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), and estimated fetal weight (EFW). All Doppler velocimetry index and fetal biometry assessments were taken three times in a sequence of 10 patients to determine intraobserver variability. Patients diagnosed with malaria were evaluated and treated with chloroquine, and follow-up was performed monthly throughout the whole pregnancy.

Ethics. This study was approved by the FMT-HVD Ethics Review Board (protocol number 2047/2011). Every pregnant woman who agreed to take part of this study signed an informed consent as recommended by National Health Council resolution no. 196/1996.

Statistical analysis. Data were analyzed on Epi Info 7.0 as well as Excel 7.0 e Minitab 15. Student's *t* and Mann-Whitney tests were used for means and ranks comparisons, respectively. Categorical data were compared using χ^2 and Fisher's exact tests whenever appropriate. The odds ratio (OR) was used to assess the strength of association between variables. Accepting alternative hypothesis considered a 95% confidence interval (95% CI) at a statistical significance level of $P < 0.05$.

RESULTS

Of 34 *P. vivax*-infected patients, 4 patients were excluded from the study: 3 patients did not attended one of the visits and 1 patient had an undetermined pregnancy outcome. In the control group, four patients were also excluded: one patient because of syphilis, one patient because of toxoplasmosis, one patient because of HIV infection, and one patient because of not attending one of the visits. Mean gestational age at the moment of recruitment was 16.3 weeks (± 1.9 SD). Primigravidae represented a total of 36.7% and 30% in malaria and control groups, respectively ($P = 0.78$). In terms of parasite load, 13.3%, 20%, 63.3%, and 3.3% presented ≥ 2 , 1+, 2+, and 3+, respectively. No patient presented a parasite load of 4+. There was no statistically significant difference between parasite load and parity ($P = 0.27$). Mean acute malaria episode length was 8.3 days (2.3 SD). Mean elapsed time between the first positive thick blood test and first negative test was 4.1 days (± 1.3 SD). In the malaria group, 40% of patients showed anemia compared with 6.6% of patients in the control group ($P = 0.006$). Anemia correlated with lower values on fetal biometry, UAs, RI, and PI in both evaluations performed in the malaria group; however, no level of statistical significance was reached (data not shown). Placental thickness greater than 10 mm above gestational age (upper limit of normality) in weeks was present in 40% and 10% of patients ($P = 0.015$) at first evaluation and 56.7% and 6.7% of patients ($P < 0.001$) on the second evaluation in malaria and control groups, respectively. There was no association

TABLE 1

Clinical and laboratorial features in *P. vivax*-infected pregnant women and a control group in a reference center for infectious diseases in the Western Brazilian Amazon

Feature	<i>P. vivax</i> -infected (N = 30)	Control group (N = 30)	P value
Maternal age (years)	25.7 ± 7.5	25.0 ± 4.6	0.34
Adolescents	9 (30.0)	6 (20.0)	0.55
Previous gestations	1 (1–4)	1 (1–3)	0.92
Parity	1 (0–4)	1 (0–4)	0.65
Primigravidae	11 (36.6)	10 (30.0)	0.94
History of LBW infant	2 (6.7)	6 (20.0)	0.25
Hematocrit (%)	32.4 ± 4.0*	35.9 ± 2.7†	< 0.001*
Hematocrit (%)	32.9 ± 2.0†	34.5 ± 1.0†	0.001†
Hemoglobin (g %)	10.8 ± 1.4*	12.0 ± 0.8*	< 0.001*
Hemoglobin (g %)	10.7 ± 0.7†	11.3 ± 0.4†	0.006†
Placental thickness‡	12 (40.0)*	3 (10.0)*	0.015*
Placental thickness‡	17 (56.7)†	2 (6.7)†	< 0.001†
Fetal heart rate (bpm)	154 (138–160)*	144 (127–160)*	0.005*
Fetal heart rate (bpm)	144 (127–160)†	142 (131–158)†	0.87†
GA at delivery (weeks)	37 (25–41)	39 (36–42)	0.039
NB weight (g)	2,948.2 ± 789.9	3,382.5 ± 289.5	0.001
NB length (cm)	46.1 ± 6.1	49.7 ± 1.3	< 0.001

Data are shown as ranks, mean ± SD, or n (%). GA = gestational age; NB = newborn.

*Mean GA = 16.3 weeks.

†GA = 26 weeks.

‡> GA + 10 mm.

between the occurrence of anemia and the increased placental thickness in both assessments in both groups (OR = 0.70, 95% CI = 0.15–3.16, $P = 0.64$ and OR = 1.42, 95% CI = 0.32–6.17, $P = 0.63$; Mantel–Haenszel; first and second evaluation, respectively). In the malaria group, 16.6% of patients showed LBW compared with 6.6% of patients in the control group ($P = 0.42$). There was no association between the occurrence of anemia and LBW (OR = 0.20, 95% CI = 0.12–4.71, $P = 0.30$; Mantel–Haenszel). Other clinical and laboratorial features are shown on Table 1.

Table 2 shows no statistically significant differences in BPD, HC, AC, FL, and estimated weight between malaria and control groups on the first assessment, but significant differences were found, with all these parameters being lower in the group with malaria on the 26th week of pregnancy evaluation. There were no statistically significant differences comparing primigravidae with multigravidae for BPD, HC, AC, FL, and estimated weight in both malaria and control groups on the first assessment (data not shown). Nonetheless, when evaluated on the 26th week of pregnancy, those differences showed statistical significance in the malaria group; all these parameters evaluated were lower in the group of primigravidae (Table 3).

Intraobserver variability was 3.2 and 3.1 for BPD, 10.4 and 11.2 mm for HC, 9.7 and 10.1 mm for AC, 0.2 and 0.2 mm for FL, and 16.5 and 17.1 g for EFW in malaria and control

groups, respectively. In both groups, intraobserver variability was lower than 11% for Doppler velocimetry indices.

The differences were not significant comparing mean UAs RI between malaria and control groups (Table 4). No patient showed zero diastole flow in both groups.

DISCUSSION

A recent study on histopathological analysis of *P. vivax*-associated placental lesions²³ concluded that this parasite may induce changes similar to those changes caused by *P. falciparum* but to a lesser degree of intensity. In that same study, syncytiotrophoblast rupture and syncytial knots as well as fibrinoid deposit and leukocyte infiltrate were observed, and parasitized erythrocytes were found only on *P. falciparum* infections. A tridimensional ultrasound scan study showed that *P. falciparum* before 24 weeks of pregnancy seems to be associated with a lower placental volume.²⁴ However, *P. vivax* infection does not seem to be associated with placental volume alterations.²⁵ Although there is little evidence that *P. vivax* may contribute to the inflammatory process in the intervillous space,²³ it is possible that the increase of placental thickness occurred because of placental inflammation. This possibility would explain, at least in part, changes in the course of pregnancy during acute malaria attacks, such as the occurrence of vaginal bleeding, uterine contraction, loss of amniotic liquid,

TABLE 2

Fetal biometry in *P. vivax*-infected pregnant women and a control group in a reference center for infectious diseases in Western Brazilian Amazon

Variable studied	GA* = 16.3 weeks			GA = 26 weeks		
	PVIP (N = 30)	CG (N = 30)	P value	PVIP (N = 30)	CG (N = 30)	P value
BPD (mm)	34.6 ± 3.5	35.2 ± 4.3	0.74	61.2 ± 2.7	63.3 ± 3.1	0.005
HC (mm)	131.4 ± 10.3	128.7 ± 9.5	0.71	231.3 ± 8.9	237.2 ± 8.6	0.01
AC (mm)	107.5 ± 13.8	109.6 ± 9.7	0.76	204.0 ± 10.0	211.6 ± 7.2	0.001
FL (mm)	21.2 ± 3.9	21.8 ± 4.9	0.77	45.2 ± 2.3	46.5 ± 2.0	0.02
EFW (g)	175.7 ± 78.9	183.7 ± 84.8	0.84	754.7 ± 99.6	830.1 ± 69.4	0.001

Data are shown as mean ± SD. CG = control group; GA = gestational age; PVIP = *P. vivax*-infected pregnant women.

*Mean GA.

TABLE 3

Fetal biometry according to parity in *P. vivax*-infected pregnant women and a control group in a reference center for infectious diseases in Western Brazilian Amazon

Variable studied	GA = 26 weeks					
	PVIP			CG		
	PMG (N = 11)	MTG (N = 19)	P value	PMG (N = 10)	MTG (N = 20)	P value
BPD (mm)	60.3 ± 2.5	63.7 ± 4.0	0.026	61.6 ± 2.7	63.1 ± 2.6	0.10
HC (mm)	228.4 ± 7.6	237.3 ± 11.3	0.04	233.0 ± 9.4	237.2 ± 7.0	0.13
AC (mm)	201.6 ± 7.2	210.1 ± 9.3	0.027	205.4 ± 11.4	209.5 ± 5.8	0.12
FL (mm)	44.6 ± 2.1	46.6 ± 2.2	0.038	45.5 ± 2.4	46.4 ± 2.0	0.23
EFW (g)	723.6 ± 77.8	823.1 ± 85.5	0.009	772.7 ± 98.0	814.2 ± 60.5	0.11

Data are shown as mean ± SD. CG = control group; GA = gestational age; MTG = multigravidae; PMG = primigravidae; PVIP = *P. vivax*-infected pregnant women.

and abortion, which may occur in about 25% of pregnant women with malaria in the Amazon region.¹⁷ However, the increase in placental thickness could reflect a compensatory mechanism because of the presence of maternal anemia,²⁶ which was present in 40% of patients with vivax malaria. However, our findings do not show this relationship, because there was no statistically significant association between the occurrence of anemia and the increased placental thickness in both evaluations. This finding supports the idea that the placental thickness observed in our study may be associated with placental inflammation. Additionally, maternal diabetes and other diseases are associated with compensatory increased placental thickness. In our study, no patient had gestational diabetes, and patients with syphilis, toxoplasmosis, and HIV were excluded. Additional studies are needed to better understand the occurrence of increased placental thickness in patients with vivax malaria.

Some studies have shown UAs Doppler velocimetry flow alterations during an acute episode of *P. falciparum* infection in pregnant women. A study undertaken in French Guiana²⁷ encompassing 23 *P. falciparum*-infected pregnant women concluded that malaria infection can induce a transitory hemodynamics distress on placental circulation and a higher placental resistance because of vascular bed degradation caused by villous degeneration. UAs Doppler velocimetry flow alterations were found in 57.1% of 55 hospitalized pregnant women during a malaria crisis in the French Guiana.²⁸

We could not find medical literature on any study on UAs Doppler velocimetry flow analysis during pregnancy in *P. vivax*-infected patients. In our study, we found that, even during an acute malaria episode, there were no significant changes in UAs Doppler velocimetry parameters. Our results suggest, therefore, that *P. vivax* infection during pregnancy may cause little or no degradation on vascular beds because of villous degeneration. These findings are in accordance with experimental models that showed that an obliteration of 30%

of placental territory leads to an increase in systole/diastole ratio on UA, and whenever 60–70% of vascularization is compromised, zero flow diastole or reverse flux occurs.²⁹ It is even possible that our results reflect an adaptive villous branching and capillarization, which can be observed in anemic pregnant women.²⁶ It is also possible that our findings are caused by a higher cardiac output, because *P. vivax*-infected fetuses had a significant higher heart rate than control group fetuses. Cardiovascular response to hypoxia is probably the most important adaptive reaction responsible for homeostasis maintenance, and it includes an increase in fetal heart rate, increase in blood pressure, and redistribution of cardiac output to vital organs.³⁰ Finally, the explanation may be a combination of some or all of those factors.

It has been shown that fetal growth rate is higher in the second trimester.²² *P. falciparum*-infected fetuses had lower BPD and HC measures compared with normal fetuses from 18 to 29 weeks of pregnancy. These differences, however, could not be seen at birth.¹⁵ In our study, we had similar findings; there was a significant difference in all fetal biometry parameters on the 26th week of pregnancy. These results favor the hypothesis that the mechanisms through which *P. vivax* exerts adverse effects on pregnancy are not fully understood. Systemic or hormonal mechanisms may have influence on intrauterine growth restriction related to *P. vivax* infection, because there is little evidence that *P. vivax* may sequester in the placenta, like *P. falciparum*.³¹

The main effects of malaria in pregnancy include maternal anemia, LBW, pre-term delivery, and increased infant and maternal mortality.³² Malaria-related anemia has a multifactorial etiology. Among different pathophysiological mechanisms, it is possible to include hemolysis, impaired erythropoiesis, inhibition of reticulocytes release, pre-mature destruction of red blood cells during the maturation phase in the bone marrow, and hypersplenism.³³ *P. falciparum*-infected erythrocytes sequester in the placenta by expressing surface antigens, mainly variant surface antigen, that bind to specific receptors, mainly chondroitin sulphate A.³² In sub-Saharan Africa, where most *P. falciparum* infections occur, the LBW effect seems to relate to nutrient transport to the fetus. A high density of parasites, chronic parasite infection in the placental blood, and the associated cellular immune response may result in consumption of glucose and oxygen that would have gone to the fetus. Histopathological studies of infected placentas have found thickening of the cytotrophoblastic membranes, which may interfere with nutrient transport.³⁴

Anemia is a common and frequently severe consequence of vivax infection.^{6,35–37} In studies from Thailand and India, women with *P. vivax* infection were more commonly anemic

TABLE 4

UAPI and UARI in *P. vivax*-infected pregnant women and a control group in a reference center for infectious diseases in Western Brazilian Amazon

Index	GA	<i>P. vivax</i> infected (N = 30)		Control group (N = 30)		P
		Mean	SD	Mean	SD	
UARI D1	16.3*	0.70	0.06	0.71	0.04	0.45
UARI D2	26.0	0.62	0.05	0.61	0.07	0.84
UAPI D1	16.3*	1.18	0.19	1.20	0.15	0.67
UAPI D2	26.0	0.96	0.14	0.91	0.19	0.21

D = Doppler scan; GA = gestational age.

*Mean GA.

compared with uninfected women.^{25,38} Some studies have reported that *P. vivax* malaria is associated with mild maternal anemia and significantly decreased birth weight,^{25,39,40} but the causative mechanisms have been unclear.⁵

There is some indirect evidence for placental binding of *P. vivax*.^{4,5,7} These data suggest pathological mechanisms independent of placental malaria, suggesting other causes of poor delivery outcomes associated with *P. vivax* infection.⁷ LBW is influenced by the gestational age at which the infection takes place and also, the number of episodes of relapsing malaria in the pregnant woman, which suggests that malaria injury may be cumulative.⁴¹ Malaria infections occurring at the beginning of pregnancy are more apt to determine LBW in as much as maximum growth occurs near 20–28 weeks of pregnancy.¹⁵ Maternal anemia is commonly considered a risk factor for poor pregnancy outcomes,⁴² mainly LBW.⁴³ Malaria-associated maternal anemia may also contribute independently to LBW,^{34,44,45} most likely through a reduction in oxygen transport to the fetus.

The impact of vivax infection on pregnancy is less clear in Brazil and Latin America as a whole. Data from Brazil confirm that malaria anemia in pregnant women with vivax is the most common complication, with few reports of LBW.⁴⁶ Our findings show a higher frequency of anemia and a mean birth weight significantly lower in the malaria group, but there were also only a few cases of LBW. However, the association between anemia and LBW was not found. Thus, it is unlikely that anemia contributed independently to the occurrence of LBW. However, the non-occurrence of the association between anemia and LBW may be because of the small sample size and the absence of other comorbidities that lead to anemia, such as HIV infection. Still, it is possible to be because of the fact that patients are coming from an area of low malaria transmission, which may have contributed to differences between our findings and those findings described in studies of Southeast Asia (areas of moderate to high disease transmission). Finally, it is possible that our findings result from the greater prevalence of *P. vivax* in the Amazon region, because it is known that infections with *P. falciparum* cause more chronic anemia as a result of successive infections by this species.

Our results suggest that *P. vivax* infection between 14 and 20 weeks of gestational age does not seem to interfere with UAs Doppler velocimetry indices, but it does affect fetal biometry both in the second semester of pregnancy and at birth. Future studies involving a larger sample should be conducted to confirm this association.

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Authors' addresses: Amantino C. Machado Filho, Elenice P. da Costa, Emely P. da Costa, Iracema S. Reis, Emanoela A. C. Fernandes, Bernardo V. Paim, Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Amazonas, Brazil, E-mails: amantino40@msn.com, pontesnice@hotmail.com, emilypontes@hotmail.com, iracema.reis@pmm.am.gov.br, emanoela_carvalho@yahoo.com.br, and bernardovpaim@yahoo.com.br. Flor E. Martínez-Espinosa, Postgraduate Program in Tropical Medicine, Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Amazonas, Brazil, E-mail: florespinosa@gmail.com.

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