

Periconceptional antibiotic use and spontaneous abortion: A prospective cohort study

Holly Michelle Crowe¹  | Elizabeth Elliott Hatch¹ | Tanran R. Wang¹ | Charles Robert Horsburgh¹ | Ellen Margrethe Mikkelsen^{2,3} | Wendy Kuohung⁴ | Lauren Anne Wise¹  | Amelia Kent Wesselink¹ 

¹Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, USA

²Department of Clinical Epidemiology, Aarhus University and Aarhus University Hospital, Aarhus N, Denmark

³Department of Clinical Medicine, Aarhus University and Aarhus University Hospital, Aarhus N, Denmark

⁴Department of Obstetrics and Gynecology, Boston University School of Medicine, Boston, Massachusetts, USA

Correspondence

Holly Michelle Crowe, Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA.

Email: hcrowe@hsph.harvard.edu

Funding information

Eunice Kennedy Shriver National Institute of Child Health and Human Development

Abstract

Background: Many reproductive-aged North Americans use antibiotics in the weeks preceding conception or during early pregnancy. Antibiotic use may influence risk of spontaneous abortion (SAB) by disrupting the reproductive tract microbiome or treating harmful infections. However, this association has not been extensively studied.

Objective: To determine the extent to which periconceptional antibiotic use is associated with the risk of SAB.

Methods: We analysed data from an internet-based preconception cohort study of pregnancy planners. Eligible participants self-identified as female, were aged 21–45 years, resided in the USA or Canada, and conceived during 12 months of follow-up ($n = 7890$). Participants completed an enrolment questionnaire during June 2013–September 2021 and bimonthly follow-up questionnaires for up to 12 months or until a reported pregnancy, whichever came first. Pregnant participants completed questionnaires in early (~8–9 weeks) and late (~32 weeks) gestation. We assessed antibiotic use, including type (penicillins, nitrofurantoin, cephalosporins and macrolides) and indication for use, during the previous 4 weeks on preconception questionnaires. Participants reported pregnancies and SAB on follow-up and pregnancy questionnaires. We used Cox proportional hazards regression models with gestational weeks as the time scale to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between periconceptional antibiotic use and SAB, controlling for potential demographic, medical, and lifestyle confounders.

Results: Nineteen percent ($n = 1537$) of pregnancies ended in SAB. Participants reported periconceptional antibiotic use in 8% of pregnancies ending in SAB and 7% not ending in SAB. Periconceptional antibiotic use was not appreciably associated with SAB (adjusted HR 1.06, 95% CI 0.88, 1.28). We observed no strong associations between antibiotic type, indication for use, or recency of exposure and SAB risk.

Conclusions: Periconceptional antibiotic use was not appreciably associated with SAB in this study. This association is likely complicated by antibiotic type and dosage, timing of conception, and the individual's overall health.

KEY WORDS

antibiotics, infection, miscarriage, penicillin, spontaneous abortion

1 | BACKGROUND

Antibiotic use is common among reproductive-aged North Americans. Approximately 40%–45% of pregnant individuals are prescribed antibiotics in the 6 months before conception.^{1,2} Although the majority of antibiotics are considered safe for use during pregnancy, certain classes of macrolides (especially clarithromycin), sulphonamides, quinolones and tetracyclines have been associated with adverse pregnancy outcomes, including congenital malformations and spontaneous abortions (SAB).^{3–6}

Spontaneous abortions is defined as pregnancy loss occurring before 20 completed weeks of gestation, and occurs in an estimated 20% of recognised pregnancies.^{7,8} SAB is a significant physical and psychological stressor and has been associated with future obstetric complications, cardiovascular disease, anxiety, depression, and post-traumatic stress.⁹ Given the widespread use of antibiotics among reproductive-age individuals, the unobservable nature of the exact timing of conception, and the high population burden of SAB, quantifying the association between periconceptional antibiotic use and SAB is of great public health importance.

Antibiotic use overall may be associated with disruption of the reproductive tract microbiome, which may be a risk factor for SAB.^{10–12} The biologic mechanisms through which specific antibiotics may be related to SAB vary by antibiotic type and are not well explicated. Although the half-life of antibiotics is generally hours to days, short-term disruptions in the periconceptional period may impact early foetal development. Clarithromycin may inhibit cardiac rhythm regulation in early embryonic development,^{3,4} quinolones may inhibit DNA replication,^{3,4,13} tetracyclines may suppress proinflammatory cytokines necessary for implantation,³ and sulphonamides may inhibit folic acid synthesis, critical for foetal development.^{3,14}

Previous studies of the association between antibiotic use and SAB have yielded inconsistent results.^{2–4,13} A 2020 systematic review and meta-analysis of 11 observational studies on the association between antibiotic use during pregnancy and SAB (7 cohort studies and 4 population-based case–control studies) found an increased risk of SAB among individuals using macrolides (RR 1.42, 95% CI 1.04, 1.93), quinolones (RR 2.48, 95% CI 1.46, 4.20), and tetracyclines (RR 2.57, 95% CI 1.95, 3.38) during pregnancy, compared with those who did not use antibiotics.³ Ten antibiotics (sulfamethizole, clarithromycin, azithromycin, ciprofloxacin, norfloxacin, levofloxacin, doxycycline, minocycline, trimethoprim, metronidazole) were associated with SAB in various included studies, while amoxicillin and nitrofurantoin were associated with a decreased risk of SAB.³ Most studies focused on antibiotic use in the first trimester of pregnancy and ascertained antibiotic use from hospital registries, prescription registries, or teratology information services.

Despite several large registry studies investigating the association between antibiotic use and SAB, confounding by indication, reverse causation, and inconsistent exposure windows continue to present challenges to ascertaining the true association.^{4,15–17} Registry studies have little to no information on relevant confounding lifestyle

Synopsis

Study question

To what extent is periconceptional antibiotic use associated with spontaneous abortion?

What's already known

Use of clarithromycin, sulphonamides, quinolones, and tetracycline antibiotics early in pregnancy has been associated with an increased risk of spontaneous abortion in some studies.

What this study adds

This study examines antibiotic use in the periconceptional period overall and by type (penicillins, nitrofurantoin, cephalosporins and macrolides), indication for use, and recency of exposure. This study adjusts for confounding by medical, reproductive, and lifestyle factors and captures spontaneous abortions occurring early in gestation. In this study, periconceptional antibiotic use was not appreciably associated with spontaneous abortion.

factors. In addition, registries are unable to identify early SABs that do not require medical care; >50% of all SABs occur before 8 weeks of gestation and are unlikely to be registered.^{8,18}

Antibiotic use during the preconception period has not been extensively studied but is highly relevant to pregnancy planners and their clinicians. Because the precise timing of conception is unknown, there is little practical difference between preconception exposures occurring close to conception and very early pregnancy exposures. In the present study, we analysed data from a preconception cohort study to examine the association between periconceptional antibiotic use and risk of SAB.

2 | METHODS

Pregnancy Study Online (PRESTO) is an ongoing, web-based cohort study of couples trying to conceive. Study methods have been described in detail elsewhere.¹⁹ Eligible participants self-identify as female, are aged 21–45 years, reside in the United States or Canada, and are not using contraception or fertility treatment. Participants complete a baseline questionnaire and follow-up questionnaires every 8 weeks for 12 months or until pregnancy, whichever comes first. On baseline and follow-up questionnaires, we ascertain data on demographics, lifestyle factors, and reproductive and medical history. Participants who report conception are invited to complete two additional questionnaires in early (~8–9 gestational weeks) and late pregnancy (~32 gestational weeks).

From June 2013 to September 2021, 15,319 eligible female participants enrolled in PRESTO and completed the baseline

questionnaire. Of these, 3910 (25%) were lost to follow-up before reporting a conception, and 3519 (23%) did not conceive during the follow-up period. The final analytic sample included 7890 pregnant participants. We added questions pertaining to antibiotic type and indication to the questionnaires in March 2016; thus, we restricted the analyses of antibiotic type and indication to participants completing the baseline questionnaire after 1 March 2016 (77% of pregnant participants).

2.1 | Assessment of exposure

We asked participants at baseline and follow-up if they had taken antibiotics in the previous 4 weeks. If a participant indicated taking antibiotics, we asked them to provide the name of the antibiotic and the reason it was prescribed in open text boxes. We classified antibiotic names by active ingredient, (penicillins, nitrofurantoin, cephalosporins, macrolides, other) and classified indications for use by type of infection (respiratory, urinary tract, pelvic/vaginal, or other). Indication categories were not mutually exclusive, and some participants reported more than one indication for a given antibiotic prescription.

We assessed antibiotic use in the prior 4 weeks every 8 weeks during the preconception period using follow-up questionnaires. Many participants reported antibiotic use during some follow-up periods and non-use during others. We hypothesized that a potential association between antibiotics and SAB would likely be transient.^{20,21} Therefore, we considered antibiotic use at the most recent questionnaire before conception to be the most etiologically relevant exposure period. We estimated the date of conception as the last menstrual period (LMP) before pregnancy +14 days and assessed exposure from the most recent questionnaire completed before conception. If the participant was unknowingly pregnant at enrolment, exposure was assessed from the baseline questionnaire. We categorised the timing of exposure assessment relative to conception as: after conception, but before pregnancy detection (10% of participants); 1–4 weeks before conception (50%); 5–8 weeks before conception (25%); and >8 weeks before conception (15%). We hypothesized that 1–8 weeks before conception would be the most etiologically relevant exposure window.

2.2 | Assessment of outcome

On follow-up questionnaires, we asked participants to report the dates and outcomes of all pregnancy tests. We asked participants who reported a pregnancy during follow-up how they detected their pregnancy (home pregnancy test, urine test at doctor's appointment, etc.) and the date. We defined SAB as pregnancy loss before 20 completed weeks of gestation, ascertained through follow-up and pregnancy questionnaires. SAB included the responses "miscarriage," "chemical pregnancy," and "blighted ovum". If a participant reported a pregnancy loss, we asked them to provide the date of the loss,

which we used to calculate completed gestational weeks at loss. We calculated gestational weeks from the date of the pregnancy LMP.

We attempted to determine pregnancy outcomes among participants lost to follow-up by contacting them by email or phone, searching social media and baby registries, using data shared by participants from fertility tracking apps, and linking participant data to birth registries in States with a high proportion of PRESTO participants (California, Florida, Massachusetts, Michigan, Ohio, Pennsylvania, Texas, and New York). If we were able to contact a participant, we asked them to provide information on pregnancy status, including date of pregnancy loss, if applicable.

2.3 | Assessment of covariates

We selected potential confounders a priori based on available literature. Covariates assessed on the baseline questionnaire included: partner's age, household income, education, geographic region of residence, body mass index (BMI), parity, irregular menstrual cycles, smoking history, efforts to improve the chances of conception (e.g. charting menstrual cycles, ovulation testing), intercourse frequency, last method of contraception (hormonal, barrier, etc.), history of SAB, history of infertility (defined as trying to conceive for 12 or more months without becoming pregnant), and history of diagnosed chlamydia. Covariates assessed at the most recent follow-up prior to the date of conception include: age, physical activity (total metabolic equivalents of task [MET]-hours per week),^{22,23} prenatal vitamin or multivitamin use, current smoking, alcohol and caffeine intake, and pain medication use in the 4 weeks prior to the questionnaire.

2.4 | Statistical analysis

We used Andersen-Gill data structure with one observation per week of gestation to account for left truncation due to differential timing of pregnancy detection.²⁴ We began follow-up at the gestational week of pregnancy detection, if available (95% of SABs), or at 4 weeks of gestation (the median gestation at first pregnancy detection in the cohort), and ended at SAB, 20 weeks of gestation, or other censoring event (date of ectopic pregnancy or induced abortion, withdrawal from study). There were 11% of participants lost to follow-up after reporting a pregnancy. As their pregnancy outcome was unknown, we censored these individuals at their date of last contact.

We used life-table methods to estimate the percentage of pregnancies ending in SAB, after accounting for censoring events. We used Cox proportional hazards regression models with gestational weeks as the time scale to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between antibiotic use and SAB, controlling for potential confounders. Results were adjusted for age (years), income (<\$50,000, \$50,000–\$99,000, \$100,000–\$149,000, >\$150,000), education (high school or less, some college, college, graduate school), BMI (kg/m^2 , continuous), multivitamin use



(yes, no), ever smoker (yes, no), parity (multiparae, primiparity), irregular cycles (yes, no), doing something to improve the chances of conception (yes, no), intercourse frequency (<1, 1, 2–3, >3 times per week), history of SAB (yes, no), history of infertility (yes, no), alcohol use (drinks per week), caffeine consumption (mg/day), smoking status (never, former, current occasional, current regular smoker), pain medication use (yes, no), history of pelvic inflammatory disease (yes, no), history of chlamydia (yes, no), use of hormonal birth control as last method of contraception (yes, no), and geography (Midwestern U.S., Northeastern U.S., Southern U.S., Western U.S., Canada).

We conducted a summary-level probabilistic bias analysis²⁵ to examine the impact of non-differential misclassification of exposure on our results. We defined distributions of bias parameters using data from an external validation study on self-reported antibiotics,²⁶ using a range of sensitivities (0.27–0.92) and specificities (0.93–0.95, the lowest plausible range given the data we observed). We performed 10,000 simulations in which we randomly drew sensitivity from a trapezoidal distribution (min = 0.27, mode 1 = 0.60, mode 2 = 0.73, max = 0.92) and specificity from a triangular distribution (min = 0.93, mode = 0.94, upper = 0.95). We calculated corrected risk ratios (to approximate hazard ratios) given the parameters and reported the median corrected risk ratio as the point estimate and the 2.5th and 97.5th percentiles of the distribution as the simulation interval.

In addition to the main analysis, we stratified by early (<8 weeks) and late (8–20 weeks) gestation, as periconceptional exposures may be more likely to be associated with earlier losses.²⁷ We also stratified by timing of exposure assessment relative to conception (after conception, but before pregnancy detection; 1–4 weeks before conception; 5–8 weeks before conception; >8 weeks before conception) to determine the extent to which antibiotic use more proximal to conception was more strongly associated with SAB. For antibiotic type and indication analyses, we classified participant-provided antibiotic names and indication for use.

2.5 | Missing data

We used multiple imputation to generate values for missing baseline and follow-up data using fully conditional specification methods.²⁸ We created 20 imputed datasets and statistically combined coefficients and standard errors across datasets. The proportion of participants missing covariate information was generally low. Household income data were missing for 3% of participants, while all other covariates were missing for <1% of participants. Missingness for antibiotic use was <1%.

2.6 | Ethics approval

This study was approved by the Institutional Review Board at the Boston University Medical Campus, and online informed consent was obtained from all participants.

3 | RESULTS

Of the 7890 pregnancies analysed, we identified 1537 SABs (19%) during 95,933 weeks of gestation. Overall, 7% of participants reported antibiotic use in the past 4 weeks on the questionnaire closest to conception, including 8% of pregnancies ending in SAB and 7% of pregnancies not ending in SAB. Participants who reported use of an antibiotic tended to have lower income and educational attainment (Table 1). They were also more likely to report irregular menstrual cycles (17% vs. 13%), a hormonal last method of contraception (41% vs. 36%), a history of SAB (32% vs. 25%), and a history of infertility (12% vs. 8%) at baseline. Antibiotic users were slightly less likely to report multivitamin use (82% vs. 86%), and more likely to report current regular smoking (7% vs. 4%), and pain medication use (77% vs. 68%) on the follow-up questionnaire closest to conception.

Any antibiotic use in the past 4 weeks on the most recent follow-up questionnaire before a recognised conception was not appreciably associated with SAB (adjusted HR 1.06, 95% CI: 0.88, 1.28). The results of the quantitative bias analysis correcting for systematic error due to misclassification of antibiotic use showed a modest positive association between antibiotic use and SAB (corrected RR 1.34, 95% simulation interval: 1.11, 1.79). There was no meaningful association between antibiotic use and SAB when stratifying by early (<8 weeks; adjusted HR 1.03, 95% CI 0.82, 1.30) or later gestation (8–20 weeks; adjusted HR 1.11, 95% CI 0.80, 1.54).

There was a modest association when antibiotic use was measured 1–4 weeks prior to conception (HR 1.20, 95% CI 0.92, 1.56) or 5–8 weeks prior to conception (HR 1.22, 95% CI 0.85, 1.75), compared with those who did not use an antibiotic (Table 2). The HR for antibiotic use after conception, but before pregnancy detection was 1.46 (95% CI 0.86, 2.47), and the HR for antibiotic use >8 weeks before conception was 0.82 (95% CI 0.50, 1.35). When we excluded individuals who conceived prior to exposure assessment from the main analysis, there was little change in the overall association between antibiotic use and SAB (HR 1.03, 95% CI 0.84, 1.25).

3.1 | Antibiotic type

Penicillins were the most frequently reported antibiotic type, comprising one-third of antibiotic prescriptions, followed by nitrofurantoin (15%), cephalosporins (11%), and macrolides (9%) (Table S1). We did not find strong associations between any specific type of antibiotics and SAB risk. The adjusted HR for use of penicillins, cephalosporins, nitrofurantoin, and macrolides were 0.95 (95% CI: 0.65, 1.39), 1.37 (95% CI: 0.78, 2.42), 1.14 (95% CI: 0.66, 1.96), and 1.16 (95% CI: 0.63, 2.17), respectively. We were unable to analyse other medication types due to the small number of users.

TABLE 1 Baseline characteristics by periconceptional antibiotic use, Pregnancy Study Online, 2013–2021.

Characteristic ^a	Antibiotic use	
	Non-use	Use
Number of participants (%)	7293 (92%)	585 (7%)
Age (year, mean)	30.1	30.1
Partner's age (year, mean)	31.8	32.2
White, non-Hispanic (%)	85.9	84.6
Annual household income, USD (%)		
<\$50,000	13.8	18.7
\$50,000–\$99,000	35.5	36.9
\$100,000–\$149,000	27.7	25.0
≥\$150,000	20.3	16.6
Education (%)		
High school or less	3.0	5.1
Some college	17.0	19.8
College	34.7	34.8
Graduate school	45.3	40.3
Geographic region (%)		
Midwest	21.8	23.5
Northeast	24.3	20.7
South	22.1	25.2
West	16.0	17.6
Canada	15.8	13.4
BMI (kg/m ² , mean)	26.8	27.5
Multiparous (%)	32.9	33.9
Irregular menstrual cycles (%)	13.4	17.0
Doing something to improve chances of conceiving (%)	81.0	81.3
Intercourse frequency (%)		
<1 time per week	20.0	19.2
≥4 times per week	14.8	20.1
Hormonal last method of contraception (%)	36.0	40.5
History of spontaneous abortion (%)	25.0	31.6
History of infertility (%)	7.6	11.9
History of chlamydia (%)	7.1	8.0
Physical activity (MET-hrs/wk, mean) ^b	35.5	33.5
Multivitamin use (%) ^b	85.7	82.4
Smoking status (%) ^b		
Never	80.8	74.9
Former	12.6	14.9
Current occasional	2.7	3.2
Current regular	4.0	7.0
Alcohol intake (drinks/week, mean) ^b	3.1	2.6
Caffeine intake (mg/day, mean) ^b	121.3	119.8
Pain medication use (%) ^b	67.6	76.7
Perceived stress score (mean) ^b	15.9	16.3

Abbreviation: USD, United States Dollar.

Note: Conception defined as 14 days after date of last menstrual period.

^aAll characteristics except for age are standardised to the age distribution of cohort at baseline.

^bAssessed at follow-up closest to conception.

3.2 | Antibiotic indication

The most common indication for antibiotic use was respiratory infections (25%), followed by urinary tract infections (24%), and pelvic or vaginal infections (13%). The remaining 38% of participants reported other indications such as surgery, dental infection, and preventive antibiotics, and other infections which could not be classified into one of the above categories (Table S2). There were enough antibiotic users to reasonably estimate the association between use of an antibiotic for respiratory, urinary tract, or pelvic/vaginal infections and SAB. The HRs for use of an antibiotic for each of these infections, compared with no antibiotic use were very similar (Table 3).

4 | COMMENT

4.1 | Principal findings

In this North American prospective cohort study, we found that antibiotic use by females in the periconceptional period was not appreciably associated with risk of SAB. These findings were consistent when examining SAB in earlier (<8 weeks) and later (8–20 weeks) gestation. There was also no clear relationship between the timing of exposure assessment and the association between antibiotic use and SAB.

4.2 | Strengths of the study

This prospective study of pregnancy planners addresses existing gaps in the literature by capturing robust data on potential confounding factors, and identifying a greater share of SABs, including those occurring early in gestation which do not result in medical treatment. In addition, the present study is less likely to be vulnerable to reverse causation, as most participants reported antibiotic use in the preconception period.

4.3 | Limitations of the data

Although antibiotics are commonly prescribed in the periconceptional period, the diversity of antibiotic types and indications led to few individuals in subcategories of antibiotic users, which precluded analysis of type and indication jointly and limited the precision of our findings.

Additionally, we used self-reported exposure information for our analyses. Misclassification of exposure is possible if participants misreport antibiotic use. Previous literature on the validity of antibiotic self-report is limited to a study of veterans. The validation study found low sensitivity and high specificity when comparing self-report of antibiotic use to a Veteran's Administration national pharmacy database.²⁶ While the PRESTO and veterans study populations may not be comparable with regard to medication recall, there is



	No. of SABs	No. of pregnancies	Unadjusted HR (95% CI)	Adjusted HR ^c (95% CI)
Never use of antibiotics	1223	6848	1.00 (Reference)	1.00 (Reference)
Antibiotic use				
1–4 weeks before conception	60	276	1.25 (0.95, 1.60)	1.20 (0.92, 1.56)
5–8 weeks before conception	32	148	1.24 (0.87, 1.77)	1.22 (0.85, 1.74)
>8 weeks before conception	17	112	0.81 (0.49, 1.32)	0.82 (0.50, 1.35)
Up to 5 weeks after conception ^b	15	58	1.41 (0.83, 2.38)	1.46 (0.86–2.47)

^aAssessed on the questionnaire closest to conception.

^bParticipant was pregnant, but unaware at study enrolment.

^cAdjusted for age, income, education, BMI, multivitamin use, parity, irregular menstrual cycles, doing something to improve chances of conception, intercourse frequency, history of SAB, alcohol intake closest to conception, smoking status closest to conception, pain medication closest to conception, history of pelvic inflammatory disease, history of chlamydia, last method of birth control, geographic region.

likely under-ascertainment and potentially some over-ascertainment of exposure in our study. When correcting for a similar magnitude of exposure classification as the veteran's study, we found that, as expected, low sensitivity and high specificity may have biased our results towards the null. However, the bimonthly follow-up questionnaires and four-week recall period reduced the likelihood of a large degree of misclassification due to this mechanism.

We did not collect information on the exact timing of medication use, so there may be some of the same reverse causation as noted in previous studies, wherein an SAB had already begun prior to initiation of antibiotics.^{15–17} However, excluding these individuals had no impact on the overall results. The CIs for the analyses stratified by timing of exposure assessment were wide, and these findings may also be due to chance.

Exposure misclassification due to misspecification of the etiologically relevant time window is also possible in this study. Proposed biological mechanisms for potential effects of antibiotics on SAB, as well as the etiologically relevant period for exposure, vary by antibiotic type. Differences in the timing of exposure assessment and specific antibiotics studies may explain some of the discrepancies between our null findings and previous literature, including a meta-analysis showing an increased risk of SAB among individuals using certain types of antibiotics in the first trimester.³

Additionally, the conflicting potential mechanisms of disruption of the reproductive tract microbiome from repeated antibiotic use (which may increase the risk of SAB), and treatment of harmful infections (which should decrease the risk of SAB) may contribute to the null results.^{11,29–32} However, we are unable to analyse these mechanisms in the present study. While we did not consider duration of antibiotic use in this study, the vaginal microbiome changes in a matter of hours under antibiotic exposure,²⁰ but is known to be quite resilient and for the most part rebounds to its original state within one to 3 weeks of cessation of

TABLE 2 Association between periconceptional^a antibiotic use and spontaneous abortion, by recency of exposure.

treatment.²⁰ Therefore, it is unlikely that exposure to antibiotics 8 weeks or more preceding pregnancy would be relevant to the risk of spontaneous abortion, although further research on this topic is warranted.³³

Misclassification of outcome is also possible in this study, as we ascertained SAB from self-report and were unable to capture losses occurring prior to pregnancy detection. However, more than 95% of PRESTO participants reported use of home pregnancy tests (mean timing of first pregnancy test was 2 days before expected menses), and the median timing of first pregnancy detection in the cohort was 4 weeks, suggesting that this study was able to capture losses very early in gestation. Non-differential misclassification of SAB would likely lead to bias towards the null, unless there was perfect specificity (which is possible, given the nature of the outcome), in which case there would be no bias. The proportion of recognised pregnancies ending in SAB in our study was similar to national estimates,⁷ indicating that a high degree of outcome misclassification is unlikely.

Although we collected robust participant data at multiple time-points to control for a range of potential demographic, lifestyle, and medical confounders, residual confounding from unmeasured or inaccurately measured factors is possible. In addition to confounding by infection severity and indication, residual confounding by unmeasured factors such as immune or inflammatory conditions is possible.

Selection bias due to differential loss to follow-up is unlikely, as loss to follow-up after a reported pregnancy was low (11.5%) and similar across antibiotic users (13.7%) and non-users (11.3%). If periconceptional antibiotic use is a strong predictor of pregnancy, our findings would be biased since our analysis conditions on recognised pregnancy.^{34,35} However, we previously found no association between antibiotic use and the probability of pregnancy in this cohort.³⁶ Therefore, this is unlikely an important source of bias in the present study.

TABLE 3 Association between periconceptional^a antibiotic use and spontaneous abortion, by weeks of gestation, antibiotic type, and indication for use.

	No. of SABs	No. of pregnancies	Unadjusted HR (95% CI)	Adjusted HR ^b (95% CI)
Periconceptional antibiotic use				
Yes	121	585	1.06 (0.88, 1.28)	1.06 (0.88, 1.28) ^c
No	1416	7305	1.00 (Reference)	1.00 (Reference)
Antibiotic use and gestational age				
<8 weeks of gestation				
Yes	81	540	1.05 (0.83, 1.32)	1.03 (0.82, 1.30)
No	955	6688	1.00 (Reference)	1.00 (Reference)
8–20 weeks of gestation				
Yes	40	467	1.10 (0.79, 1.53)	1.11 (0.80, 1.54)
No	461	5700	1.00 (Reference)	1.00 (Reference)
Antibiotic type				
None	1128	5599	1.00 (Reference)	1.00 (Reference)
Penicillins	28	148	0.93 (0.64, 1.36)	0.95 (0.65, 1.39)
Nitrofurantoin	15	66	1.17 (0.67, 2.03)	1.14 (0.66, 1.96)
Cephalosporins	14	49	1.43 (0.81, 2.52)	1.37 (0.78, 2.42)
Macrolides	10	41	1.22 (0.66, 2.27)	1.16 (0.63, 2.17)
Antibiotic indication for use				
No antibiotic use	1128	5599	1.00 (Reference)	1.00 (Reference)
Urinary tract infection	30	130	1.13 (0.75, 1.70)	1.14 (0.77, 1.70)
Respiratory infection	32	134	1.14 (0.80, 1.62)	1.14 (0.79, 1.63)
Pelvic/vaginal infection	16	71	1.19 (0.73, 1.94)	1.15 (0.71, 1.89)

^aAssessed on the questionnaire closest to conception.

^bAdjusted for age, income, education, BMI, multivitamin use, parity, irregular menstrual cycles, doing something to improve chances of conception, intercourse frequency, history of SAB, alcohol intake closest to conception, smoking status closest to conception, pain medication closest to conception, history of pelvic inflammatory disease, history of chlamydia, last method of birth control, geographic region.

^cBias-corrected estimate 1.34 (95% simulation interval 1.11, 1.79).

4.4 | Interpretation

Previous studies have shown a positive association between some antibiotics and SAB, and an inverse association between others when examining prenatal antibiotic use and SABs occurring after 8 weeks of gestation. However, our study found no strong associations between antibiotics and SAB, when examining by type, indication, or timing relative to conception. Our study concentrated on the period before pregnancy detection and captured very early losses. As individuals may initiate lifestyle changes and seek to decrease medication use after pregnancy detection, and early losses make up the majority of SAB, this study provides additional information on periconceptional antibiotic use and SAB.

We also observed little association between use of cephalosporins, nitrofurantoin, or macrolides and increased risk of SAB. Previous literature has shown no association between female use of cephalosporins and SAB.³ Any increased risk of SAB among nitrofurantoin users may be attributable to confounding by indication, as nitrofurantoin is commonly used to treat urinary tract infections,

which may have a modest association with increased risk of SAB independent of antibiotic class.³⁷ While previous studies have shown an association between macrolides, specifically clarithromycin, and SAB, there were too few clarithromycin users in the present study to examine the association with SAB.

We did not observe a strong association between respiratory, pelvic, or vaginal infection and SAB. While pelvic or vaginal infections may be more biologically plausibly related to SAB than respiratory infections, the absence of strong associations implies that confounding by indication (infection type) did not have a strong impact on our findings. Confounding by infection in general cannot be ruled out in this study. While some participants reported preventive antibiotic use (e.g. to prevent infection following a routine medical or dental procedure, to prevent diarrhoea when travelling), there were too few preventive antibiotic users to allow for comparison between preventive antibiotic use and use to treat an active infection. We also did not have data on the severity of infection, and more severe maternal infections have been found to be associated with an increased risk of SAB.²⁹



5 | CONCLUSIONS

In summary, antibiotic use in the periconceptional period or very early in gestation was not appreciably associated with SAB. The relationship between antibiotic use in the periconceptional period and SAB is likely complicated by the nature and timing of the infection for which the antibiotic was prescribed, type, dosage, and frequency of medication, timing of conception, and the participant's overall health.

AUTHOR CONTRIBUTIONS

EEH and LAW designed the study. EEH, EEM, EEH, and LAW planned and initiated Pregnancy Online. HMC and TRW analysed the data. All authors interpreted the results. HMC drafted the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

FUNDING INFORMATION

This study was supported through funds provided by the National Institute of Child Health and Human Development, National Institutes of Health (R01-HD086742, R21-HD072326). HC is supported by the National Research Service Award (T32 HD 104612).

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Holly Michelle Crowe  <https://orcid.org/0000-0001-9164-532X>

Lauren Anne Wise  <https://orcid.org/0000-0003-2138-3752>

Amelia Kent Wesselink  <https://orcid.org/0000-0002-3929-4451>

REFERENCES

1. Daw JR, Mintzes B, Law MR, Hanley GE, Morgan SG. Prescription drug use in pregnancy: a retrospective, population-based study in British Columbia, Canada (2001–2006). *Clin Ther.* 2012;34(1):239-249.e2. doi:10.1016/J.CLINTHERA.2011.11.025
2. Stokholm J, Schjørring S, Pedersen L, et al. Prevalence and predictors of antibiotic administration during pregnancy and birth. *PLoS One.* 2013;8(12):e82932. doi:10.1371/journal.pone.0082932
3. Omranipoor A, Kashanian M, Dehghani M, Sadeghi M, Baradaran HR. Association of antibiotics therapy during pregnancy with spontaneous miscarriage: a systematic review and meta-analysis. *Arch Gynecol Obstet.* 2020;302(1):5-22. doi:10.1007/S00404-020-05569-4
4. Muanda FT, Sheehy O, Berard A. Use of antibiotics during pregnancy and risk of spontaneous abortion. *CMAJ.* 2017;189(17):E625-E633. doi:10.1503/CMAJ.161020
5. Ziv A, Masarwa R, Perlman A, Ziv D, Matok I. Pregnancy outcomes following exposure to quinolone antibiotics – a systematic-review and meta-analysis. *Pharm Res.* 2018;35(5):109. doi:10.1007/s11095-018-2383-8
6. Schembri S, Williamson PA, Short PM, et al. Cardiovascular events after clarithromycin use in lower respiratory tract infections: analysis of two prospective cohort studies. *BMJ.* 2013;346:f1235. doi:10.1136/bmj.f1235
7. Strumpf E, Lang A, Austin N, et al. Prevalence and clinical, social, and health care predictors of miscarriage. *BMC Pregnancy Childbirth.* 2021;21(1):1-9. doi:10.1186/S12884-021-03682-Z/TABLES/2
8. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med.* 1988;319(4):189-194. doi:10.1056/NEJM198807283190401
9. Quenby S, Gallos ID, Dhillon-Smith RK, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. *Lancet.* 2021;397(10285):1658-1667.
10. Vaughn SJ, Moreno I, Simon C, Lathi RB. Comparing the uterine microbiome in recurrent pregnancy loss to parous fertile controls. *Fertil Steril.* 2019;111(4):e17-e18. doi:10.1016/J.FERTNSTERT.2019.02.058
11. Moreno I, Simon C. Relevance of assessing the uterine microbiota in infertility. *Fertil Steril.* 2018;110(3):337-343. doi:10.1016/J.FERTNSTERT.2018.04.041
12. Verstraeten H, Vilchez-Vargas R, Desimpel F, et al. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. *PeerJ.* 2016;4:e1602. doi:10.7717/peerj.1602
13. Hargreaves CA, Rogers S, Hills F, Rahman F, Howell RJS, Homa ST. Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in vitro. *Hum Reprod.* 1998;13(7):1878-1886. doi:10.1093/humrep/13.7.1878
14. Li P, Qin X, Tao F, Huang K. Maternal exposure to sulfonamides and adverse pregnancy outcomes: a systematic review and meta-analysis. *PLoS One.* 2020;15(12):e0242523. doi:10.1371/JOURNAL.PONE.0242523
15. Muanda FT, Sheehy O, Bérard A. Use of trimethoprim-sulfamethoxazole during pregnancy and risk of spontaneous abortion: a nested case control study. *Br J Clin Pharmacol.* 2018;84(6):1198-1205. doi:10.1111/bcp.13542
16. Jepsen P, Skriver MV, Floyd A, Lipworth L, Schønheyder HC, Sørensen HT. A population-based study of maternal use of amoxicillin and pregnancy outcome in Denmark. *Br J Clin Pharmacol.* 2003;55(2):216-221. doi:10.1046/J.1365-2125.2003.01750.X
17. Ratanajamit C, Skriver MV, Nørgaard M, Jepsen P, Schønheyder HC, Sørensen HT. Adverse pregnancy outcome in users of sulfamethizole during pregnancy: a population-based observational study. *J Antimicrob Chemother.* 2003;52(5):837-841. doi:10.1093/JAC/DKG438
18. Griebel CP, Halvorsen J, Golemon TB, Day AA. Management of spontaneous abortion. *Am Fam Physician.* 2005;72(7):1243-1250.
19. Wise LA, Rothman KJ, Mikkelsen EM, et al. Design and conduct of an internet-based preconception cohort study in North America: pregnancy study online. *Paediatr Perinat Epidemiol.* 2015;29(4):360-371. doi:10.1111/ppe.12201
20. Mayer BT, Srinivasan S, Fiedler TL, Marrazzo JM, Fredricks DN, Schiffer JT. Rapid and profound shifts in the vaginal microbiota following antibiotic treatment for bacterial vaginosis. *J Infect Dis.* 2015;212(5):793-802. doi:10.1093/INFDIS/JIV079
21. dos Santos L, Santiago G, Cools P, Verstraeten H, et al. Longitudinal study of the dynamics of vaginal microflora during two consecutive menstrual cycles. *PLoS One.* 2011;6(11):e28180. doi:10.1371/JOURNAL.PONE.0028180
22. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* 2000;32(Supplement):S498-S516. doi:10.1097/00005768-200009001-00009
23. Ainsworth BE, Haskell WL, Herrmann SD, et al. Compendium of physical activities: a second update of codes and MET

values. *Med Sci Sports Exerc.* 2011;2011:1575-1581. doi:10.1249/MSS.0b013e31821ece12

24. Cox DR. Regression models and life-tables. *J R Stat Soc Ser B.* 1972;34(2):187-202. doi:10.1111/j.2517-6161.1972.tb00899.x

25. Lash TL, Fox MP, Fink AK. *Applying Quantitative Bias Analysis to Epidemiologic Data.* Springer; 2009.

26. Metlay JP, Hardy C, Strom BL. Agreement between patient self-report and a veterans affairs national pharmacy database for identifying recent exposures to antibiotics. *Pharmacoepidemiol Drug Saf.* 2003;12(1):9-15. doi:10.1002/PDS.772

27. Lassi ZS, Imam AM, Dean SV, Bhutta ZA. Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. *Reprod Health.* 2014;11(3):1-12. doi:10.1186/1742-4755-11-S3-S6/FIGURES/8

28. Liu Y, De A. Multiple imputation by fully conditional specification for dealing with missing data in a large epidemiologic study. *Int J Stat Med Res.* 2015;4(3):287-295. doi:10.6000/1929-6029.2015.04.03.7

29. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SEM, Horne AW. The role of infection in miscarriage. *Hum Reprod Update.* 2016;22(1):116-133. doi:10.1093/HUMUPD/DMV041

30. Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol.* 2019;18(1):40-50. doi:10.1002/rmb2.12249

31. Mor A, Driggers PH, Segars JH. Molecular characterization of the human microbiome from a reproductive perspective. *Fertil Steril.* 2015;104:1344-1350. doi:10.1016/j.fertnstert.2015.10.008

32. García-Velasco JA, Menabrito M, Catalán IB. What fertility specialists should know about the vaginal microbiome: a review. *Reprod Biomed Online.* 2017;35(1):103-112. doi:10.1016/j.rbmo.2017.04.005

33. Ahrens P, O'Brien Andersen L, Lilje B, et al. Changes in the vaginal microbiota following antibiotic treatment for *Mycoplasma genitalium*, *chlamydia trachomatis* and bacterial vaginosis. *PLoS One.* 2020;15(7):e0236036. doi:10.1371/JOURNAL.PONE.0236036

34. Snowden JM, Bovbjerg ML, Dissanayake M, Basso O. The curse of the perinatal epidemiologist: inferring causation amidst selection. *Curr Epidemiol Reports.* 2018;5(4):379-387. doi:10.1007/S40471-018-0172-X

35. Liew Z, Olsen J, Cui X, Ritz B, Arah OA. Bias from conditioning on live birth in pregnancy cohorts: an illustration based on neurodevelopment in children after prenatal exposure to organic pollutants. *Int J Epidemiol.* 2015;44:345-354. doi:10.1093/ije/dyu249

36. Crowe HM, Wesselink AK, Wise LA, et al. Antibiotics and fecundability among female pregnancy planners: a prospective cohort study. *Hum Reprod.* 2021;36(10):2761-2768. doi:10.1093/HUMREP/DEAB173

37. Andersen JT, Petersen M, Jimenez-Solem E, et al. Clarithromycin in early pregnancy and the risk of miscarriage and malformation: a register based nationwide cohort study. *PLoS One.* 2013;8(1):e53327. doi:10.1371/journal.pone.0053327

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Crowe HM, Hatch EE, Wang TR, et al. Periconceptional antibiotic use and spontaneous abortion: A prospective cohort study. *Paediatr Perinat Epidemiol.* 2022;00:1-9. doi: [10.1111/ppe.12931](https://doi.org/10.1111/ppe.12931)