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Cohort profile: Effect of malaria in early pregnancy on fetal growth in Benin (RECIPAL pre-conceptional cohort).

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Cohort profile: Effect of malaria in early pregnancy on fetal growth in Benin (RECIPAL pre-conceptional cohort).

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Abstract

Purpose: RECIPAL is an original pre-conceptional cohort designed to assess the consequences of malaria during the first trimester of pregnancy, which is a poorly investigated period in Africa and during which malaria may be detrimental to the fetus.

Participants: For this purpose, a total of 1214 women of reproductive age living in Sô-Ava and Akassato districts (south Benin) were followed-up monthly from June 2014 to December 2016 until 411 of them became pregnant. A large range of health determinants was collected both before and during pregnancy from the first weeks of gestation to delivery. Five Doppler-ultrasound scans were performed for early dating of the pregnancy and longitudinal fetal growth assessment.

Findings to date: Pregnant women were identified at a mean of 6.9 weeks of gestation (wg). Preliminary results confirmed the high prevalence of malaria in the first trimester of pregnancy, with more than 25.4% of women presenting at least one microscopic malarial infection during this period. Most infections occurred before 6 wg. The prevalence of low birthweight, small-birthweight-for-gestational age (according to INTERGROWTH21-st charts) and preterm birth was 9.3%, 18.3%, and 12.6%, respectively.

Future plans: RECIPAL represents at this time a unique resource that will provide information on multiple infectious (including malaria), biological, nutritional and environmental determinants in relation to health outcomes in women of reproductive age, pregnant women and their newborns. It will contribute to better define future recommendations for the prevention of malaria in early pregnancy and maternal malnutrition in Africa. It confirms that it is possible to constitute a pre-conceptional pregnancy cohort in Africa and provides valuable information for researchers starting cohorts in the future.

Strengths and limitations of this study

- A unique cohort combining highly detailed, high-quality health-related information on the pregnancies of ≈ 400 women recruited in the pre-conception period, with a substantial related bio-bank of plasmas, placental and other samples.

- Pre-conceptional design allowing the early identification and follow-up of pregnant women: screening of women for both microscopic and submicroscopic malaria from the first weeks of pregnancy; accurate estimation of gestational age by using early obstetrical ultrasound scan and collection of some important factors influencing fetal growth such as pre-pregnancy nutritional status and gestational hypertensive disorders.

- Collection of valuable information for the implementation of future pre-conceptional cohorts in Africa.

- High attrition in the pre-conceptional cohort, with a possible impact on the external validity of some findings.

- Reduced sample size at delivery due to a high proportion of spontaneous abortion, with a possible lack of power for analyses on birth outcomes.

Introduction

Malaria in pregnancy is associated with a wide range of deleterious effects in women and their offspring. In Sub-Sahara African (SSA) countries, preventive strategies are based on long-lasting insecticide treated bed nets (LLITNs) and intermittent preventive treatment in pregnancy (IPTp). IPTp consists in the administration of sulfadoxine-pyrimethamine (SP) at each antenatal care (ANC) visit from the 2nd trimester of pregnancy onwards [1]. LLITN are distributed at the first ANC visit, which generally occurs around 4-5 months of pregnancy [2]. Therefore, pregnant women remain unprotected or insufficiently protected during the first months of pregnancy, and particularly during the first trimester. However, this period may be a high-risk period for the fetus if pregnancy-associated malaria parasites accumulate into the placenta during trophoblast differentiation and vascular remodelling of the uterus [3,4]. Impaired placentation may then contribute to fetal growth restriction.

Few studies have investigated malaria in early pregnancy and while some have shown an adverse effect on birth outcomes, this has not been consistent [5]. In Burkina Faso and in Benin, malaria infection in the first trimester [6] or before 4-5 months of pregnancy [7,8] has been associated with a higher risk of low birthweight (LBW) or a decrease in birthweight. Conversely, such association was not reported in Uganda and Malawi [9,10] but the number of women screened in the first trimester was low in these two studies. One of the problems in interpreting this existing literature relates to the challenges of recruiting women early in pregnancy; most of the studies were not designed to assess the consequences of malaria early in pregnancy and therefore women were generally recruited late in the first trimester, which may lead to misclassification errors and underestimation of exposure.

The RECIPAL study aimed to assess the effect of malaria (both microscopic and submicroscopic) in the first trimester of pregnancy on both the mother and the fetus by

following a cohort of pregnant women recruited before conception. In addition, it aimed to assess the influence of the woman's nutritional status in the relationship between malaria in early pregnancy and birth outcomes.

Cohort description

Study setting

The study cohort was conducted in the districts of Sô-Ava and Abomey-Calavi, located in Southern Benin (Figure 1). In the area, malaria is hyperendemic with a mean entomological inoculation rate of 2.1 infected anopheles bites/person/100 nights [11]. The main mosquito vectors of malaria are *Anopheles gambiae* ss and *Anopheles funestus* [12]. Four sub-districts (Sô-Ava, Vekky and Houedo in Sô-Ava District, and Akassato in Abomey-Calavi District) were selected for the study according to the population density and mean number of ANC visits and deliveries per month in the main public maternity clinic. Thirty-five out of a total of 36 villages were selected for the study according to their proximity to the maternity clinics.

Study design, subject identification, recruitment and enrolment procedures

Briefly, women of reproductive age (WRA) were recruited at community-level and followed monthly for a maximum period of 24 months until becoming pregnant, they constituted the RECIPAL initial cohort. The sub-sample of women who became pregnant was then followed-up monthly at the maternity clinic from early pregnancy to delivery; they constituted the RECIPAL final cohort.

A sample size of 466 births was estimated sufficient to evidence a 2-fold odds ratio for the association between malaria and poor birth outcomes [4], assuming a prevalence of 25% of women with microscopic malaria in the 1st trimester of pregnancy and 30% of poor birth outcomes (LBW, small-for-gestational age (SGA), preterm birth (PTB) or stillbirth) [8,13,14]

with a 80% power and significant level at 0.05. We estimated that following 2,000 WRA (initial cohort) would have allowed identifying 510 pregnant women (final cohort) during the time of the study (based on a total fertility rate of 200/1,000/year in Benin (http://esa.un.org/unpd/wpp/index.htm) and allowing 15% of loss to follow-up).

After explanation of the study to the local political and administrative authorities, three concomitant procedures were used to recruit WRA. First, repeated sensitization events were organized in each of the 35 selected villages for presenting the study to all inhabitants. If interested, women were invited to register with the head of the community and were visited at home the day after for their inclusion. That procedure constituted the main way of recruitment throughout the study. Second, leaders of the community, religious authorities and mass-media were involved in order to help us mobilizing women during public meetings (masses, women's association meetings, etc.). The last way of recruitment consisted of going door-to-door to identify eligible and interested women with the assistance a network of community-health workers.

To be included, women had met the following criteria: negative urinary pregnancy test, 18 to 45 years old, no current contraception, no previous fecundity issues, willingness to become pregnant, no planned travel for more than 2 months within the next 18 months, acceptance of RECIPAL protocol and signed written informed consent. Both oral and written communication was used for providing information on the project to the women and their husbands. The consent form was translated into the local language for women who were illiterate.

From June 2014 to December 2016, 1302 WRA were willing to participate in the study. Among them, 1214 (93.2%) met the inclusion criteria and were recruited, 88 (6.8%) were not included mainly because of a positive urinary pregnancy test at inclusion (63.6%) or past

infertility issues (14.8%). Based on both the 2013 national census and 2011 Demographic Health Survey in Benin, we estimated that women included in the project represented 6% of the total number of WRA living in the study area.

RECIPAL study was approved by the ethics committees of the Institut des Sciences Biomédicales Appliquées (ISBA) in Benin and from the IRD in France as well as by the Ministry of Health in Benin.

Cohort follow-up

Pre-conceptional follow-up

After enrolment, WRA were visited at home monthly by specifically trained-investigators assisted by local community-health workers. Follow-up consisted mainly of recording the first day of last menstrual period (LMP) and performing a urinary pregnancy test every month. To minimize the risk of loss of confidentiality during the tracking of women for follow-up, the study team was instructed to speak only to people—including family members—to whom women have granted them permission to speak. After 12 months, women who were still followed received medical advices to help conceive and they were invited to attend the maternity clinic for a medical examination in case of symptoms of genital infection. After 24 months, the follow-up ended and women who did not conceive were invited to attend a gynaecologist for fertility issues assessment.

Gestational follow-up

Once pregnant, women were followed-up at the maternity clinic for monthly ANC visits. In addition, they were encouraged to attend the maternity clinic any time in case of any symptoms. Both the maternity staff (for usual ANC follow-up and clinical examination) and study investigators (for all specific issues related to RECIPAL) were involved in women's

follow-up. Throughout the study, women were offered free transport to the maternity clinic; in Sô-Ava District a free river shuttle was set up. At 37 weeks gestation, women were visited at home weekly and transportation to the study maternity clinic was scheduled for delivery.

According to Beninese national recommendations, a kit including iron and folic acid tablets for one month, mebendazole for deworming, the 1st dose of SP for IPTp and a LLITN was given to each pregnant woman at the first ANC visit. The maternity staff was encouraged to administer at least three doses of IPTp from the 2nd trimester onwards as recommended by the Beninese National Malaria Control Program, as well as iron and folic acid supplementation throughout the pregnancy. RECIPAL women benefited from free management of any diseases related to the pregnancy—detected either as part of RECIPAL follow-up or during unscheduled visits, free ANC visits and delivery. Women infected with malaria were treated with quinine in the first trimester of pregnancy and arthemeter-lumefantrine from the 2nd trimester onwards. Management of preterm or growth-restricted newborns were also in charge of the project.

Cohort attrition

Figure 2 presents the flowchart diagram of the study. Among the 1214 WRA enrolled in the initial cohort, 411 (33.9%) became pregnant, 359 (29.6%) completed the 24 month-follow-up without conceiving and 444 women (36.6%) did not complete the study mainly because of informed consent withdrawal (272/444) or migration outside the study area (138/444) that occurred after 5.2 and 6.3 months of follow-up in average, respectively.

Among the 411 pregnant women, 207 (50.4%) have already delivered, 16.5% (68/411) had a spontaneous abortion (n=68) or a non-viable pregnancy (n=2), 52 did not complete the study because of informed consent withdrawal (37/411, 9%) or migration (15/411, 3.6%), and 72 women are still followed (Figure 2). Spontaneous abortions, informed consent withdrawals

and migrations occurred at 8.9 ($\pm$ 3.9), 13.2 ($\pm$ 8.1) and 14.2 ($\pm$ 8.6) weeks of gestation (wg) in average (SD), respectively.

Data collection

Pre-conceptional data

Demographic, socioeconomic and household characteristics were collected at inclusion (Table 1). At this occasion, screening for malaria, urinary schistosomiasis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was performed. Haemoglobin (Hb) and markers of inflammation levels were determined. The first day of LMP was recorded, and a urinary pregnancy test (One-Step®, International Holding Corp, Germany) was performed each month. Anthropometric measurements were collected every three months and dietary intakes were assessed twice during follow-up (see below for details). After 12 months, for those women who were still followed, they were screened for urinary schistosomiasis again in case of macroscopic haematuria.

Gestational data

At each ANC visit, clinical, anthropometric and obstetrical data, as well as LLITN use, IPTp administration, alcohol consumption, and iron, folic acid and other micronutrient supplementation intake were collected (Table 2). In addition, malaria screening was performed, and proteinuria, glycosuria and urinary infection were detected using a urine dipstick test (Combi-screen®, Analyticon, Germany). Dietary intakes were assessed once every trimester. Five Doppler-ultrasound scans (US) were performed (see below for details). A sample of venous blood was collected in the 1st and 3rd trimesters for Hb, markers of inflammation and lead levels determination.

At delivery, newborns were weighed within 1 hour after birth on an electronic digital scale with an accuracy of 2 grams (SECA 757, SECA Ltd., Germany). Newborn's length (SECA infantometer 416, Germany) and head circumference (SECA 201, Germany) were recorded to the nearest millimetre. Malaria screening was performed on maternal venous blood, placental and cord blood (Table 2). A placental biopsy was performed for malaria histopathology.

The types and origins of the biological samples collected before and during pregnancy are listed in Table 3. Preparation and storage are performed by two laboratory technicians under the supervision of a senior biologist. Total blood, serum, plasma urine samples are stored in -20°C or -80°C freezers with a daily control of the temperature.

Specifics components

Malaria diagnosis. Before conception and during pregnancy, malaria screening was performed using both microscopy (thick blood smear) and polymerase chain reaction (PCR). Blood smears were stained with Giemsa and parasitaemia was quantified by the Lambaréné method [15]. A molecular diagnostic approach using a combination of real-time PCR assays comprising genus-specific primers and probes for the gene encoding the small (18S) of *Plasmodium*r RNA and an ultra-sensitive *P. falciparum*-specific detection system [16] were used to screen samples containing *Plasmodium* parasites. In addition, any time during pregnancy, a rapid diagnostic test (Pf + pan rapid test SD Bioline Ag®, IDA Foundation, Netherlands; BioSynex®, France) was performed in case of symptoms suggestive of malaria for immediate diagnosis and treatment.

Ultrasound follow-up. The first US for dating the pregnancy was performed between 9 and 13 wg based on LMP recorded during the pre-conceptional follow-up (supplementary file S1). The gestational age (GA) was estimated in days based on crown-rump length measurement using the Robinson's chart [17]. At the end, GA estimation was based either on LMP if the

difference between the two measurements (LMP/US) was less than 7 days or on US if the difference is > 7 days. Following INTERGROWTH-21st methodology, four additional scans were performed for fetal biometry assessment, and uterine and umbilical blood flow measurements [18]. Each parameter was measured twice and then averaged; a third measure was performed in case of discrepancy between the two first measurements. A quality control was performed on 10% of the pictures.

Anthropometric measurements and dietary intake assessment. Anthropometric measurements were collected using standard procedures (Tables 1 and 2) [19]. During household visits, women's body weight was measured with a 200 g precision with calibrated electronic scales (Tefal, France). At the facility-level, women were weighed with a Tanita MC-780 body composition device (Tanita Corporation, Tokyo, Japan). Height was measured to the nearest millimeter with a SECA 206 (Hamburg, Germany) gauge. The left mid-upper-arm-circumference (MUAC) was measured with a SECA 201 (Hamburg, Germany) ergonomic circumference measuring tape. Skinfold thickness measurements were made to the nearest millimeter at the triceps and biceps sites using a Holtain (Crymyeh, UK) skinfold caliper. The set of measurements was repeated twice and then the measurements were averaged. Body composition, assessed by bioelectrical impedance analysis method, was measured by the multi-frequency body composition analyser Tanita MC-780 with a four-electrode arrangement paired on hands and feet.

Dietary intakes were assessed by 24h-recalls using the standard multiple-pass methodology. A comprehensive list of the most common food items and recipes was developed from a preliminary study of the diet habits of the study area. This list was used to identify foods mentioned by the women as consumed during the interview. Amounts of food consumed were estimated by household measures consisted of pre-calibrated utensils, food pictures of different portion sizes, monetary value of known food portions sold on the study area markets.

A food composition table and recipes database are under construction for conversion of household units to gram, and to determine macro- and micro-nutrients intake by the women.

Characteristics of the study populations

At enrolment, WRA were 27 years old in average (Table 4). Nulligravidae represented 8.8% of the cohort. More than half of the women were anaemic, one quarter was infected with schistosomiasis and 38% had an abnormal BMI. The prevalence of microscopic malaria was 5.7%, with two geographical clusters in Sô-Ava and Vekky sub-districts (Figure 3). The median duration of follow-up in the initial cohort was 5.9 months (mean=7.1, range: 0.49–23.7). Compared to women included in the initial cohort, women who did not complete the 24-month follow-up were significantly different in terms of residence area, ethnicity, household density and schistosomiasis status (Table 4).

Pregnant women were identified at a mean of 6.9 wg and benefited from 7.5 scheduled ANC visits in average (Table 4). The prevalence of microscopic malaria infection was 25.4%, 19.4% and 16.1% during the 1st, 2nd and 3rd trimester of pregnancy, respectively. The prevalence of SGA was 11.5% and 18.3% using Schmiegelow's and INTERGROWTH 21st charts, respectively (Table 5). The proportion of PTB and LBW was 12.6% and 9.3%, respectively. Overall, 29.5% (61/207) of newborns presented at least one poor birth outcome defined as LBW, PTB, SGA or stillbirth.

Findings to date

Fertility and time-to-pregnancy

The median time-to-pregnancy was 12.3 months (Figure 4). The fertility rate was 5.4 pregnancies/100 persons-month (95% confidence interval [CI] = 4.9-5.9). The probability (95% CI) of conceiving after 5, 10, 15 and 20 months of follow-up were 24.5% (21.9-27.4),

42.1% (38.6-45.9), 56.6% (52.1-61.1) and 62.8% (57.6-68), respectively. Using multivariate survival analysis, factors associated with a lower risk of conceiving were maternal age more than 35 years, a low education level, being polygamous, living in Vekky sub-district, long-term couple (> 8 years), infrequent sexual activity, overweight and urinary schistosomiasis infection (Yovo, personal communication).

Malaria in the first trimester of pregnancy

The incidence rate of malaria infection during the first trimester was 27.5 cases per 100 persons-month. Using a multi-level logistic regression, we showed that women infected with malaria before pregnancy were significantly more likely to be infected with malaria during the 1st trimester of pregnancy (adjusted relative risk (aRR)= 2.72, 95% CI: 1.26-5.85). Besides, malaria was significantly more frequent in the first weeks of pregnancy ($\leq$ 6 wg) than later in the first trimester (aRR= 1.77, 95% CI: 1.07-2.94) (Accrombessi, personal communication).

Strengths and limitations

The pre-conceptional design—and early identification of pregnant women—allowed (i) the screening of women for malaria from the very beginning of pregnancy; (ii) the accurate estimation of GA by using US before 14 wg; (iii) the determination of pre-pregnancy nutritional status[20] and hypertensive disorders screening [21], which highly influence fetal growth; and (iv) the minimization of selection bias embedded in the design of women recruited at facility-level. Besides, PTB and IUGR could be accurately assessed thanks to the precise estimation of GA and longitudinal fetal growth assessment.

In addition to malaria, RECIPAL will provide valuable information on WRA in Africa in terms of fertility and nutrition, which are seldom evaluated and may help to define new pre-

conceptional interventions for improving maternal and child health [22]. Also, it yields logistical and ethical information for the implementation of future pre-conceptional cohorts.

Because of constraints related to the study design (long duration of the pre-conceptional follow-up and weariness of the women) and African socio-cultural realities (rumour about blood collection, suspicion regarding medical care provided free of charge, etc.), a noticeable proportion (22%) of women withdrew their informed consent before the end of the 24 month-follow-up. Overall, the initial cohort attrition was 37%. Since some baseline characteristics in women who completed the pre-conceptional follow-up and those who did not were different, this might impact the external validity of some of results. During pregnancy, there was a high proportion (16.5%) of spontaneous abortions, which contributed to the final cohort attrition. At the end, birth outcomes could be evaluated in only 68% of women, with a possible lack of power for some upcoming analyses.

Conclusion

If the deleterious effects of malaria in the first trimester of pregnancy are confirmed, our results may argue in favor of starting preventive measures from the very beginning of pregnancy or even before pregnancy. Dihydroartemisinin-piperaquine has been shown to be safe during the first trimester of pregnancy and, therefore, may be a good option for the replacement of SP [23]. Besides, a vaccine against pregnancy-associated malaria parasites, which could elicit protective immunity prior to pregnancy to best protect pregnant women during early pregnancy may be proposed as a complementary strategy. Such a vaccine is currently under evaluation [24].

Collaboration

The "Mother and child face to tropical infections" research unit (MERIT), French National Research Institute for Sustainable Development (IRD)/Paris Descartes University was the promoter of the study. RECIPAL was a collaborative project between MERIT, another IRD research unit (Nutripass, Montpellier), EPOPé research team (Inserm), the Ecole des Hautes Etudes en Santé Publique (EHESP) and two Beninese collaborators from Abomey-Calavi University (the Faculty of Health Sciences and the Faculty of Agronomy Sciences).

The researchers associated with this study are open for the sharing and reuse of the data but an end user data use agreement will be required for accessing the data. Collaborations are encouraged, although data sets are not currently publicly available. Any researcher interested in exploring RECIPAL data should contact directly the principal investigator, Valerie Briand (e-mail: valerie.briand@ird.fr).

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Table 1. Clinical, nutritional and biological data collected during the pre-conceptional follow-up. The RECIPAL study, 2014-2017.

	Inclusion	D1	M1	M2,M3	M4	M5,M6	M7	M8,M9	M10	M11,M12	M13	M14,M15	M16	M17,M18	M19	M20,M21	M22
General characteristics																	
Place of visit	‡	¤	¤	‡	‡	‡	‡	‡	¤	‡	‡	‡	‡	‡	‡	‡	‡
Eligibility criteria	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Informed consent	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GPS location	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Socio-demographic characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Housing characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Economic characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clinical and nutritional data																	
Obstetrical history	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Height	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weight, MUAC, skinfold thickness	-	†	†	-	†	-	†	-	†	-	†	-	†	-	†	-	†
Body composition (BIA)	-	†	†	-	-	-	-	-	†	-	-	-	-	-	-	-	-
Dietary 24-h recall	-	†	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-
Amenorrhea/LMP	-	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†
Biological data																	
Urinary pregnancy test	†	-	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†
Malaria screening (TBS, PCR)	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hb, ferritin, folic acid levels	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CRP, AGP, sTfR levels	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlamydia trachomatis	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neisseria gonorrhoeae																	
Urinary schistosomiasis*	-	†	†	-	-	-	-	-	-	†	-	-	-	-	-	-	-
Urine storage**	-	†	†	-	-	-	†	-	†	-	-	-	-	-	-	-	-

(¤) Visit performed at the study maternity clinic; (‡) Household visit; Information collected/assessed (†) or not (-) at each specific time-point.

* C. trachomatis and N. gonorrhoeae screening (using PCR and serology), as well as schistosomiasis screening (Nytrel® filter) started 9 and 12 months after study initiation, respectively.

** Urine storage for further analysis

Abbreviations: D: day; M: Months; MUAC: Mid-Upper-Arm-Circumference; BIA: Bio impedance analysis; LMP: Last menstrual period; TBS: Thick blood smear; PCR: polymerase chain reaction; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein; Hb: Haemoglobin.

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Table 2. Clinical, nutritional and biological data collected during pregnancy. The RECIPAL study, 2014-2017.

	ANCv 1	ANCv 2	ANCv 3	ANCv 4	ANCv 5	ANCv 6	ANCv 7	ANCv8	Delivery
Clinical and nutritional data									
Gestational age (weeks), mean (SD)	6.8 ± 2.5	11.6 ± 2.9	16.7 ±2.9	22.1 ± 3.7	27.6 ± 3.8	32.7 ± 3.5	36.2 ± 2.5	38.4 ± 1.4	39.1 ± 3.0
Past medical and obstetrical history*‡	†	-	-	-	-	-	-	-	-
Alcohol consumption in the last 24 hours	†	†	†	†	†	†	†	†	-
Iron and folate intake in the last 24 hours	-	†	†	†	†	†	†	†	-
Use of ITN the night before the visit	†	†	†	†	†	†	†	†	-
Axillary temperature	†	†	†	†	†	†	†	†	†
Blood pressure	†	†	†	†	†	†	†	†	†
Gestational age (based on LMP or first US)	†	†	†	†	†	†	†	†	†
Anthropometric measurements									
- Weight	†	†	†	†	†	†	†	†	†
- Mid-Upper-Arm-Circumference	†	-	-	†	-	†	-	-	-
- Skinfold thickness (bicipitalandtricipital)	†	-	-	†	-	†	-	-	-
Body composition (Bio impendance analysis)	†	†	†	†	†	†	†	†	-
Dietary 24-h recall	-	†	-	-	†	-	†	-	-
Biological data									
Blood and rhesus group	†	-	-	-	-	-	-	-	-
HIV 1 screening	†	-	-	-	-	-	-	-	-
Peripheral malaria (TBS, PCR)	†	†	†	†	†	†	†	†	†
Placental malaria (TBS, PCR, histology)	-	-	-	-	-	-	-	-	†
Cord blood malaria (TBS, PCR)	-	-	-	-	-	-	-	-	†
Hb level	-	†	-	-	-	†	-	-	-
Serum ferritin, sTfR, CRP, AGP, folic acid levels	-	†	-	-	-	†	-	-	-
Lead level	-	†	-	-	-	-	-	-	-
Urinary infection and proteinuria (dipstick test)	†	†	†	†	†	†	†	†	-
Helminthic intestinal infection (PCR)	†	-	-	-	-	-	-	-	-
Urinary schistosomiasis	-	-	-	-	-	-	†	-	-

* Sickle cell disease, diabetes or other chronic medical affection; history of preterm delivery or hypertensive disorders.

‡History of cigarette smoking during pregnancy collected at delivery.

Information collected/assessed (†) or not (-) at each specific time-point.

Abbreviations: ANCv: Antenatal care visit; ITN: Insecticide-treated bed net; IPTp: Intermittent preventive treatment in pregnancy; LMP: Last menstrual period; TBS: Thick blood smear; PCR: polymerase chain reaction; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein, Hb: Haemoglobin.

Table 3. Biological samples stored before conception, during pregnancy and at birth. The RECIPAL study, 2014-2018

	Pre-pregnancy	Pregnancy$	Delivery/Birth	Planned analyses
Total blood				
Total blood (200 µL)	-	Mother (x2)	-	Erythrocytic folic acid
Total blood (500 µL)	-	Mother (x2)	Mother-placenta-cord	Metals level
Blood spot +4°C (50 µL)	Mother (x1)	Mother (x8)[‡]	Mother-placenta-cord	*Plasmodium* detection (PCR)
Blood spot -20°C (100 µL)	Mother (x1)	Mother (x8)[‡]	Mother-placenta-cord	Immunological markers
Red cells (Trizol, 100 µL)	Mother (x1)	Mother (x8)	Mother-placenta-cord	*Plasmodium*phenotyping
Buffy coat PAXgene (300 µL)	-	Mother (x2)	Mother-placenta-cord	Immunological markers
Plasma/serum				
Plasma-20°C [†]	Mother (x1)	Mother (x2)	Mother-placenta-cord	Micro RNAs, cytokines and chemokines
Plasma -80°C[†]	Mother (x1)	Mother (x2)	Mother-placenta-cord	Micro RNAs, cytokines and chemokines
Serum (100µL)	Mother (x1)	-	-	*C. trachomatis* and *N. gonorrhoeae* detection
Serum (400 µL)	-	Mother (x2)		Vitamin A
Serum (100µL)[†]	Mother (x1)	Mother (x2)	-	Ferritin, CRP, sTfR, AGP
Urine(1.8 mL)	Mother (x2)	Mother (x3)	-	Pollutant/pesticide
Stool (-80°C)	Mother (x1)	-	-	Helminthic infection
Placenta				
Biopsy with formalin	-	-	Placenta	Histopathology for malaria
Biopsy -20°C	-	-	Placenta	Immunological markers
Biopsy with RNA Later	-	-	Placenta	Immunological markers

The frequency of sample collection is indicated in brackets.

$ During pregnancy, samples were collected twice (during the 1st and 3rd trimester of pregnancy), three times (during each trimester), or eight times (during each ANC visit).

‡ In case of unscheduled visit, an additional blood spot was collected.

† Quantity of plasma stored before, during pregnancy and at delivery was 200, 600-800, 600 µl, respectively.

Abbreviations: TBS: Thick blood smear; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein, PCR: Polymerase Chain Reaction.

Table 4. Characteristics of women included in the initial and final RECIPAL cohorts, Southern Benin, 2014-2018.

		Initial cohort (N=1214 WRA)	WRA with incomplete follow-up (N=444)	P value (1214 vs 444)	WRA with complete follow-up but no pregnancy (N=359)	Final cohort Pregnant women (N=411)	P value (411 vs 359)
General characteristics							
Sub-district (%)	Sô-Ava	23.0	23.0		12.5	32.1	
	Vekky	34.8	51.4	< 0.001	15.9	33.8	< 0.001
	Houedo	15.3	7.4		33.4	7.8	
	Akassato	26.9	18.2		38.2	26.3	
Ethnic group (%)	Toffin	71.2	77.9		60.7	72.9	
	Aizo	16.1	12.6	0.001	22.3	14.4	0.002
	Fon	7.1	5.2		8.6	7.8	
	Others	5.6	4.3		8.4	4.9	
Age (years)	Mean (± SD)	27.8 (± 5.5)	28.0 (± 5.6)	0.31	28.8 (± 5.6)	26.8 (± 5.0)	< 0.001
Marital status (%)	Polygamy	36.7	38.9		42.9	28.7	
	Monogamy	60.0	57.9	0.45	56.8	65.2	< 0.001
	Cohabitation	3.3	3.1		0.3	6.1	
Education (%)	Illiterate	73.2	75.7	0.14	73.3	70.6	0.41
Household density	Mean (± SD)	5.7 (± 3.0)	6.0 (± 3.4)	0.009	5.2 (±2.3)	5.9 (±3.0)	0.001
Gravidity (%)	Nulligravidae	8.8	10.8				
	Primigravidae	13.8	13.1	0.17	12.9	8.0	0.03
	Multigravidae	77.4	76.1		87.1	92.0	
Number of live birth(s)	Mean (± SD)	2.8 (± 2.0)	2.6 (± 2.0)	0.07	2.9 (± 2.1)	2.7 (± 1.9)	0.16
Short stature (%)	Maternal length < 150 cm	8.0	7.2	0.46	8.9	8.0	0.65
Mid-Upper-Arm-Circumference (%)	< 23 cm	6.7	6.1	0.62	7.5	6.3	0.51
Pre-conceptionalcharacteristics							
Body mass index (%)	< 18.5kg/m²	8.2	6.9		8.1	9.7	
	18.5–24kg/m²	61.8	61.1	0.36	59.2	64.5	0.10
	≥ 25kg/m²	30.0	31.0		32.7	25.8	
Schistosomiasis infection (%) [£]	Yes	24.7	31.2	0.03	19.6	27.0	0.04
Anaemia (%)[§]	Yes	52.5	56.3	0.07	46.5	54.2	0.03
Microscopic malaria (%)	Yes	5.7	7.5	0.06	3.4	5.9	0.10

Table 4. Characteristics of women included in the initial and final RECIPAL cohorts, Southern Benin, 2014-2018 (*continued*).

		Initial cohort (N=1214 WRA)	WRA with incomplete follow-up (N=444)	P value* (1214 vs 444)	WRA with complete follow-up but no pregnancy (N=359)	Final cohort Pregnant women (N=411)	P value* (411 vs 359)
Gestational characteristics							
Median time to pregnancy (months)	Mean (± SD)	-	-	-	-	12.3	-
Fertility incidence rate(95% CI)[μ]		-	-	-	-	5.4 (4.9–5.9)	-
Gestational age at 1st ANC visit (weeks)	Mean (± SD)	-	-	-	-	6.9(± 2.5)	-
Gravidity (%)	Primigravidae	-	-	-	-	8.0	-
	Multigravidae	-	-	-	-	92.0	-
Number of IPTp doses (%)[†]	≥ 2	-	-	-	-	65.7	-
Inadequate gestational weight gain (%) [†]	Yes	-	-	-	-	57.9	-
Number of ANC visits (scheduled) [†]	Mean (± SD)	-	-	-	-	7.5 (± 1.1)	-
Number of ANC visits (unscheduled) [†]	Mean (± SD)	-	-	-	-	2.1 (± 1.3)	-
HIV status (%) [†]	Yes	-	-	-	-	1.7	-
Gestational hypertension (%) [†]	Yes	-	-	-	-	12.1	-
Anaemia (%) [§†]	During pregnancy	-	-	-	-	68.6	-
	In the 1st trimester	-	-	-	-	48.5	-
	In the 3rd trimester	-	-	-	-	57.0	-
Microscopic malaria (%) [†]	During pregnancy	-	-	-	-	44.9	-
	In the 1st trimester	-	-	-	-	25.4	-
	In the 2nd trimester	-	-	-	-	19.4	-
	In the 3rd trimester	-	-	-	-	16.1	-
Placental malaria infection (%) [†]	Yes	-	-	-	-	7.8	-

* T-student and chi2 test were used for comparing continuous and categorical variables, respectively.
£ Results based on a sub-sample of women (n=718).
§ According to WHO thresholds (12 g/dL for non-pregnant women and 11 g/dL for pregnant).
μ Incidence rate defined as the number of pregnancies / persons-month at risk.
† Results based on the 207 women who have already delivered.
Abbreviations: WRA: women of reproductive age; SD: Standard deviation; ANC: Antenatal care.

Table 5. Preliminary data description of newborns at birth (sub-sample of the cohort). The RECIPAL study (2014-2018).

Newborn characteristics[†]		N	Mean (± SD) or Proportion (%)
Gender	Male	205	55.2
Stillbirth	Per 1000 live births	206	14.8
Preterm birth (< 37 weeks)	Yes	207	12.6
Small-birthweight-for-gestational age [‡§]	Yes	191	11.5
Small-birthweight-for-gestational age [¥ §]	Yes	191	18.3
Birthweight (g)[§]		193	3043.9 (± 432.8)
	< 2500	193	9.3
Poor birth outcome [£, §]	Yes	207	29.5

† Data based on the first 207 deliveries.
[§]Twins excluded
‡ Small-birthweight-for-gestational age: < 10th percentile of birthweight for gestational age using Schmiegelow's charts.
¥ Small-birthweight-for-gestational age: < 10th percentile of birthweight for gestational age using;
£ Stillbirth, preterm birth, small-birthweight-for-gestational age (using Intergrowth 21[st]charts) or low birthweight

Funding

Author's contribution

Acknowledgements

Conflict of interest. None declared

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Figure legends

Figure 1. Geographical location of Sô-Ava and Abomey-Calavi Districts, and of the 35 villages of the RECIPAL study, Southern Benin, 2014-2018.

Figure 2. Flowchart diagram of RECIPAL study.

* 24-month follow-up without conceiving

Figure 3. Malaria status (infected *vs.* non infected) in women of reproductive age at inclusion in RECIPAL (initial cohort), Southern Benin, 2014-2018.

Figure 4. Kaplan-Meier failure estimate of the probability of conceiving; 1214 women of reproductiveage included in the initial RECIPAL cohort, Southern Benin, 2014-2018.

Probability of conceiving (**solid line**) and its 95% confidence interval (**grey lines**).Number of pregnancy events are in brackets. Number of censored women at 5, 10, 15, 20 and 24 months were : 223, 260, 88, 50 and 30, respectively.

Supporting information

S1Fig. Ultrasound follow-up (RECIPAL study)

HC: Head circumference; AC: Abdominal circumference; FL: Femur length; BPD: Biparietal diameter; OFD: occipito frontal diameter; ATD: Abdominal transverse diameter; APAD: Antero-posterior abdominal diameter.

* Uterine, and umbilical blood flows were measured at 21-25 wg and from 28 wg, respectively

Figure 1. Geographical location of Sô-Ava and Abomey-Calavi Districts, and of the 35 villages of the RECIPAL study, Southern Benin, 2014-2018.
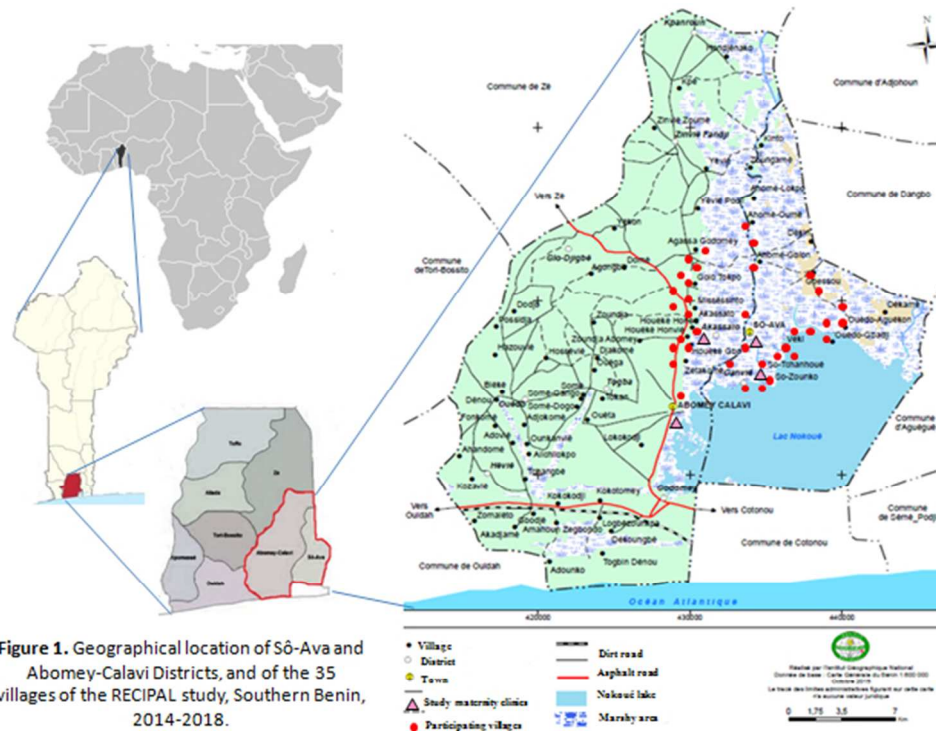
Figure 1. Geographical location of Sô-Ava and Abomey-Calavi Districts, and of the 35 villages of the RECIPAL study, Southern Benin, 2014-2018.
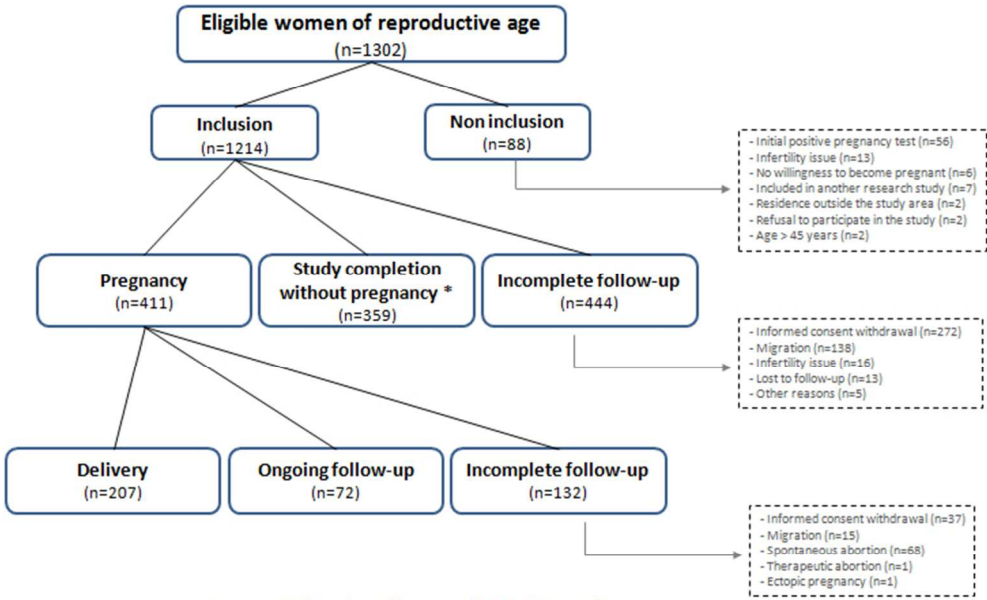
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Figure 2. Flowchart diagram of RECIPAL study

Figure 2. Flowchart diagram of RECIPAL study.
* 24-month follow-up without conceiving

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Figure 3. Malaria status (infected *vs.* non infected) in women of reproductive age at inclusion in RECIPAL (initial cohort), Southern Benin, 2014-2018
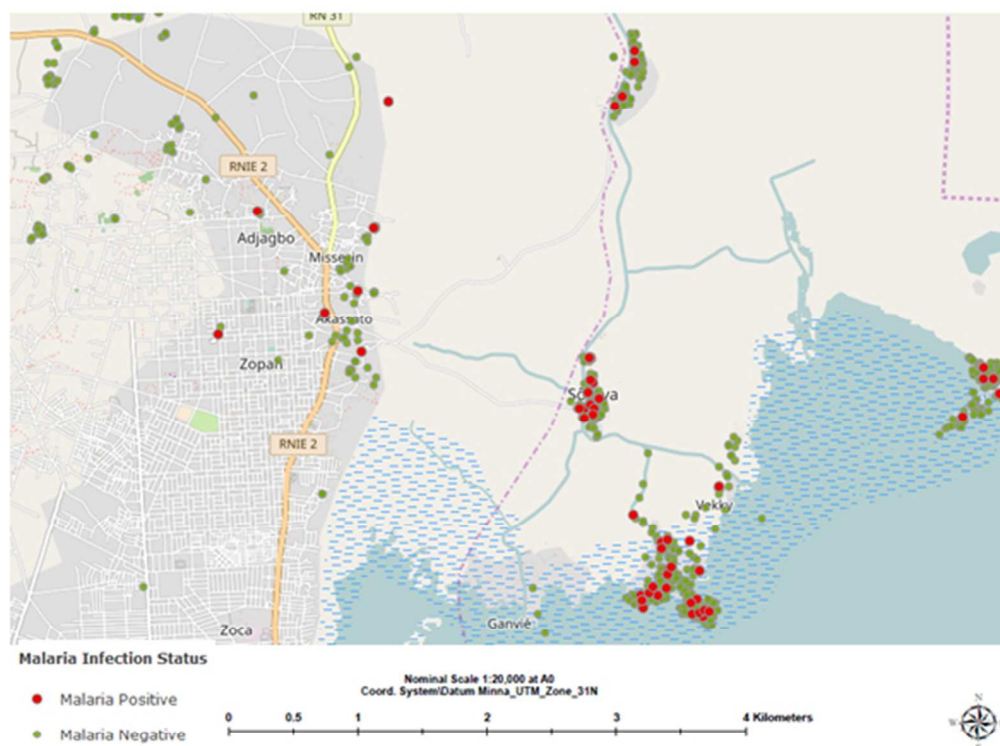
Figure 3. Malaria status (infected vs. non infected) in women of reproductive age at inclusion in RECIPAL (initial cohort), Southern Benin, 2014-2018.
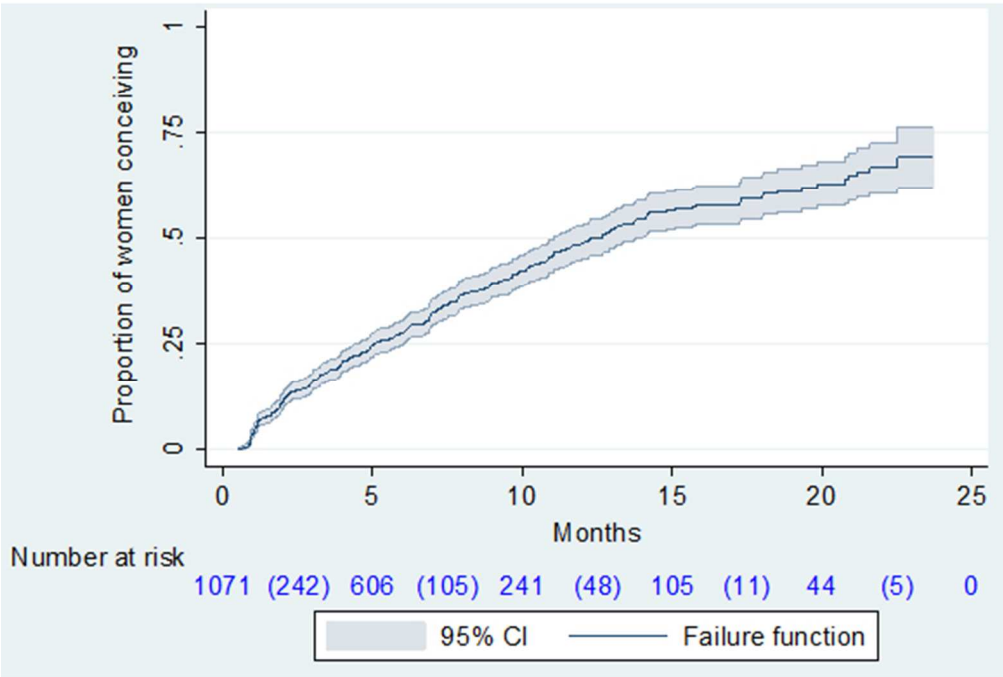
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Figure 4. Kaplan-Meier failure estimate of the probability of conceiving; 1214 women of reproductive age included in the initial RECIPAL cohort, Southern Benin, 2014-2018.

Figure 4. Kaplan-Meier failure estimate of the probability of conceiving; 1214 women of reproductiveage included in the initial RECIPAL cohort, Southern Benin, 2014-2018.
Probability of conceiving (solid line) and its 95% confidence interval (grey lines).Number of pregnancy events are in brackets. Number of censored women at 5, 10, 15, 20 and 24 months were : 223, 260, 88, 50 and 30, respectively.

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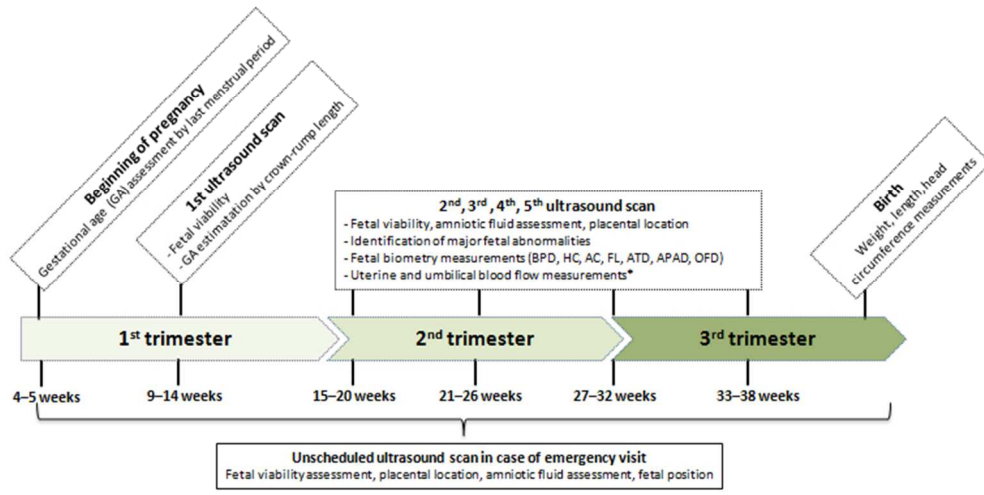
Supplementary file S1. Ultrasound follow-up (RECIPAL study)

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (page 2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 6)
Objectives	3	State specific objectives, including any pre-specified hypotheses (page 6)
Methods		
Study design	4	Present key elements of study design early in the paper (page 7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 7, 8, 9, 10, 11)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 8)
		Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls (Not applicable)
		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants (Not applicable)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed (Not applicable)
		Case-control study—For matched studies, give matching criteria and the number of controls per case (Not applicable)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (page 12, 13)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 12, 13)
Bias	9	Describe any efforts to address potential sources of bias (page 15)
Study size	10	Explain how the study size was arrived at (page 7, 8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Not applicable for cohort profiles publication)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Not applicable for cohort profiles publication)
		(*b*) Describe any methods used to examine subgroups and interactions (Not applicable for cohort profiles publication)
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed (page 10 and 14)
		Case-control study—If applicable, explain how matching of cases and controls was addressed (Not applicable)
		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy (Not applicable)

(*e*) Describe any sensitivity analyses (Not applicable)

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (page 10)
		(b) Give reasons for non-participation at each stage (page 10)
		(c) Consider use of a flow diagram (Figure 2)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Table 4)
		(b) Indicate number of participants with missing data for each variable of interest (page 10)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (Table 1 and 2)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time (page 14)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure (Not applicable)
		Cross-sectional study—Report numbers of outcome events or summary measures (Not applicable)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (page 14, 15)
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)

Discussion (Not applicable for cohort profiles publication)

Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (page 24)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Cohort profile: Effect of malaria in early pregnancy on fetal growth in Benin (RECIPAL pre-conceptional cohort).

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Title

Cohort profile: Effect of malaria in early pregnancy on fetal growth in Benin (RECIPAL pre-conceptional cohort).

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Abstract

Purpose: RECIPAL is an original pre-conceptional cohort designed to assess the consequences of malaria during the first trimester of pregnancy, which is a poorly investigated period in Africa and during which malaria may be detrimental to the fetus.

Participants: For this purpose, a total of 1214 women of reproductive age living in Sô-Ava and Akassato districts (south Benin) were followed-up monthly from June 2014 to December 2016 until 411 of them became pregnant. A large range of health determinants was collected both before and during pregnancy from the first weeks of gestation to delivery. Five Doppler-ultrasound scans were performed for early dating of the pregnancy and longitudinal fetal growth assessment.

Findings to date: Pregnant women were identified at a mean of 6.9 weeks of gestation (wg). Preliminary results confirmed the high prevalence of malaria in the first trimester of pregnancy, with more than 25.4% of women presenting at least one microscopic malarial infection during this period. Most infections occurred before 6 wg. The prevalence of low birthweight, small-birthweight-for-gestational age (according to INTERGROWTH21-st charts) and preterm birth was 9.3%, 18.3%, and 12.6%, respectively.

Future plans: RECIPALrepresents at this time a unique resource that will provide information on multiple infectious (including malaria), biological, nutritional and environmental determinants in relation to health outcomes in women of reproductive age, pregnant women and their newborns. It will contribute to better define future recommendations for the prevention of malaria in early pregnancy and maternal malnutrition in Africa. It confirms that it is possible to constitute a pre-conceptional pregnancy cohort in Africa and provides valuable information for researchers starting cohorts in the future.

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Strengths and limitations of this study
• A unique cohort combining highly detailed, high-quality health-related information on the pregnancies of ≈ 400 women recruited in the pre-conception period, with a substantial related bio-bank of plasmas, placental and other samples.
• Pre-conceptional design allowing the early identification and follow-up of pregnant women: screening of women for both microscopic and submicroscopic malaria from the first weeks of pregnancy;accurate estimation of gestational ageby using early obstetrical ultrasound scan andcollectionofsome important factors influencing fetal growth such as pre-pregnancy nutritional status and gestational hypertensive disorders.
• Collection of valuable information for the implementation of future pre-conceptional cohorts in Africa.
• High attrition in the pre-conceptional cohort, with a possible impact on the external validity of some findings.
• Reduced sample size at delivery due to a high proportion of spontaneous abortion, with a possible lack of power for analyses on birth outcomes.

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Introduction

Malaria in pregnancy is associated with a wide range of deleterious effects in women and their offspring. In Sub-Sahara African (SSA) countries, preventive strategies are based on long-lasting insecticide treated bed nets (LLITNs) and intermittent preventive treatment in pregnancy (IPTp). IPTp consists in the administration of sulfadoxine-pyrimethamine (SP) at each antenatal care (ANC) visit from the 2nd trimester of pregnancy onwards [1]. LLITN are distributed at the first ANC visit, which generally occurs around 4-5 months of pregnancy [2]. Therefore, pregnant women remain unprotected or insufficiently protected during the first months of pregnancy, and particularly during the first trimester. However, this period may be a high-risk period for the fetus if pregnancy-associated malaria parasites accumulate into the placenta during trophoblast differentiation and vascular remodelling of the uterus [3,4]. Impaired placentation may then contribute to fetal growth restriction and low birthweight (LBW).

Few studies have investigated malaria in early pregnancy and while some have shown an adverse effect on birth outcomes, this has not been consistent [5]. In Burkina Faso and in Benin, malaria infection in the first trimester [6] or before 4-5 months of pregnancy [7,8] has been associated with a higher risk of LBW or a decrease in birthweight. Conversely, such association was not reported in Uganda and Malawi [9,10] but the number of women screened in the first trimester was low in these two studies. One of the problems in interpreting this existing literature relates to the challenges of recruiting women early in pregnancy; most of the studies were not designed to assess the consequences of malaria early in pregnancy and therefore women were generally recruited late in the first trimester, which may lead to misclassification errors and underestimation of exposure. Besides, using LBW as a proxy for fetal growth restriction (FGR) can lead to the overestimation of FGR prevalence and possible

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bias when estimating the effect of malaria. Finally, maternal nutrition is one of the main factors influencing fetal growth in developing countries [11]. Therefore, adjusting for this factor when assessing the effect of early malaria on FGR is important. As well, a recent study has suggested a higher risk of FGR due to malaria in undernourished women [12].

The RECIPAL study aimed to assess the effect of malaria (both microscopic and submicroscopic) in the first trimester of pregnancy on both the mother and the fetus, with a focus on fetal growth, by following a cohort of pregnant women recruited before conception. Moreover, it also aimed at assessing the influence of the woman's nutritional status in the relationship between malaria in early pregnancy and birth outcomes.

Cohort description

Study setting

The study cohort is currently conducted in the districts of Sô-Ava and Abomey-Calavi, located in Southern Benin (Figure 1). Women's recruitment has been completed by December 2016, and pregnancy follow-up is still ongoing. In the area, malaria is hyperendemic with a mean entomological inoculation rate of 2.1 infected anopheles bites/person/100 nights [13]. The main mosquito vectors of malaria are *Anopheles gambiae* ss and *Anopheles funestus* [14]. Four sub-districts (Sô-Ava, Vekky and Houedo in Sô-Ava District, and Akassato in Abomey-Calavi District) were selected for the study according to the population density and mean number of ANC visits and deliveries per month in the four main public maternity clinics of Sô-Ava and Abomey-Calavi districts. In these four sub-districts, the population size was estimated to 124,994 people in 2013, including 17.4% (21,779) women of reproductive age (WRA) [15]. Thirty-five out of a total of 36 villages were then selected for the study according to their proximity to the maternity clinics.

Study design, subject identification, recruitment and enrolment procedures

Briefly, WRAs were recruited at community-level and followed monthly for a maximum period of 24 months until becoming pregnant, they constituted the RECIPAL initial cohort. The sub-sample of women who became pregnant was then followed-up monthly at the maternity clinic from early pregnancy to delivery; they constituted the RECIPAL final cohort.

A sample size of 466 births was estimated sufficient to evidence a 2-fold odds ratio for the association between malaria and poor birth outcomes [4], assuming a prevalence of 25% of women with microscopic malaria in the 1st trimester of pregnancy and 30% of poor birth outcomes (LBW, small-for-gestational age (SGA), preterm birth (PTB) or stillbirth) [8,16,17] with a 80% power, significant level at 0.05. We estimated that following 2,000 WRA (initial cohort) would have allowed identifying 510 pregnant women (final cohort) during a planned 18 month-period for recruitment in the initial cohort (based on a total fertility rate of 200/1,000/year in Benin (http://esa.un.org/unpd/wpp/index.htm) and allowing 15% of loss to follow-up after recruitment in the initial cohort.

After explanation of the study to the local political and administrative authorities, three concomitant procedures were used to recruit WRA. First, repeated sensitization events were organized in each of the 35 selected villages for presenting the study to all inhabitants. If interested, women were invited to register with the head of the community and were visited at home the day after for their inclusion. That procedure constituted the main way of recruitment throughout the study. Second, leaders of the community, religious authorities and mass-media were involved in order to help us mobilizing women during public meetings (masses, women's association meetings, etc.). The last way of recruitment consisted of going door-to-door to identify eligible and interested women with the assistance a network of community-health workers (more details are provided in Supplemental file S1).

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To be included, women had met the following criteria: negative urinary pregnancy test, 18 to 45 years old, no current contraception, no previous fecundity issues, willingness to become pregnant, no planned travel for more than 2 months within the next 18 months, acceptance of RECIPAL protocol and signed written informed consent. Both oral and written communication was used for providing information on the project to the women and their husbands. The consent form was translated into the local language for women who were illiterate.

From June 2014 to December 2016, 1302 WRA were willing to participate in the study. Among them, 1214 (93.2%) met the inclusion criteria and were recruited, 88 (6.8%) were not included mainly because of a positive urinary pregnancy test at inclusion (63.6%) or past infertility issues (14.8%). Based on both the 2013 national census and the 2011-2012 Demographic Health Survey (DHS-IV) in Benin, we estimated that women included in the project represented 6% of the total number of WRA living in the study area. Compared with women included in Dansou *et al.* study [18], which used individual data of a representative sample of WRA included in the Beninese DHS-IV, WRA included in RECIPAL had a higher level of poverty. Age, education level, household wealth, gravidity and media exposure were, however, similar (Supplemental file S2).

RECIPAL study was approved by the ethics committees of the Institut des Sciences Biomédicales Appliquées (ISBA) in Benin and from the IRD in France as well as by the Ministry of Health in Benin.

Cohort follow-up

Pre-conceptional follow-up

After enrolment, WRAs were visited at home monthly by specifically trained-investigators assisted by local community-health workers. Follow-up consisted mainly of recording the first

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day of last menstrual period (LMP) and performing a urinary pregnancy test every month. To minimize the risk of loss of confidentiality during the tracking of women for follow-up, the study team was instructed to speak only to people—including family members—to whom women have granted them permission to speak. After 12 months, women who were still followed received medical advices to help conceive and they were invited to attend the maternity clinic for a medical examination in case of symptoms of genital infection. After 24 months, the follow-up ended and women who did not conceive were invited to attend a gynaecologist for fertility issues assessment. More details on the follow-up procedures have been provided in Supplemental File S3.

Gestational follow-up

Once pregnant, women were followed-up at the maternity clinic for monthly ANC visits. In addition, they were encouraged to attend the maternity clinic any time in case of any symptoms. Both the maternity staff (for usual ANC follow-up and clinical examination) and study investigators (for all specific issues related to RECIPAL) were involved in women's follow-up. Throughout the study, women were offered free transport to the maternity clinic; in Sô-Ava District a free river shuttle was set up. At 37 weeks gestation, women were visited at home weekly and transportation to the study maternity clinic was scheduled for delivery.

According to Beninese national recommendations, a kit including iron and folic acid tablets for one month, mebendazole for deworming, the 1st dose of SP for IPTp and a LLITN was given to each pregnant woman at the first ANC visit. The maternity staff was encouraged to administer at least three doses of IPTp from the 2nd trimester onwards as recommended by the Beninese National Malaria Control Program, as well as iron and folic acid supplementation throughout the pregnancy. RECIPAL women benefited from free management of any diseases related to the pregnancy—detected either as part of RECIPAL follow-up or during

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unscheduled visits, free ANC visits and delivery. Women infected with malaria were treated with quinine in the first trimester of pregnancy and artemether-lumefantrine from the 2nd trimester.

Infant follow-up

The infant follow-up was not initially planned as part of the RECIPAL project. It is currently carried out as part of the SEPSIS project (bioMérieux-funding, UMR216/9198 coordination), which aims to assess immune dysfunctions associated with the risk of sepsis in preterm and growth-restricted newborns as well as in those exposed to malaria *in utero.* All infants born from the RECIPAL women from April 2016 have been included and followed until 3 months of age.

Cohort attrition

Figure 2 presents the flowchart diagram of the study. Among the 1214 WRA enrolled in the initial cohort, 411 (33.9%) became pregnant, 359 (29.6%) completed the follow-up without conceiving and 444 women (36.6%) did not complete the study mainly because of informed consent withdrawal (272/444) or migration outside the study area (138/444) that occurred after 5.2 and 6.3 months of follow-up in average, respectively.

Among the 411 pregnant women, 207 (50.4%) have already delivered, 16.5% (68/411) had a spontaneous abortion (n=68) or a non-viable pregnancy (n=2), 62 did not complete the study because of informed consent withdrawal (45/411, 10.9%) or migration (17/411, 4.1%), and 72 women are still followed (Figure 2). Spontaneous abortions, informed consent withdrawals and migrations occurred at 8.9 (± 3.9), 13.2 (± 8.1) and 14.2 (± 8.6) weeks of gestation (wg) in average (SD), respectively. More details on the reasons for cohort attrition are provided in Supplemental file S4.

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Data collection

Pre-conceptional data

Demographic, socioeconomic and household characteristics were collected at inclusion (Table 1). At this occasion, screening for malaria, urinary schistosomiasis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was performed. Haemoglobin (Hb) and markers of inflammation levels were determined. The first day of LMP was recorded, and a urinary pregnancy test (One-Step®, International Holding Corp, Germany) was performed each month. Anthropometric measurements were collected every three months and dietary intakes were assessed twice during follow-up.

Gestational data

At each ANC visit, clinical, anthropometric and obstetrical data, as well as LLITN use, IPTp administration, alcohol consumption, and iron, folic acid and other micronutrient supplementation intake were collected (Table 2). In addition, malaria screening was performed, and proteinuria, glycosuria and urinary infection were detected using a urine dipstick test (Combi-screen®, Analyticon, Germany). Dietary intakes were assessed once every trimester. Five Doppler-ultrasound scans (US) were performed. A sample of venous blood was collected in the 1st and 3rd trimesters for Hb, markers of inflammation and lead levels determination.

At delivery, newborns were weighed within 1 hour after birth on an electronic digital scale with an accuracy of 2 grams (SECA 757, SECA Ltd., Germany). Newborn's length (SECA infantometer 416, Germany) and head circumference (SECA 201, Germany) were recorded to the nearest millimetre. Malaria screening was performed on maternal venous blood, placental and cord blood (Table 2). A placental biopsy was performed for malaria histopathology. Follow-up and quality control procedures are provided in Supplemental file S3.

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The types and origins of the biological samples collected before and during pregnancy are listed in Table 3. Total blood, serum, plasma urine samples are stored in -20°C or -80°C freezers with a daily monitoring of the temperature.

Specifics components

Malaria diagnosis. Before conception and during pregnancy, malaria screening was performed using both microscopy (thick blood smear) and polymerase chain reaction (PCR). Blood smears were stained with Giemsa and parasitaemia was quantified by the Lambaréné method [19]. A molecular diagnostic approach using a combination of real-time PCR assays comprising genus-specific primers and probes for the gene encoding the small (18S) of *Plasmodium* rRNA and an ultra-sensitive *P. falciparum*-specific detection system [20] were used to screen samples containing *Plasmodium* parasites. In addition, any time during pregnancy, a rapid diagnostic test (Pf + pan rapid test SD Bioline Ag®, IDA Foundation, Netherlands; BioSynex®, France) was performed in case of symptoms suggestive of malaria for immediate diagnosis and treatment.

Ultrasound follow-up. The first US for dating the pregnancy was performed between 9 and 13 wg based on LMP recorded during the pre-conceptional follow-up (supplementary file S5). The gestational age (GA) was estimated in days based on crown-rump length measurement using the Robinson's chart [21]. At the end, GA estimation was based either on LMP if the difference between the two measurements (LMP/US) was less than 7 days or on US if the difference is > 7 days. Following INTERGROWTH-21st methodology, four additional scans were performed for fetal biometry assessment, and uterine and umbilical blood flow measurements [22]. Each parameter was measured twice and then averaged; a third measure was performed in case of discrepancy between the two first measurements. A quality control was performed on 10% of the pictures.

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Anthropometric measurements and dietary intake assessment. Anthropometric measurements were collected using standard procedures (Tables 1 and 2) [23]. During household visits, women's body weight was measured with a 200 g precision with calibrated electronic scales (Tefal, France). At the facility-level, women were weighed with a Tanita MC-780 body composition device (Tanita Corporation, Tokyo, Japan). Height was measured to the nearest millimeter with a SECA 206 (Hamburg, Germany) gauge. The left mid-upper-arm-circumference was measured with a SECA 201 (Hamburg, Germany) ergonomic circumference measuring tape. Skinfold thickness measurements were made to the nearest millimeter at the triceps and biceps sites using a Holtain (Crymyeh, UK) skinfold caliper. The set of measurements was repeated twice—by the same field investigator—and then the measurements were averaged. Body composition, assessed by bioelectrical impedance analysis method, was measured by the multi-frequency body composition analyser Tanita MC-780 with a four-electrode arrangement paired on hands and feet. A quality control of anthropometric data was performed periodically by a senior research scientist in nutrition.

Dietary intakes were assessed by 24h-recalls using the standard multiple-pass methodology [24]. A comprehensive list of the most common food items and recipes was developed from a preliminary study of the diet habits of the study area. This list was used to identify foods mentioned by the women as consumed during the interview. Amounts of food consumed were estimated by household measures consisted of pre-calibrated utensils, food pictures of different portion sizes, monetary value of known food portions sold on the study area markets. A food composition table and recipes database are under construction for conversion of household units to gram, and to determine macro- and micro-nutrients intake by the women.

Characteristics of the study populations

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At enrolment, WRA were 27 years old in average (Table 4). Nulligravidae represented 8.8% of the cohort. More than half of the women were anaemic, one quarter was infected with schistosomiasis and 38% had an abnormal BMI. The prevalence of microscopic malaria was 5.7%, with two geographical clusters in Sô-Ava and Vekky sub-districts (Figure 3). The median duration of follow-up in the initial cohort was 5.9 months (mean=7.1, range: 0.49–23.7). Compared to women included in the initial cohort, women who did not complete the follow-up were significantly different in terms of residence area, ethnicity, household density and schistosomiasis status (Table 4).

Pregnant women were identified at a mean of 6.9 wg and benefited from 7.5 scheduled ANC visits in average (Table 4). The prevalence of microscopic malaria infection was 25.4%, 19.4% and 16.1% during the 1st, 2nd and 3rd trimester of pregnancy, respectively. The prevalence of SGA was 11.5% and 18.3% using Schmiegelow's and INTERGROWTH 21st charts, respectively (Table 5). The proportion of PTB and LBW was 12.6% and 9.3%, respectively. Overall, 29.5% (61/207) of newborns presented at least one poor birth outcome defined as LBW, PTB, SGA or stillbirth.

Findings to date

Fertility and time-to-pregnancy

The median time-to-pregnancy was 12.3 months (Figure 4). The fertility rate was 5.4 pregnancies/100 persons-month (95% confidence interval [CI] =4.9-5.9). The probability (95% CI) of conceiving after 5, 10, 15 and 20 months of follow-up were 24.5% (21.9-27.4), 42.1% (38.6-45.9), 56.6% (52.1-61.1) and 62.8% (57.6-68), respectively. Using multivariate survival analysis, factors associated with a lower risk of conceiving were maternal age more than 35 years, a low education level, being polygamous, living in Vekky sub-district, long-

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term couple (> 8 years), infrequent sexual activity, overweight and urinary schistosomiasis infection (Yovo, personal communication).

Malaria in the first trimester of pregnancy

The incidence rate of malaria infection during the first trimester was 19.7 cases per 100 persons-month (95% CI 15.8-24.5). Using a multi-level logistic regression, we showed that women infected with malaria before conception were more likely to be infected during the 1st trimester (aOR: 2.68, 95% CI 1.24, 5.78). Gestational age was also negatively correlated with malaria infection (aOR: 0.64, 95% CI 0.41, 0.98) (Accrombessi, personal communication).

Strengths and limitations

The pre-conceptional design—and early identification of pregnant women—allowed (i) the screening of women for malaria from the very beginning of pregnancy; (ii) the accurate estimation of GA by using US before 14 wg; (iii) the determination of pre-pregnancy nutritional status [25] and hypertensive disorders screening [26], which highly influence fetal growth; and (iv) the minimization of selection bias embedded in the design of women recruited at facility-level. Besides, PTB and IUGR could be accurately assessed thanks to the precise estimation of GA and longitudinal fetal growth assessment.

In addition to malaria, RECIPAL will provide valuable information on WRA in Africa in terms of fertility and nutrition, which are seldom evaluated and may help to define new pre-conceptional interventions for improving maternal and child health [27]. Also, it yields logistical and ethical information for the implementation of future pre-conceptional cohorts.

Only107 (8%) nulligravidae were included in the study. The main reason is that RECIPAL was implemented shortly after a study, which has recruited nulligravid WRAs living in the same area. This study aimed at assessing maternal immune responses in early pregnancy in

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order to help designing a phase 1 vaccine trial against malaria. It included 278 nulligravid WRAs, of whom 60 became pregnant [28]. Both studies were conducted concomitantly for several months and women already included in the first study could not be eligible for RECIPAL. Because primigravidae were under-represented in the final RECIPAL cohort, it is likely that the prevalence of maternal conditions related to primigravidity were underestimated. Although we cannot completely exclude selection bias, it should not have strongly influenced the association between malaria in early pregnancy and FGR.

Finally, because of constraints related to the study design (long duration of the pre-conceptional follow-up and weariness of the women) and African socio-cultural realities (rumour about blood collection, suspicion regarding medical care provided free of charge, etc.), a noticeable proportion (22%) of women withdrew their informed consent before the end of the 24 month-follow-up. Overall, the initial cohort attrition was 37%. Since some baseline characteristics in women who completed the pre-conceptional follow-up and those who did not were different, this might impact the external validity of some of results. During pregnancy, there was a high proportion (16.5%) of spontaneous abortions, which contributed to the final cohort attrition. At the end, birth outcomes could be evaluated in only 68% of women, with a possible lack of power for some upcoming analyses.

Conclusion

If the deleterious effects of malaria in the first trimester of pregnancy are confirmed, our results may argue in favor of starting preventive measures from the very beginning of pregnancy or even before pregnancy. Artemisinin-based Combination Therapies (ACTs) have been shown to be safe during the first trimester of pregnancy and, therefore, may be a good option for the replacement of SP [29]. Besides, a vaccine against pregnancy-associated malaria parasites, which could elicit protective immunity prior to pregnancy to best protect

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pregnant women during early pregnancy may be proposed as a complementary strategy. Such a vaccine is currently under evaluation [30].

Collaboration

The "Mother and child face to tropical infections" research unit (MERIT), French National Research Institute for Sustainable Development (IRD)/Paris Descartes University was the promoter of the study. RECIPAL was a collaborative project between MERIT, another IRD research unit (Nutripass, Montpellier), EPOPé research team (Inserm), the Ecole des Hautes Etudes en Santé Publique (EHESP) and two Beninese collaborators from Abomey-CalaviUniversity (the Faculty of Health Sciences and the Faculty of Agronomy Sciences).

The researchers associated with this study are open for the sharing and reuse of the data but an end user data use agreement will be required for accessing the data. Collaborations are encouraged, although data sets are not currently publicly available. Any researcher interested in exploring RECIPAL data should contact directly the principal investigator, Valerie Briand (e-mail: valerie.briand@ird.fr).

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Author's contribution

Project management: MA, NF, AM, MC and VB (principal investigator).

Field, Epidemiology and data collection: MA, EY, GC, GA, PA, NF and VB.

Nutritional data collection: YMP, AG, NFF, DD, MA and EY.

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Biology and molecular analyses: NF, NTN and SH.

Obstetrical data collection: MA, EY, NJ and VB.

Statistical analysis: MA, GC, MC, JZ, FBL, YMP, AG, NFF, DD and VB

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Table 1. Clinical, nutritional and biological data collected during the pre-conceptional follow-up. The RECIPAL study, 2014-2017.

	Inclusion	D1	M1	M2,M3	M4	M5,M6	M7	M8,M9	M10	M11,M12	M13	M14,M15	M16	M17,M18	M19	M20,M21	M22
General characteristics																	
Place of visit	‡	¤	¤	‡	‡	‡	‡	‡	¤	‡	‡	‡	‡	‡	‡	‡	‡
Eligibility criteria	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Informed consent	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GPS location	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Socio-demographic characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Housing characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Economic characteristics	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clinical and nutritional data																	
Obstetrical history	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Height	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weight, MUAC, skinfold thickness	-	†	†	-	†	-	†	-	†	-	†	-	†	-	†	-	†
Body composition (BIA)	-	†	†	-	-	-	-	-	†	-	-	-	-	-	-	-	-
Dietary 24-h recall	-	†	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-
Amenorrhea/LMP	-	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†
Biological data																	
Urinary pregnancy test	†	-	†	†	†	†	†	†	†	†	†	†	†	†	†	†	†
Malaria screening (TBS, PCR)	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hb, ferritin, folic acid levels	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CRP, AGP, sTfR levels	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Chlamydia trachomatis**	-	†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Neisseria gonorrhoeae**																	
Urinary schistosomiasis*	-	†	†	-	-	-	-	-	-	†	-	-	-	-	-	-	-
Urine storage**	-	†	†	-	-	-	†	-	†	-	-	-	-	-	-	-	-

(¤) Visit performed at the study maternity clinic; (‡) Household visit; Information collected/assessed (†) or not (-) at each specific time-point.

* C. trachomatis and N. gonorrhoeae screening (using PCR and serology), as well as schistosomiasis screening (Nytrel® filter) started 9 and 12 months after study initiation, respectively.

** Urine storage for further analysis

Abbreviations: D: day; M: Months; MUAC: Mid-Upper-Arm-Circumference; BIA: Bio impedance analysis; LMP: Last menstrual period; TBS: Thick blood smear; PCR: polymerase chain reaction; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein; Hb: Haemoglobin.

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Table 2. Clinical, nutritional and biological data collected during pregnancy. The RECIPAL study, 2014-2017.

	ANCv 1	ANCv 2	ANCv 3	ANCv 4	ANCv 5	ANCv 6	ANCv 7	ANCv8	Delivery
Clinical and nutritional data									
Gestational age (weeks), mean (SD)	6.8 ± 2.5	11.6 ± 2.9	16.7 ±2.9	22.1 ± 3.7	27.6 ± 3.8	32.7 ± 3.5	36.2 ± 2.5	38.4 ± 1.4	39.1 ± 3.0
Past medical and obstetrical history*‡	†	-	-	-	-	-	-	-	-
Alcohol consumption in the last 24 hours	†	†	†	†	†	†	†	†	-
Iron and folate intake in the last 24 hours	-	†	†	†	†	†	†	†	-
Use of ITN the night before the visit	†	†	†	†	†	†	†	†	-
Axillary temperature, blood pressure	†	†	†	†	†	†	†	†	†
Gestational age (based on LMP or first US)	†	†	†	†	†	†	†	†	†
Anthropometric measurements									
- Weight	†	†	†	†	†	†	†	†	†
- Mid-Upper-Arm-Circumference	†	-	†	-	†	-	-	-	-
- Skinfold thickness (bicipitalandtricipital)	†	-	-	-	-	†	-	-	-
Body composition (Bio impendance analysis)	†	†	†	†	†	†	†	†	-
Dietary 24-h recall	-	†	-	-	†	-	†	-	-
Biological data									
Blood and rhesus group	†	-	-	-	-	-	-	-	-
HIV 1 screening	†	-	-	-	-	-	-	-	-
Peripheral malaria (TBS, PCR)	†	†	†	†	†	†	†	†	†
Placental malaria (TBS, PCR, histology)	-	-	-	-	-	-	-	-	†
Cord blood malaria (TBS, PCR)	-	-	-	-	-	-	-	-	†
Hb level	-	†	-	-	-	†	-	-	-
Serum ferritin, sTfR, CRP, AGP, folic acid levels	-	†	-	-	-	†	-	-	-
Lead level	-	†	-	-	-	†	-	-	-
Urinary infection and proteinuria (dipstick test)	†	†	†	†	†	†	†	†	-
Helminthic intestinal infection (PCR)	†	-	-	-	-	-	-	-	-
Urinary schistosomiasis	-	-	-	-	-	-	†	-	-
Urine storage**	-	†	-	†	-	†	-	-	-

* Sickle cell disease, diabetes or other chronic medical affection; history of preterm delivery or hypertensive disorders.

‡ History of cigarette smoking during pregnancy collected at delivery; ** Urine storage for further analysis

Information collected/assessed (†) or not (-) at each specific time-point.

Abbreviations: ANCv: Antenatal care visit; ITN: Insecticide-treated bed net; IPTp: Intermittent preventive treatment in pregnancy; LMP: Last menstrual period; TBS: Thick blood smear; PCR: polymerase chain reaction; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein, Hb: Haemoglobin.

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Table 3. Biological samples stored before conception, during pregnancy and at birth. The RECIPAL study, 2014-2017

	Pre-pregnancy	Pregnancy$	Delivery/Birth	Planned analyses
Total blood				
Total blood (200 µL)	-	Mother (x2)	-	Erythrocytic folic acid
Total blood (500 µL)	-	Mother (x2)	Mother-placenta-cord	Metals level
Blood spot +4°C (50 µL)	Mother (x1)	Mother (x8)[‡]	Mother-placenta-cord	*Plasmodium* detection (PCR)
Blood spot -20°C (100 µL)	Mother (x1)	Mother (x8)[‡]	Mother-placenta-cord	Immunological markers
Red cells (Trizol, 100 µL)	Mother (x1)	Mother (x8)	Mother-placenta-cord	*Plasmodium* phenotyping
Buffy coat PAXgene (300 µL)	-	Mother (x2)	Mother-placenta-cord	Immunological markers
Plasma/serum				
Plasma-20°C [†]	Mother (x1)	Mother (x2)	Mother-placenta-cord	Micro RNAs, cytokines and chemokines
Plasma -80°C[†]	Mother (x1)	Mother (x2)[†]	Mother-placenta-cord	Micro RNAs, cytokines and chemokines
Serum (100µL)	Mother (x1)	-	-	*C. trachomatis* and *N. gonorrhoeae* detection
Serum (400 µL)	-	Mother (x2)		Vitamin A
Serum (100µL)[†]	Mother (x1)	Mother (x2)	-	Ferritin, CRP, sTfR, AGP
Urine (1.8 mL)	Mother (x2)	Mother (x3)	-	Pollutant/pesticide
Stool (-80°C)	Mother (x1)	-	-	Helminthic infection
Placenta				
Biopsy with formalin	-	-	Placenta	Histopathology for malaria
Biopsy -20°C	-	-	Placenta	Immunological markers
Biopsy with RNA Later	-	-	Placenta	Immunological markers

The frequency of sample collection is indicated in brackets.

$ During pregnancy, samples were collected twice (during the 1st and 3rd trimester of pregnancy), three times (during each trimester), or eight times (during each ANC visit).

‡In case of unscheduled visit, an additional blood spot was collected.

†Quantity of plasma stored before, during pregnancy and at delivery was 200, 600-800, 600 µl, respectively.

Abbreviations: TBS: Thick blood smear; sTfR: Soluble Transferrin Receptor; CRP: C-Reactive protein; AGP: alpha1-Acid Glycoprotein, PCR: Polymerase Chain Reaction.

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Table 4. Characteristics of women included in the initial and final RECIPAL cohorts, Southern Benin, 2014-2017.

Characteristics		Initial cohort (N=1214 WRA)	WRA with incomplete follow-up (N=444)	P value (1214 vs 444)	WRA with complete follow-up but no pregnancy (N=359)	Final cohort Pregnant women (N=411)	P value (411 vs 359)
General characteristics							
Sub-district, n (%)	Sô-Ava	279 (23.0)	102 (23.0)		45 (12.5)	132 (32.1)	
	Vekky	423 (34.8)	228 (51.4)	< 0.001	57 (15.9)	138 (33.8)	< 0.001
	Houedo	186 (15.3)	33 (7.4)		120 (33.4)	33 (7.8)	
	Akassato	326 (26.9)	81 (18.2)		137 (38.2)	108 (26.3)	
Ethnic group, n (%)	Toffin	864 (71.2)	346 (77.9)		218 (60.7)	300 (72.9)	
	Aizo	195 (16.1)	56 (12.6)	0.001	80 (22.3)	59 (14.4)	0.002
	Fon	86 (7.1)	23 (5.2)		31 (8.6)	32 (7.8)	
	Others	69 (5.6)	19 (4.3)		30 (8.4)	20 (4.9)	
Age (years)	Mean (± SD)	27.8 (± 5.5)	28.0 (± 5.6)	0.31	28.8 (± 5.6)	26.8 (± 5.0)	< 0.001
Marital status, n (%)	Polygamy	445 (36.7)	173 (38.9)		154 (42.9)	118 (28.7)	
	Monogamy	729 (60.0)	257 (57.9)	0.45	204 (56.8)	268 (65.2)	< 0.001
	Cohabitation	40 (3.3)	14 (3.1)		1 (0.3)	25 (6.1)	
Education, n (%)	Illiterate	889 (73.2)	336 (75.7)	0.14	263 (73.3)	290 (70.6)	0.41
Household density	Mean (± SD)	5.7 (± 3.0)	6.0 (± 3.4)	0.009	5.2 (±2.3)	5.9 (±3.0)	0.001
Gravidity, n (%)	Nulligravidae	107 (8.8)	48 (10.8)		26 (7.2)	-	
	Primigravidae	167 (13.8)	58 (13.1)	0.17	43 (12.0)	33 (8.0)	0.22
	Multigravidae	940 (77.4)	338 (76.1)		290 (80.8)	378 (92.0)	
Number of live birth(s)	Mean (± SD)	2.8 (± 2.0)	2.6 (± 2.0)	0.07	2.9 (± 2.1)	2.7 (± 1.9)	0.16
Short stature, n (%)	Maternal length < 150 cm	92 (8.0)	27 (6.1)	0.46	32 (8.9)	33 (8.0)	0.65
Mid-Upper-Arm-Circumference, n (%)	< 23 cm	76 (6.3)	23 (5.2)	0.62	27 (7.5)	26 (6.3)	0.51
Pre-conceptional characteristics							
Body mass index, n (%)	< 18.5kg/m²	95 (8.3)	26 (6.9)		29 (8.1)	40 (9.7)	
	18.5–24kg/m²	706 (61.7)	229 (61.1)	0.36	212 (59.2)	265 (64.5)	0.10
	≥ 25kg/m²	343 (30.0)	120 (32.0)		117 (32.7)	106 (25.8)	
Schistosomiasis infection, n (%) [£]	Yes	177 (24.7)	50 (31.2)	0.03	63 (19.6)	64 (27.0)	0.04
Anaemia, n (%) [§]	Yes	596 (49.1)	210 (47.3)	0.07	165 (46.0)	221 (53.8)	0.03
Microscopic malaria, n (%) [‡]	Yes	64 (5.7)	28 (7.5)	0.06	12 (3.4)	24 (5.9)	0.10

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Table 4. Characteristics of women included in the initial and final RECIPAL cohorts, Southern Benin, 2014-2017 (*continued*).

Characteristics		Initial cohort (N=1214 WRA)	WRA with incomplete follow-up (N=444)	P value* (1214 vs 444)	WRA with complete follow-up but no pregnancy (N=359)	Final cohort Pregnant women (N=411)	P value* (411 vs 359)
Gestational characteristics							
Median time to pregnancy (months)	Median	-	-	-	-	12.3	-
Fertility incidence rate(95% CI)[μ]		-	-	-	-	5.4 (4.9–5.9)	-
Gestational age at 1st ANC visit (weeks)	Mean (± SD)	-	-	-	-	6.9 (± 2.5)	-
Gravidity, n (%)	Primigravidae	-	-	-	-	33 (8.0)	-
	Multigravidae	-	-	-	-	378 (92.0)	-
Number of IPTp doses, n (%)[†]	≥ 2	-	-	-	-	136 (65.7)	-
Inadequate gestational weight gain[#], n (%) [†]	Yes	-	-	-	-	120 (57.9)	-
Number of ANC visits (scheduled) [†]	Mean (± SD)	-	-	-	-	7.5 (± 1.1)	-
Number of ANC visits (unscheduled) [†]	Mean (± SD)	-	-	-	-	2.1 (± 1.3)	-
HIV status, n (%) [†]	Positive	-	-	-	-	4 (1.9)	-
Gestational hypertension, n (%) [†]	Yes	-	-	-	-	7 (3.4)	-
Anaemia, n (%) [§] [†]	During pregnancy[χ]	-	-	-	-	142 (68.6)	-
	In the 1st trimester	-	-	-	-	98 (48.5)	-
	In the 3rd trimester	-	-	-	-	118 (57.0)	-
Microscopic malaria, n (%) [†]	During pregnancy[χ]	-	-	-	-	93 (44.9)	-
	In the 1st trimester[χ]	-	-	-	-	51 (25.4)	-
	In the 2nd trimester[χ]	-	-	-	-	40 (19.4)	-
	In the 3rd trimester[χ]	-	-	-	-	33 (16.1)	-
Placental malaria infection, n (%) [†]	Yes	-	-	-	-	13 (7.8)	-

* T-student and chi2 test were used for comparing continuous and categorical variables, respectively.

£ Schistosomiasis infection status before conception has been assessed in 718 WRA (Initial cohort): 160 with incomplete pre-conceptional follow-up, 321 with complete pre-conceptional follow-up without pregnancy, and 237 included in the final cohort.

§ According to WHO thresholds (12 g/dL for non-pregnant women and 11 g/dL for pregnant).

‡ Before conception, 78 missing and 5 missing values in the initial and final cohort, respectively. During pregnancy, 6, 1 and 2 missing values at 1st, 2nd and 3rd trimester of pregnancy, respectively; 40 missing values for placental malaria

μIncidence rate defined as the number of pregnancies / persons-month at risk.

A gestational weight gain was considered inadequate when the total weight gain during pregnancy was below to 12.5kg, 11.5kgs, 7kg and 5kg in the underweight women (pre-pregnancy BMI < 18.5 kg/m²), normal weighted women (pre-pregnancy BMI between 18.5-24.9 kg/m²), overweight women (pre-pregnancy BMI between 25.0-29.9 kg/m²) and obese women (pre-pregnancy BMI ≥ 30 kg/m²)before conception, respectively.

† Results based on the 207 women who have already delivered.

χ At least one episode

Abbreviations: WRA: women of reproductive age; SD: Standard deviation; ANC: Antenatal care; BMI: Body mass index

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Table 5. Preliminary data description of newborns at birth (sub-sample of the cohort). The RECIPAL study (2014-2017).

Newborn characteristics[†]		N	Mean (± SD) or Proportion (%)
Gender	Male	205	55.2
Stillbirth	Per 1000 live births	206	14.8
Preterm birth (< 37 weeks)	Yes	207	12.6
Small-birthweight-for-gestational age [‡§]	Yes	191	11.5
Small-birthweight-for-gestational age [¥ §]	Yes	191	18.3
Birthweight (g)[§]		193	3043.9 (± 432.8)
	< 2500	193	9.3
Poor birth outcome [£, §]	Yes	207	29.5

† Data based on the first 207 deliveries.
[§]Twins excluded
‡ Small-birthweight-for-gestational age: < 10th percentile of birthweight for gestational age using Schmiegelow's charts.
¥ Small-birthweight-for-gestational age: < 10th percentile of birthweight for gestational age using Intergrowth 21[st]charts;
£ Stillbirth, preterm birth, small-birthweight-for-gestational age (using Intergrowth 21[st]charts) or low birthweight

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Figure legends

Figure 1. Geographical location of Sô-Ava and Abomey-Calavi Districts, and of the 35 villages of the RECIPAL study, Southern Benin, 2014-2017.

Figure 2. Flowchart diagram of RECIPAL study.

* Study completion: Follow-up from enrolment until the end of the study (24-month follow-up without pregnancy for women recruited before December 2014 or monthly follow-up without pregnancy for women recruited between December 2014 and December 2016), excluding consent withdrawal, migration and lost to follow-up.

Figure 3. Malaria status (infected *vs.* non infected) in women of reproductive age at inclusion in RECIPAL (initial cohort), Southern Benin, 2014-2017. For online interactive map, check this link: Malaria Infection Status of Women at Inclusion of Cohort

Figure 4. Kaplan-Meier failure estimate of the probability of conceiving; 1214 women of reproductiveage included in the initial RECIPAL cohort, Southern Benin, 2014-2017.

Probability of conceiving (**solid line**) and its 95% confidence interval (**grey lines**).Number of pregnancy events are in brackets. Number of censored women at 5, 10, 15, 20 and 24 months were : 223, 260, 88, 50 and 30, respectively.

Supporting information

Supplemental file S5. Ultrasound follow-up (RECIPAL study)

HC: Head circumference; AC: Abdominal circumference; FL: Femur length; BPD: Biparietal diameter; OFD: occipito frontal diameter; ATD: Abdominal transverse diameter; APAD: Antero-posterior abdominal diameter.

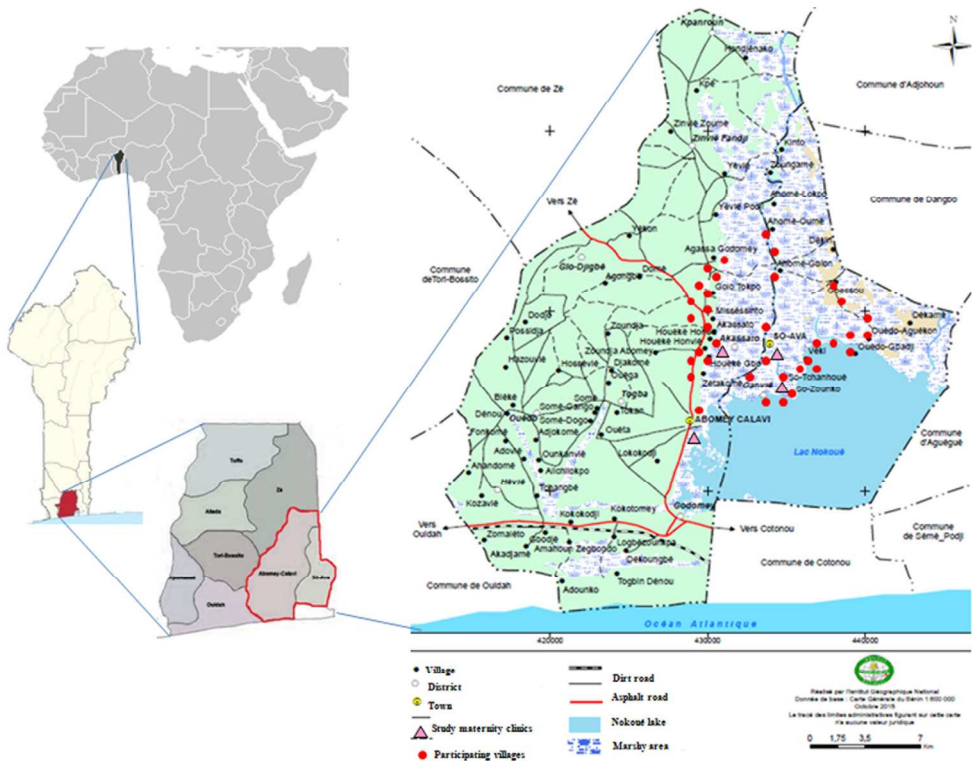* Uterine, and umbilical blood flows were measured at 21-25 wg and from 28 wg, respectively

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Figure 1. Geographical location of Sô-Ava and Abomey-Calavi Districts, and of the 35 villages of the RECIPAL study, Southern Benin, 2014-2017.
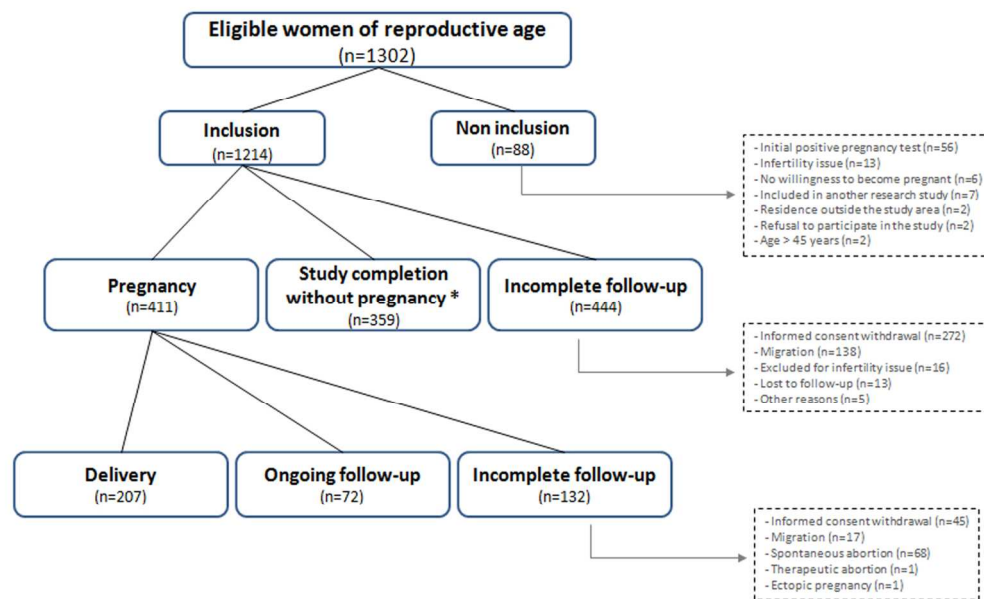
162x123mm (300 x 300 DPI)

Figure 2. Flowchart diagram of RECIPAL study.

* Study completion: Follow-up from enrolment until the end of the study (24-month follow-up without pregnancy for women recruited before December 2014 or monthly follow-up without pregnancy for women recruited between December 2014 and December 2016), excluding consent withdrawal, migration and lost to follow-up.
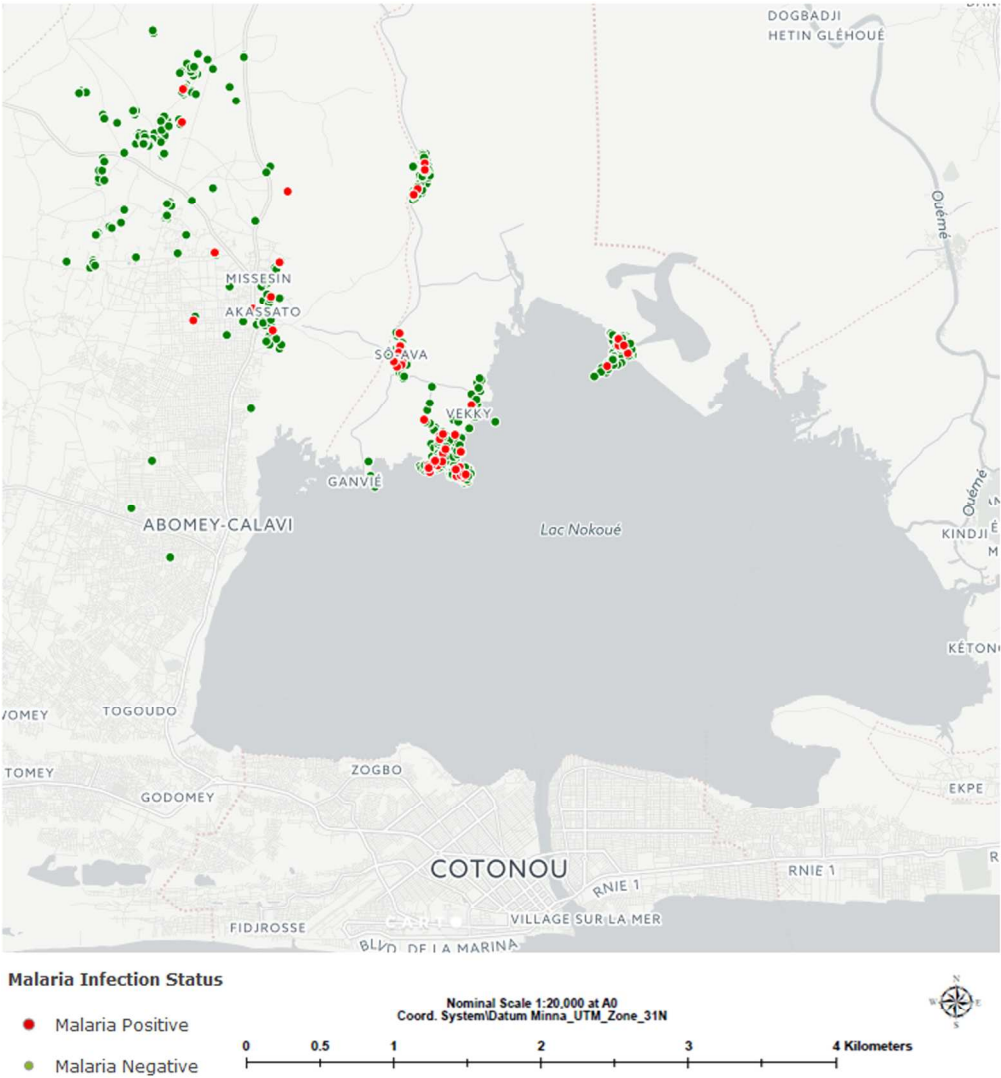
133x81mm (300 x 300 DPI)

Figure 3. Malaria status (infected vs. non infected) in women of reproductive age at inclusion in RECIPAL (initial cohort), Southern Benin, 2014-2017.
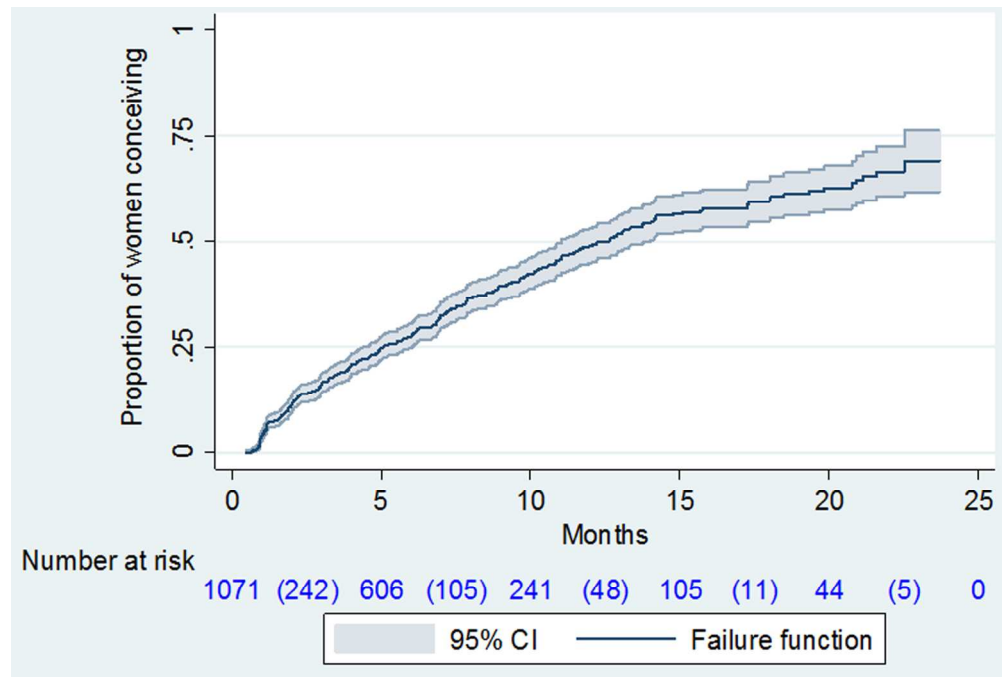
215x238mm (300 x 300 DPI)

Figure 4. Kaplan-Meier failure estimate of the probability of conceiving; 1214 women of reproductiveage included in the initial RECIPAL cohort, Southern Benin, 2014-2017.

Probability of conceiving (solid line) and its 95% confidence interval (grey lines).Number of pregnancy events are in brackets. Number of censored women at 5, 10, 15, 20 and 24 months were : 223, 260, 88, 50 and 30, respectively.

132x89mm (300 x 300 DPI)

Supplemental file S1: Procedures of enrolment of women of reproductive age in the RECIPAL study

All women of reproductive age (WRA), whatever their gravidity, living in the study area were eligible to be included in the study. To be recruited, they should met the following criteria: negative urinary pregnancy test, 18 to 45 years old, no current contraception, no previous fertility issues, in willingness to become pregnant, no planned travel for more than 2 months within the next 18 months, acceptance of RECIPAL protocol and signed written informed consent. Possible fertility issues were: primary or secondary amenorrhea; women unable – but wishing – to become pregnant for 10 years or more; and past history of three or more successive spontaneous abortions.

Three different procedures were used concomitantly to enrol WRAs:

✋ **First procedure**: Sensitization events were organized in each of the 35 selected villages for presenting the study to all inhabitants. If interested, women were invited to register with the head of the village and were visited at home the day after for their inclusion. The field investigators were in constant contact with the head of the villages to inquire about women registration and to know whether women or other inhabitants had shown interest in the study. Sensitization events were repeated several times throughout the study in villages with a low level of recruitment. Moreover, information sessions were organized in the different certified and accredited heath centres in the area, whether or not participating in the study, in order to increase community involvement to and acceptability of the study.

✋ **Second procedure**: Specific sensitization events were organized along with the community leaders (local elected and health authorities, crowned head families), religious authorities (traditional, muslim and christian) and mass-media (mainly through radio messages) for supporting the study and mobilizing WRAs during masses, women's or young's association meetings, church sessions, etc. Women's population was also informed about the project during health facility activities such as antenatal care visits and routine EPI's (expanded programme on immunization) sessions. Then, WRAs interested in the project could meet the study team (including field investigators and community health workers) to be registered.

✋ **Third procedure**: The last way of recruitment consisted of going door-to-door to identify eligible and interested women with the assistance of a network of community health workers. Community health workers are members of the community, they are selected by the community and are answerable to the communities for their activities. They must be endorsed by the health centre, but do not necessarily belong to it, and have a shorter training than professional workers. "Trackers", who are community members usually solicited by the heath centres for advanced EPI's sessions and national vaccination campaigns, were identified and involved in WRAs identification and enrolment after being trained.

Supplemental file S2: Characteristics of women of reproductive age from RECIPAL cohort in comparison with data extracted from the 2011/2012 Beninese Demographic and Health Survey

		RECIPAL cohort[§] (2014-2017)		Dansou *et al.*[*§] (2017)
		N	**% (n)**	**%**
Age (years)	≤ 19	1214	3.2 (38)	4.5
	20-24	1214	24.2 (294)	19.0
	25-29	1214	33.6 (408)	25.8
	30-34	1214	22.3 (271)	23.8
	≥ 35	1214	16.7 (203)	24.2
Education attainment	No education	1214	73.2	73.1
Household wealth quintiles[†]	Poorest	1214	35.2 (427)	22.3
	Poorer	1214	23.1 (281)	21.6
	Middle	1214	17.4 (211)	21.6
	Richer	1214	12.4 (150)	19.5
	Richest	1214	11.9 (145)	15.0
Gravidity	0	1214	8.8 (107)	-
	1	1214	13.8 (167)	18.4
	2-4	1214	47.6 (578)	51.5
	≥ 5	1214	29.8 (362)	30.1
Mass media exposure	None	1214	44.1 (536)	45.2
	Average	1214	37.5 (455)	32.6
	Higher	1214	18.4 (223)	22.2

* N= 8,701. Data extracted from the 2011-2012 national Beninese Demographic and Health Survey (DHS-IV);

Dansou J, Adekunle AO, Arowojolu AO. Factors associated with antenatal care services utilisation patterns amongst reproductive age women in Benin Republic: An analysis of 2011/2012 Benin Republic's Demographic and Health Survey data. Niger Postgrad Med J 2017;24:67–74

§ Women aged 18 to 45 years and 15 to 49 years old in the RECIPAL study and DHS-IV, respectively.

† Household wealth was approximated using a synthetic score combining occupation and ownership of assets in RECIPAL study, and by using principal component analysis in Dansou's article; it was then categorized according to the quintiles.

Supplemental file S3: Follow-up and quality control procedures in RECIPAL study

1. **Follow-up procedures**

Women of reproductive age (WRA) were recruited at community-level and followed monthly for a maximum period of 24 months; they constituted the **RECIPAL initial cohort**. The sub-sample of women who became pregnant was then followed-up monthly at the maternity clinic from early pregnancy to delivery; they constituted the **RECIPAL final cohort**. Data were collected by five specifically-trained field investigators, helped by three community health workers (CHW) recruited for the project.

Pre-conceptional follow-up

Each field investigator was responsible for the follow-up of a sub-sample of the cohort (~ 243 WRAs by investigator). Home visits were scheduled by the field-investigators (~159 visits/month/investigator) under site coordinator and deputy coordinator supervision. At each visit, the next visit was planned with the woman agreement and according to RECIPAL protocol. Few days before the visit, the investigator contacted the woman by telephone to confirm the date and time of the visit. A list of unreachable women was then established and sent to the CHWs, who visited the women at home.

The following procedures was applied when a woman remained unreachable:

- In case of absence because of daily activities, the field investigator planned another visit within the next few days and asked a family member or neighbor to warn the woman about the visit. In cases of over deadlines, the CHWs revisited the woman on the same day;

- When a travel was planned for less than 7 days, the investigator called the woman and planned a visit when she came back;

- When a travel was planned for more than 7 days, a urinary pregnancy test was performed before departure: if positive, the first antenatal care visit was performed before departure; if negative, a new visit was planned when the woman came back (whether in the two coming months);

- A **monthly visit** was considered as "**missing**" when not completed within 21 days after the planned date despite several requests from the field investigator.
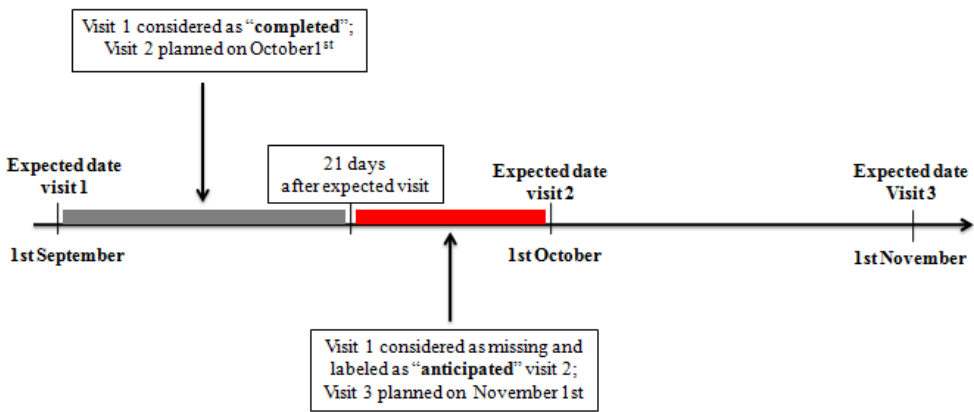
Women were excluded from the study in the following cases:

- Three successive missing visits;
- Two successive missing visits with a positive urinary pregnancy test when the woman came back.

Gestational follow-up

ANC visits were scheduled by midwives after discussion with the RECIPAL team. They were scheduled every month or every six weeks depending on whether an ultrasound scan was planned at this visit (see Suppl. file S5). In case of missing visit, the procedures were the same than during the pre-conceptional follow-up.

2. Follow-up completion

A WRA **completed** the pre-conceptional follow-up when she was followed until the end of the study (from 4 to 24 months of follow-up depending on when she was recruited). A pregnant woman **completed** the pregnancy follow-up when she was followed until delivery. Both follow-ups were considered **incomplete** in case of (i) lost to follow-up (two or three missing visits during the pre-conceptional period, or no information for more than 3 months during pregnancy); (ii) informed consent withdrawal; (iii) migration outside the study area; (iv) no data available at delivery because of adverse pregnancy outcomes such as spontaneous abortion or ectopic gestation.

3. Quality control

The quality control of the clinical data was carried out in two stages. At the first stage, the two supervisors verified on a weekly basis that data collection was exhaustive (i.e., concordant number of expected and collected forms). A control of missing and abnormal data was carried out on all participant forms. The second stage was carried out after data have been entered. The validation of double-entering was realized by the data manager and an audit report of the database, including lists of discrepancies, duplications and missing or abnormal data was sent regularly to the supervisors who were responsible for correcting any errors. Regarding the ultrasound data, a quality control of 10% of the pictures was performed monthly by a senior sonographer from Oxford University, United Kingdom.

Supplemental file S4: Main reasons of study drop out in RECIPAL cohort

Recruitment of WRAs was stopped in September 2016, when only 1,214 WRAs had been included. The recruitment period lasted 9 months more than was planned in the protocol (27 months vs. 18 months), but it could not be extended further because of financial reasons. Besides, because of constraints related to both the study design and the local socio-cultural context, a noticeable proportion, 36.5 % (444/1214) of WRAs did not complete the pre-conceptional follow-up. At the end, both the number of WRAs and the number of pregnant women included in the RECIPAL study were far lower than was planned. Difficulties in recruiting women, and the reasons of drop out before and during pregnancy were the following:

✋ Constraints related to the study design

To be included in RECIPAL, women should not have planned to travel for more than 2 months within the next 18 months. However, many women travelled during their follow-up. Women with three successive missing visits or with two successive missing visits with a positive pregnancy test at their return were excluded from the study (see Supplemental file S3). As described in the flow chart diagram, among women with incomplete pre-conceptional follow-up, 31.1% (138/444) moved outside the study area for more than two months and had to be excluded. Sô-Ava district is a lakeside city with fishing as principal activity. During the period of high fishing activity (~ 3 to 4 months/year), many women travel by ship to Makoko city in Nigeria for fish trade. In RECIPAL, 63.2% (86/138) of women who travelled went to Nigeria as final destination.

One of the main constraints related to the study design was the long duration of the pre-conceptional follow-up. Among the WRAs who withdrew their informed consent, 36% (98/272) of them reported study fatigue and disappointment of not being pregnant. No drugs or interventions were administered/implemented by the project to help women to conceive, only medical advices and clinical examination in case of symptoms suggestive of genital infection were provided.

During pregnancy, the main constraint was related to the number of ANC visits. Indeed, nine ANC visits were scheduled. At each visit, a thick blood smear was performed using capillary blood, except at the 2nd and 6th ANC visit when venous blood was collected. The mean number of ANC visits (including both scheduled and unscheduled visits) was 8.1 (SD ±2.8;

range: 1-15). Although most women were globally satisfied with the RECIPAL follow-up, some of them withdrew their informed consent because of too frequent ANC visits.

✋ African socio-cultural realities

Sixty-four percent of women (174/272) refused to continue participating in the study because of rumours about blood and placental samples collection, as well as suspicion regarding medical care provided free of charge for the woman. Indeed, at the beginning of the study false rumours circulated about the quantity and number of times blood was collected, and its use for religious purposes. Despite regular sensitization campaigns in the villages, project endorsement by the local (health) authorities and religious leaders, as well as organization of visits to our laboratory, rumours could not be completely eradicated. The fact that a high proportion of women were illiterate (> 70%) and from Toffin ethnicity (fears of foreign populations) contributed to the spread of rumours.

Despite the usefulness of the placenta for biomedical research, personal, socio-economic and cultural factors undermine the willingness of mothers to freely donate their placentas for research purpose, especially in SAA [1]. In Africa, including Benin, there are strong emotional, religious and cultural ties to how placentas are disposed. Indeed, a careless handling of the placenta is associated with a risk of infertility, illness and death in the newborn baby [2,3]. In RECIPAL, after a small piece of placenta was drawn for malaria histology and 4mL placental blood was collected for malaria screening, the placenta was immediately returned to the woman. Information—on why and how placenta will be collected—was provided to the woman and her family throughout the follow-up. Despite these precautions, placenta collection remained one of the main reasons for consent withdrawal.

The social pressure exerted by the family members was another issue in RECIPAL study. As far as possible, women's partners and family members (particularly, the women's mother-in-law) were involved at the various stages of follow-up. For many women, family agreement was required for their participation in the study and for monthly follow-up at the maternity clinic until delivery. Besides, in the RECIPAL study, women benefited from free management of any diseases related to the pregnancy—detected either as part of scheduled follow-up or during emergency visits—, free ANC visits and delivery. Eighteen percent (8/45) of consent withdrawals were due to the fact that the family-in-law judged the woman's partner unable to take care of their daughter correctly because of free antenatal care.
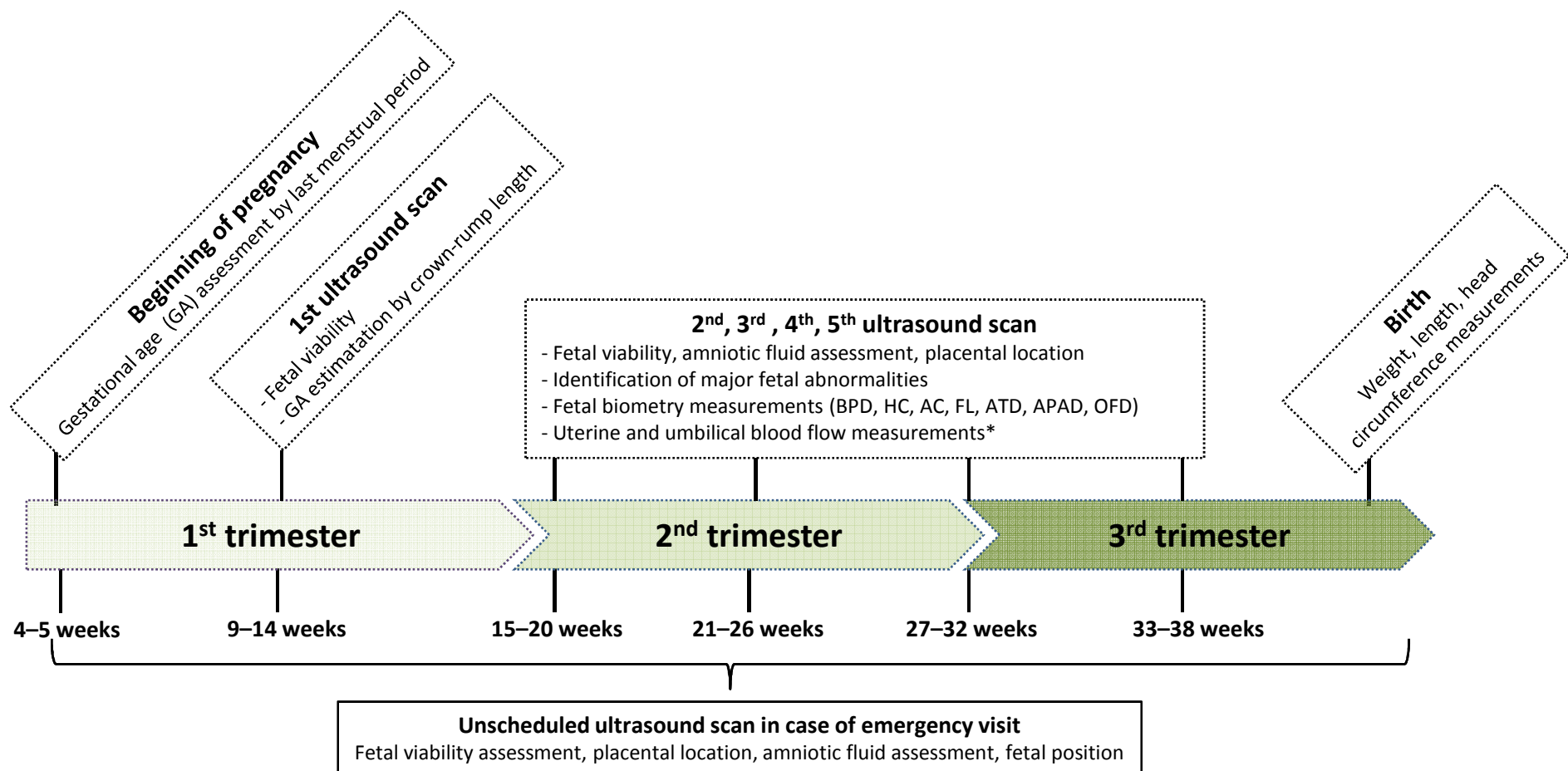
✋ Pregnancy cohort attrition

In the final cohort, 32.1% (132/411) of pregnant women did not complete the follow-up, mainly because of spontaneous abortion (51.5%, 68/132) and informed consent withdrawal (34.1%, 45/132). Reasons for consent withdrawal have been given above. There are few data available on spontaneous abortions, particularly in SSA countries, where they are neither detected nor reported. In RECIPAL, the prevalence of spontaneous abortions was unexpectedly high (16.5%, 68/411), with more than half (50.7%, 34/68) of cases occurring before 6 weeks of gestation.

References:

1   Abudu EK, Inyang-Etoh EC, Eziagu UB. Pregnant women perception of placenta donation for biomedical research- experience at a Nigerian Tertiary Health Care Institution.*Savannah J Med Res Pract* 2015;**4**:8–14.

2   Bazuaye G, Enabudoso E. Willingness of Pregnant Women in Benin City Nigeria to Donate Placenta Cord Blood for Stem Cell Transplantation. *Ann Biomed Sci* 2011;**10**.

3   Herlihy JM, Shaikh A, Mazimba A, *et al*. Local Perceptions, Cultural Beliefs and Practices That Shape Umbilical Cord Care: A Qualitative Study in Southern Province, Zambia. *PLoS ONE* 2013;**8**.

Beginning of pregnancy
Gestational age (GA) assessment by last menstrual period

1st ultrasound scan
- Fetal viability
- GA estimation by crown-rump length

2nd, 3rd, 4th, 5th ultrasound scan
- Fetal viability, amniotic fluid assessment, placental location
- Identification of major fetal abnormalities
- Fetal biometry measurements (BPD, HC, AC, FL, ATD, APAD, OFD)
- Uterine and umbilical blood flow measurements*

Birth
Weight, length, head circumference measurements

| 1st trimester | 2nd trimester | 3rd trimester |

4–5 weeks   9–14 weeks   15–20 weeks   21–26 weeks   27–32 weeks   33–38 weeks

Unscheduled ultrasound scan in case of emergency visit
Fetal viability assessment, placental location, amniotic fluid assessment, fetal position

Supplemental file S5. Ultrasound follow-up (RECIPAL study)

HC: Head circumference; AC: Abdominal circumference; FL: Femur length; BPD: Biparietal diameter; OFD: occipito frontal diameter;
ATD: Abdominal transverse diameter; APAD: Antero-posterior abdominal diameter.
* Uterine, and umbilical blood flows were measured at 21-25 wg and from 28 wg, respectively

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (page 2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 6)
Objectives	3	State specific objectives, including any pre-specified hypotheses (page 6)
Methods		
Study design	4	Present key elements of study design early in the paper (page 7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 7, 8, 9, 10, 11)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 8)
		Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls (Not applicable)
		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants (Not applicable)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed (Not applicable)
		Case-control study—For matched studies, give matching criteria and the number of controls per case (Not applicable)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (page 12, 13)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 12, 13)
Bias	9	Describe any efforts to address potential sources of bias (page 15)
Study size	10	Explain how the study size was arrived at (page 7, 8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Not applicable for cohort profiles publication)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Not applicable for cohort profiles publication)
		(*b*) Describe any methods used to examine subgroups and interactions (Not applicable for cohort profiles publication)
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed (page 10 and 14)
		Case-control study—If applicable, explain how matching of cases and controls was addressed (Not applicable)
		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy (Not applicable)

(*e*) Describe any sensitivity analyses (Not applicable)

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (page 10)
		(b) Give reasons for non-participation at each stage (page 10)
		(c) Consider use of a flow diagram (Figure 2)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Table 4)
		(b) Indicate number of participants with missing data for each variable of interest (page 10)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (Table 1 and 2)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time (page 14)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure (Not applicable)
		*Cross-sectional study—*Report numbers of outcome events or summary measures (Not applicable)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (page 14, 15)
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion (Not applicable for cohort profiles publication)		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (page 24)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.