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Rilpivirine plasma and cervico-vaginal concentrations in women during pregnancy and postpartum

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Abstract

Background—Concentrations of antiretrovirals (ARVs) in the genital tract play a key role in pre-exposure prophylaxis. This study aims to describe rilpivirine (Edurant®) concentrations in the genital tract in pregnant and postpartum women.

Methods—International Maternal Pediatric Adolescent AIDS Clinical Trials Protocol P1026s is an ongoing, prospective study of antiretroviral pharmacokinetics (PK) in HIV infected pregnant women that include a cohort receiving rilpivirine combination regimen. Intensive PK evaluations were performed at steady state during the second and third trimester, and postpartum. Plasma and

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directly-aspirated cervicovaginal fluid (CVF) samples were collected at 4 timepoints around an observed dose, and measured using high-performance liquid chromatography with ultraviolet detection, (plasma; lower limit of quantification (LLQ) = 10ng/mL) or liquid chromatography-tandem mass spectrometry (CVF; LLQ = 1ng/mL).

Results—A total of 24 women were included in the analysis. For all time points combined, median (Interquartile range, IQR) rilpivirine concentrations were 70ng/ml (23–121) in CVF and 92 ng/ml (49–147) in plasma. The CVF to plasma $AUC_{(0-4)}$ ratios were significantly higher in the 2^{nd} (0.90, 90% CI 0.61–1.46) and 3^{rd} trimesters of pregnancy compared to postpartum (0.40, 90% CI 0.19–0.87). Three of 189 (1.6%) plasma samples in two women were below the LLQ as well as the corresponding CVF concentrations. Seventeen additional CVF concentrations (10.6%) were below LLQ in 13 participants. No major safety concerns were noted.

Conclusions—Rilpivirine concentrations were higher in the CVF during pregnancy compared to postpartum. CVF Rilpivirine is likely to achieve inhibitory concentrations effective for preventing peripartum HIV transmission.

Keywords

Rilpivirine; pregnancy; cervicovaginal fluid; post-partum

INTRODUCTION:[1]

Many ARVs have been shown to reduce mother to child transmission of HIV.[1,2] Genital tract concentrations of ARVs may play a key role in preventing perinatal HIV transmission by reducing viral replication in this compartment. [3] This is critical because several studies have demonstrated that HIV viral load in the female genital tract is independently associated with the risk of mother-to-child HIV transmission [1,2,4–6]. While there has been considerable research describing ARV concentrations in the genital tracts of men and non-pregnant women, studies in pregnant women have been limited. [7,8]. Although pharmacokinetic analyses of mucosal tissue drug concentrations typically involve invasive biopsies, these techniques limit the number of samples that can safely be obtained from pregnant women, increases cost and difficulty associated with sample collection and makes storage and processing for drug quantification difficult. Therefore, recent studies use CVF as surrogates to cervicovaginal tissue biopsy [9].

The physiological changes during pregnancy impact the pharmacokinetics of most ARVs, and some ARV's may require dose adjustment during pregnancy in order to maintain optimal pharmacokinetic exposure [8]. The extent of penetration through the genital tract in non-pregnant women has been previously shown to be constant regardless of the number of doses given, reflecting a constant relationship between systemic and genital drug exposure [10] In the only published study reporting female genital tract ARV concentrations during pregnancy, genital tract/plasma ratios for zidovudine and lopinavir were significantly lower than those in non-pregnant women, suggesting that genital tract drug concentrations from non-pregnant women cannot be extrapolated to pregnant women.[8,10]

Pregnancy PK data have been described for some of the newer antiretroviral agents. Currently available data suggest that with standard adult dosing, plasma concentrations of some ARVs (especially protease inhibitors) are reduced during the second and/or third trimesters [6,11,12]. Rilpivirine is the newest of five non-nucleoside reverse transcriptase inhibitors (NNRTIs) approved by the Food and Drug Administration [13]. Rilpivirine is recognized for its ability to inhibit HIV-1 replication, adaptability to reverse transcriptase (RT) mutations, high oral bioavailability and long half-life, which allows for 25mg once-daily oral dosing in antiretroviral naïve adults with HIV-1 RNA copies less than 100,000 copies/mL [14,15]. In a PK study of rilpivirine in pregnant women, area under the curve (AUC) during the second and third trimester were reduced by 20–33% compared to postpartum [16,17]. However, genital tract concentrations of rilpivirine have not been previously studied or described in pregnant women. The primary objective of this study was to investigate the concentrations of rilpivirine in the female genital tract and to compare the concentrations between pregnancy and postpartum.

METHODS

Data were collected as part of International Maternal Pediatric Adolescent AIDS Clinical Trials Protocol P1026s, an ongoing, multicenter, non-blinded, prospective Phase IV study of the pharmacokinetics and safety of selected ARVs in HIV infected pregnant women that included an arm for pregnant women at US sites receiving rilpivirine.[17] The study is registered in ClinicalTrials.gov [NCT00042289]. For eligibility, HIV-infected women (≥ 20 weeks gestation until 12 weeks postpartum), not on tuberculosis treatment, and receiving rilpivirine were included in the rilpivirine arm. Local institutional review boards approved P1026s at all participating sites, and the study followed all relevant human subject research guidelines. All participants provided signed informed consent before participation. HIV-infected pregnant women receiving rilpivirine 25 mg orally once daily as part of clinical care before the beginning of the 35th week of pregnancy and expected to continue on treatment until at least six weeks postpartum were eligible to enroll in the rilpivirine arm of P1026s. All antiretroviral medications were prescribed by primary care providers and dispensed by local pharmacies, as per the sites' standard of care. Maternal exclusion criteria were current use of medications known to interfere with rilpivirine metabolism, including dexamethasone, omeprazole, and phenytoin, multiple gestation, or clinical or laboratory toxicity that, per site investigator, would require a change in the antiretroviral regimen. Mothers and their infants continued in the study until 6 months after delivery. Infant HIV status was evaluated at 24 weeks of life by physical examination and chart abstraction.

Clinical and laboratory monitoring

Maternal demographic and clinical information were extracted from the medical record, including maternal HIV-1 RNA, CD4+ lymphocyte count, maternal age, ethnicity, weight and concomitant medications. Background regimen were similar for all women throughout the evaluation period. Plasma HIV-1 RNA assays were performed locally. Study mothers and infants were followed for clinical and laboratory toxicities through six months after delivery. Neonatal gestational age at the time of delivery, birth weight and HIV infection status data were collected from the infant's medical record. Physical examinations were performed on neonates after delivery, and infant laboratory evaluations were performed only as clinically indicated.

Sample collection and drug assays

Cervicovaginal fluid and plasma samples were collected pre-dose and at 1, 2, and 4 hours post-dose during second trimester (20–26 weeks of gestation), third trimester (30–38 weeks of gestation), and postpartum (6–12 weeks after delivery) visits. Cervicovaginal fluid samples were collected by direct aspiration and blood samples by venipuncture. On the day of sampling, rilpivirine was given as an observed dose with a meal consisting of at least 500 calories.

Cervicovaginal secretions were collected directly using a soft plastic aspirator (UNC Center for AIDS Research Vaginal Specimen Aspirator; CarTika Medical, Inc) or by other methods, such as a swipe with a gloved finger, for women who had difficulty using the aspirator. Cervicovaginal fluid samples were collected by the participant or by the clinician. Aspirates were placed into 2mL pre-weighed vials that were then reweighed at the sites. For the rilpivirine samples, mean sample weight was 0.13 grams, with range of 0.01 to 0.93 grams. The samples were stored at −70°C or colder. Rilpivirine concentrations in cervicovaginal fluid were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the University of North Carolina Center for AIDS Research's Clinical Pharmacology and Analytical Chemistry Core with a lower limit of quantitation of 1 ng/mL. The CVF assay utilized stable isotopically-labeled rilpivirine-d_6 as an internal standard and was linear over the concentration range of 1–500ng/mL. Rilpivirine in CVF was analyzed in three runs with an average correlation coefficient of 0.9976. Quality control samples over the three runs demonstrated between-assay precision (%CV) from 3.87% to 6.58% coefficient of variation (CV), and accuracy ranging from −2.13% to 11.50% deviation, All calibration standards and quality control samples were within 15% of their nominal value.

Plasma rilpivirine concentrations were measured by high-performance liquid chromatography with ultraviolet detection at the University of California, San Diego Pediatric Pharmacology Laboratory. Mean recovery of drug from plasma was 99.1 %. The plasma method was linear over the concentration range of 10–2560 ng/mL, with a lower limit of quantitation of 10 ng/mL. Linearity was evaluated over three days, and had an average correlation coefficient of 0.9992 from 3 curves. For all validation samples, the between-assay precision and accuracy ranged from 4.06% to 9.04% coefficient of variation (CV), and −9.39% to 7.62% deviation, respectively. Rilpivirine was stable in plasma stored at −70°C for 2 years.

Pharmacokinetic and statistical analysis

Cervicovaginal fluid and plasma rilpivirine concentrations were analyzed using standard descriptive statistics and are presented as medians with interquartile range. Areas under the concentration time curve (AUC) for cervicovaginal fluid and plasma from pre-dose concentration (C_0) to 4 hours post dose (AUC_{0-4}) was estimated using the trapezoidal rule. The ratio of cervicovaginal fluid AUC to plasma AUC at each study visit were determined. Within-participant comparisons (e.g., between second versus third trimester) was performed for continuous outcome measures using the Wilcoxon signed-rank test and for dichotomous outcome measures using McNemar's test. Between-participant comparisons was performed for continuous outcome measures using the Wilcoxon rank-sum test and for dichotomous outcome measures using the chi-square or Fisher exact test. 90% confidence limits for the geometric mean ratio of the PK exposure parameters were calculated to describe the range of values that are consistent with the observed data to assess whether there was a clinically significant difference in exposure. The 90% rather than 95% confidence interval was used to match the usual practice in the pharmacokinetic literature. Pairwise comparisons of cervicovaginal AUC, plasma AUC and their ratio within each subject during the second trimester and third trimester compared to postpartum were performed using a two-sided Wilcoxon signed rank test with $p < 0.01$ considered statistically significant.

RESULTS

The study enrolled 24 women with cervicovaginal fluid and plasma concentration data available for ten women in the second trimester, seventeen in the third trimester and nineteen postpartum. Maternal demographic and clinical characteristics of the participants and the pregnancy outcomes are described in Table 1. Twelve of 24 (50.0%) of the mothers were black, eleven of the mothers were Hispanic (46%), and one woman was white (non-Hispanic), with a median age of 27 years (interquartile range [IQR], 17 to 38). No congenital anomalies were identified by prenatal ultrasound or physical examination at the time of birth. The mean gestational age at the time of sampling in the 2^{nd} trimester was 24 weeks (interquartile range [IQR], 22 to 26 weeks). The mean gestational age at the time of sampling in the 3^{nd} trimester was 34 weeks (interquartile range [IQR], 29 to 35 weeks), and median postpartum sampling time was 9 weeks after delivery (interquartile range [IQR], 6 to 12 weeks postpartum).

Maternal plasma HIV-1 RNA was noted to be less than or equal to 50 copies (≤ 50 copies/mL) in 7 of 10 (70%) participants during the second trimester, 13 of 17 (82%) participants during the third trimester and 13 of 18 (72%) participants postpartum. All infants in the cohort were uninfected. Three of 189 (1.6%) plasma samples in two women were below the quantitative limit for rilpivirine; the corresponding CVF concentrations were also below quantitation for rilpivirine. Seventeen additional CVF concentrations out of 189 (10.6%) were below quantitation in 13 women. When all time points were combined, median (IQR) rilpivirine concentrations were 70 ng/mL (23 – 121) in cervicovaginal fluids and 92 ng/mL (49–147) in plasma. Median cervicovaginal fluid and plasma rilpivirine concentrations for each study visit are shown in Figure 1.

Median (IQR) rilpivirine cervicovaginal fluid and plasma AUC_{0-4} and their ratio for the second trimester, third trimester and postpartum visits are presented in Table 2. Median $AUC_{(0-4)}$ of rilpivirine in cervicovaginal fluid ranged from 419 ng*hr/mL in the second trimester of pregnancy to 217 ng*hr/mL postpartum. Plasma $AUC_{(0-4)}$ of rilpivirine ranged from 409 ng*hr/mL in the second trimester of pregnancy to 327 ng*hr/mL in third trimester and then 410 ng*hr/mL in postpartum. The cervicovaginal fluid to plasma AUC_{0-4} ratios were significantly higher in the 2nd and 3rd trimesters of pregnancy compared to postpartum due to differential increase in rilpivirine concentrations in cervicovaginal fluid compared to plasma (Figure 1, Table 2) ($p<0.05$ for second trimester vs postpartum, and $p=0.04$ for third trimester versus postpartum. Cervicovaginal and plasma AUC_{0-4} were moderately correlated antepartum and weakly correlated postpartum (Figure 2).

DISCUSSION

This study included pregnant and postpartum women who are HIV infected and being treated with combination regimen that included a 25 mg rilpivirine tablet (Edurant®). For all time points combined, median (IQR) rilpivirine concentrations were higher in plasma (70ng/mL in cervicovaginal fluid and 92 ng/ml in plasma). Although rilpivirine plasma AUC concentrations decreased by 19.9% between the 2nd and 3rd trimesters of pregnancy, the cervicovaginal to plasma $AUC_{(0-4)}$ ratios of rilpivirine were significantly higher in the 2nd and 3rd trimesters of pregnancy compared to postpartum due to increased rilpivirine concentrations in cervicovaginal fluid. A prior study in non-pregnant women describing the pharmacokinetics of rilpivirine in CVF of women taking daily rilpivirine showed that rilpivirine concentrations in the CVF were sufficient for inhibiting HIV infection in this compartment.[18] A similar effect of pregnancy on rilpivirine exposure was seen in analysis of the full pharmacokinetic profiles from the P1026s rilpivirine arm [17]. The decrease in rilpivirine plasma AUC in our study between the 2nd and 3rd trimesters of pregnancy was less than 20%, and was not associated with virological failure or with mother to child transmission of HIV.

Quantifying drug concentrations in body compartments with which the neonate has exposure during pregnancy and the postpartum period (genital tract, cord blood plasma, and amniotic fluid) may aid in selecting drug regimens, and may have implications for mother to child transmission of HIV, as well as for pre-exposure prophylaxis. Findings from this study show that the cervicovaginal to plasma AUC_{0-4} ratios were statistically significantly higher in the 2nd and 3rd trimesters of pregnancy compared to postpartum (0.90, 0.74 and 0.40 respectively). However, in a pharmacokinetic compartmental analysis of genital tract, umbilical cord blood and amniotic fluid exposures study of seven older antiretroviral drugs (lamivudine, zidovudine, tenofovir, nelfinavir, lopinavir-ritonavir, nevirapine) during pregnancy and postpartum in HIV Type 1-infected women, no statistically significant differences in genital tract penetration were observed for any of the ARVs between the second and third trimesters, with only nelfinavir genital tract penetration being significantly higher postpartum compared to the second trimester and third trimester respectively [8]. Tissue- specific pharmacokinetics may help explain these findings.

Tissue-specific pharmacokinetics of drugs for HIV prevention may help us distinguish populations that are more likely to benefit from their use [19]. For example, in a 2009 pharmacokinetic study, maraviroc cervicovaginal fluid concentrations 72 hours after dose and plasma concentrations 12 hours after dose were similar [20]. In our study, in addition to the cervicovaginal to plasma AUC_{0-4} ratios being statistically significantly higher in the 2nd and 3rd trimesters of pregnancy compared to postpartum, the median CVF rilpivirine concentration was noted to be more than 100 fold above the protein-free EC_{90} for rilpivirine (0.66 ng/mL). Protein binding needs to be taken into account, as the free rilpivirine concentration in cervicovaginal fluid needs to exceed the EC90, not just the EC50, to develop full antiviral effect [21]. It is plausible that these finding might not be a true reflection of rilpivirine concentrations in the genital tract because binding proteins are altered in the female genital tract compared to plasma during pregnancy [22]. Hence, protein free EC_{90} may be more reflective of inhibitory concentrations of rilpivirine (as rilpivirine is 99.7% bound to plasma proteins)[23]. Accumulation of rilpivirine in cervicovaginal fluid may also be driven by physicochemical properties, in particular by lipophilicity [23]. Since rilpivirine is highly lipophilic (pKa of 5.6, log P = 4.86) and has high accumulation levels in multiple cell types, it shows high cellular penetration [13].

Differential drug metabolism and drug transport in the female genital tract relative to plasma can also be a possible explanation for the difference in rilpivirine concentrations in cervicovaginal fluid versus plasma. Recent studies of mRNA expression of CYP enzymes in cervical tissues demonstrated that cytochrome P450 CYP activity present in cervical and vaginal tissue are markedly different from those expressed in the liver [24]. Rilpivirine is primarily metabolized by cytochrome P450 (CYP)3A, and drugs that induce or inhibit CYP3A may thus affect the clearance of rilpivirine. Co-administration of rilpivirine and drugs that induce CYP3A may result in decreased plasma concentrations of rilpivirine and loss of virologic response and possible resistance to rilpivirine. Co-administration of rilpivirine and drugs that inhibit CYP3A may result in increased plasma concentrations of rilpivirine.

Our study has several strengths. This is the first study to report the cervicovaginal concentrations of rilpivirine in pregnancy. The participants in our study were followed longitudinally over time, and the collection of clinical findings related to rilpivirine exposure occurred at regular time intervals, so recall error or bias, systematic bias and confounding by genetic, sociodemographic and other individual characteristics were minimized. Any random measurement error that arises from the study would tend to diminish apparent effect size, causing estimates to be conservative. There was a high rate of follow up for mothers and neonates. The collection of samples followed a strict protocol with observed dosing to minimize errors due to sample collection.

This study had its limitations. First, the population studied within this network is mainly black or Hispanic, with only a limited number of non-Hispanic white patients included, so that limitations of generalizability may exist. Second, although methods for collecting samples were standardized, there may be still be limitations associated with some technical aspects of collecting cervicovaginal specimens from patients due to human related factors. For example, differences in collecting cervicovaginal samples with or without a vaginal speculum; swiping with a gloved finger versus using an aspirator for women who had problems with the aspirator. Third, there is still limited information available regarding protein binding in cervicovaginal fluid, which may limit interpretation of such data in the context of related plasma concentration data, although it is possible to make some educated guesses based on what is known about the concentrations of albumin and alpha1-acid glycoprotein (*AAG*) in cervicovaginal fluid and what proteins rilpivirine binds to. In addition, we did not measure unbound concentrations, thus limiting our ability to assess pharmacologically active rilpivirine. Fourth, we did not study the effects of genital inflammation on rilpivirine levels. Vaginal microbiome has been shown to affect the levels of ARVs when used topically, and bacterial vaginosis is common during pregnancy and postpartum. It is possible that genital inflammation in these women might have contributed to lower levels of rilpivirine in the CVF.[25] Fifth, we did not assess the effect of co-administration of rilpivirine with other medications in pregnancy, especially antacids (women on antacids were excluded from the study). In prior pharmacokinetic studies, rilpivirine AUC concentration decreased by 76% with famotidine 40 mg, and by 40% with omeprazole (proton pump inhibitors cannot be coadministered with rilpivirine and H_2 antagonists can be used only if taken more than 12 h before or 4 h after rilpivirine).[26,27] Sixth, a major drawback of our study includes a small sample size.

In conclusion, our findings confirm that concentrations of rilpivirine in the genital tract correlate with plasma concentrations, with higher concentrations in the cervicovaginal fluid during pregnancy than during the postpartum period. This could be pertinent not only to viral suppression during pregnancy and delivery and prevention of mother to child transmission of HIV (PMTCT), but, could also be an important new prevention drug for uninfected pregnant women for pre-exposure prophylaxis.

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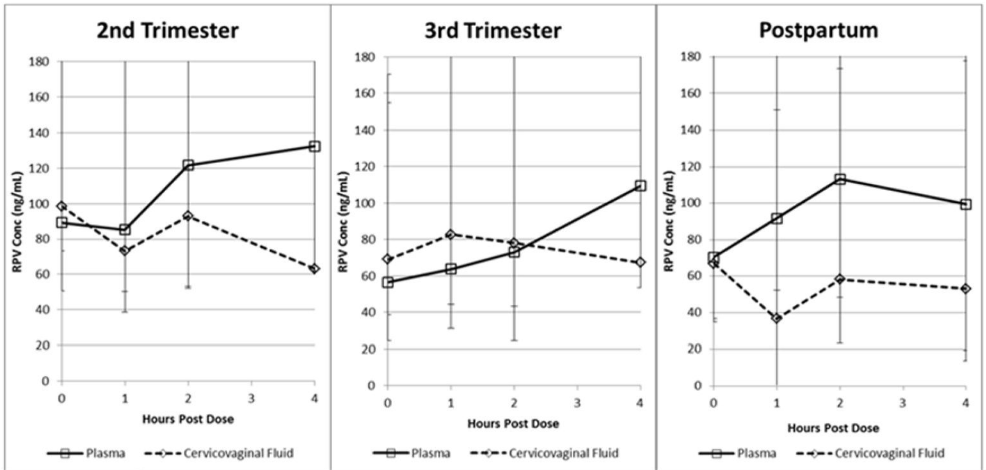

	2nd Trimester (n=10)	3rd Trimester (n=17)	Post-partum (n=19)
CVF AUC (ng*hr/mL)	418.8 (176.1-78.0)	325.3 (185.1-491.0)	216.5 (60.8-456.4)
Plasma (ng*hr/mL)	409.0 (263.4-626.8)	327.1 (184.6-645.5)	410.1 (238.4-737.8)
CVF:Plasma Ratio	0.90 (0.61-1.46)§	0.74 (0.49-1.32)¶	0.40 (0.19-0.87)

Figure 1. Rilpivirine Curves (2nd trimester, 3rd trimester, postpartum)

Figure 1 shows the rilpivirine curves, including the cervicovaginal fluid AUC, plasma concentration and CVF/plasma ratios across the 2nd trimester, 3rd trimester and postpartum. 2nd trimester vs postpartum, p=0.02; 3rd trimester vs postpartum, p=0.06

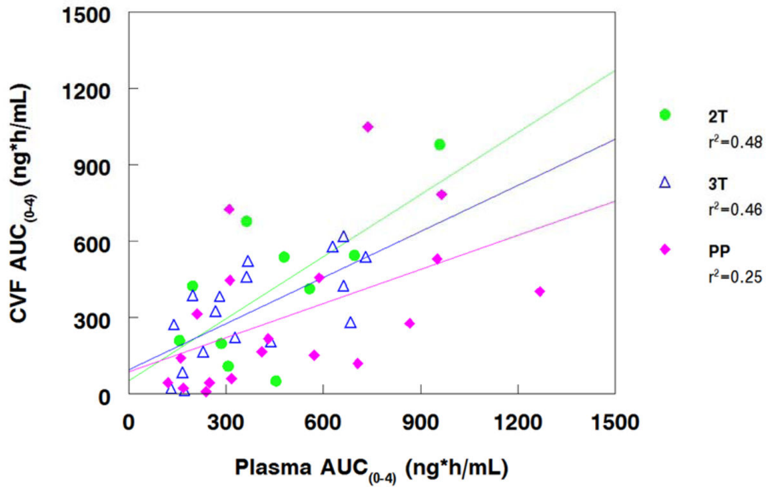

Figure 2. CVF and Plasma AUC Correlations

Figure 2 shows the cervicovaginal fluid and plasma AUC correlations

Table 1

Participant demographics – Table 1 describes the demographics of participants that were recruited into the rilpivirine pharmacokinetic study.

Age at delivery (years)	26.8 (17.2–37.6)
Weight at delivery (kg)	92 (60.9–131.8)
Race/Ethnicity	
White	1 (4%)
Black	12 (50%)
Hispanic	11 (46%)
2nd Trimester	
Gestational age	23.6 (21.7 – 26.7)
HIV-1 RNA (≤ 50 copies/mL)	7/10 (70%)
CD4+ cells (cells/mm^3)	565 (293–828)
3rd Trimester	
Gestational age	33.6 (29.3–35.0)
HIV-1 RNA (≤ 50 copies/mL)	14/17 (82%)
CD4+ cells (cells/mm^3)	554 (297–1147)
Postpartum	
Weeks after delivery	9.3 (6.1–12.4)
HIV-1 RNA (≤ 50 copies/mL)	13/18 (72%)
CD4+ cells (cells/mm^3)	693 (185–1180)
Pregnancy outcomes	
Gestational age (weeks)	38.9 (32.3–41.4)
Birth weight (grams)	3075 (1570–4570)
Infection status	
Uninfected by best available data	24/24 (100%)

Interquartile ranges (IQR) are in brackets. 90% confidence intervals (CIs) were used for analysis.

Table 2

Rilpivirine cervicovaginal fluid (CVF) and plasma pharmacokinetics – Table 2 describes the cervicovaginal fluid parameters in the 2nd and 3rd trimesters, including postpartum. Area under the curves (AUCs) were described across all the 2 trimesters and postpartum.

Parameter median (IQR)	2nd trimester	3rd trimester	Postpartum
CVF AUC (ng*hr/mL)	419 (176–578)	325 (185–491)	217 (61–456)
Plasma AUC (ng*hr/mL)	409 (263–627)	327 (185–646)	410 (238–738)
CVF:Plasma AUC Ratio	0.90 (0.61–1.46)*	0.74 (0.49–1.32)**	0.40 (0.19–0.87)

AUC – Area under the curve

*2nd trimester versus postpartum (p=0.02)

**3rd trimester versus postpartum (p=0.04).