EPIDEMIOLOGY

Preconception use of antibiotics and fecundability: a Danish prospective cohort study

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Objective: To assess the association between preconception antibiotic use and fecundability, the per menstrual cycle probability of conception.
Design: SnartForaeldre.dk, a Danish prospective cohort study of women trying to conceive (2007–2020).
Setting: Not applicable.
Subject(s): 9462 female participants, median age 29 years at enrollment.
Exposure: Antibiotic use was defined by filled prescriptions retrieved from the Danish National Prescription Registry, using Anatomical Therapeutic Chemical codes, and modeled as time-varying (menstrual cycle-varying) exposure.
Main Outcome Measure(s): Pregnancy status was reported on female follow-up questionnaires every 8 weeks for up to 12 months or until conception. Fecundability ratios (FR) and 95% confidence intervals (CI) were computed using proportional probabilities regression models, with adjustment for age, partner age, education, smoking, folic acid supplementation, body mass index, parity, cycle regularity, timing of intercourse, and sexually transmitted infections.
Result(s): During all cycles of observation, the percentage of participants filing at least 1 antibiotic prescription was 11.9%; 8.6% had a prescription for penicillins, 2.1% for sulfonamides, and 1.8% for macrolides. Based on life-table methods, 86.5% of participants conceived within 12 cycles of follow-up. Recent preconception antibiotic use was associated with reduced fecundability (≥ 1 prescription vs. none: adjusted FR = 0.86; 95% CI, 0.76–0.99). For participants using penicillins, sulfonamides, or macrolides, the adjusted FRs were 0.97 (95% CI, 0.83–1.12), 0.68 (95% CI, 0.47–0.98), and 0.59 (95% CI, 0.37–0.93), respectively.
Conclusion(s): Preconception use of antibiotics, specifically sulfonamides and macrolides, was associated with decreased fecundability compared with no use. The observed associations may be explained plausibly by confounding by indication, as we lacked data on indications for the prescribed antibiotics. Consequently, we cannot separate the effect of the medication from the effect of the underlying infection. (Fertil Steril® 2023;■:■–■. ©2023 by American Society for Reproductive Medicine.)
Key Words: Preconception, antibiotic use, and fecundability

Antibiotics are used commonly by women of reproductive age, including those trying to conceive. In the period within 30 days before conception, 4.9% of Danish women filled an antibiotic prescription (1).

The use of antibiotics, such as amoxicillin, gentamicin, or salinomycin, has been associated with decreased fertility in rodents (2–4) and may have adverse effects on semen parameters, and male reproductive tissue in humans (5). The purported biological mechanisms may involve oxidative

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stress, alterations in folate metabolism (3, 6, 7), and imbalances in the microbiome (8, 9). On the other hand, by clearing sexually transmitted infections in the reproductive organs, antibiotics may improve fertility.

One study of Danish pharmacy assistants exposed to antibiotics in the workplace reported increased odds of being unable to conceive compared with unexposed colleagues (10). In contrast, a case-control study and a prospective cohort study of pregnancy planners found little association between self-reported antibiotic exposure and reduced fecundability (11, 12). Given the frequent use of antibiotics (13) and the fact that approximately 15% of couples trying to conceive experience infertility (14), defined as time to pregnancy (TTP) >12 months, a potential association between antibiotic use and TTP would have important public health implications.

In a prospective cohort study of Danish pregnancy planners, we examined the association between the use of antibiotics defined by filled prescriptions, overall, and by type of antibiotic (penicillin, sulfonamide, and macrolide), with fecundability, the per-cycle probability of conception. We hypothesize that antibiotic use, overall and by type of antibiotic is associated with decreased fecundability.

METHODS

The cohort comprises females trying to conceive and enrolled in the "SnartGravid.dk" [Soon Pregnant] (SG) study and its successor, the ongoing "SnartForaeldre.dk" [Soon Parents] (SF) study. The SG study began in 2007 and was extended in 2011 to collect dietary data and include male partners (SF). Recruitment and data collection procedures are described in detail elsewhere (15, 16). In short, participants were recruited from across Denmark, primarily using web bloggers, online advertisements, and invitations in a national official digital post system, called e-Boks. To confirm eligibility, participants completed a screener questionnaire and a consent form before receiving the baseline questionnaire. Participants were invited to answer follow-up questionnaires bimonthly until pregnancy occurred or end of observation (12 months), whichever came first.

Study Population

We enrolled Danish female residents who were aged 18–49 years, trying to conceive, in a relationship with a male partner, and not using fertility treatment. To confirm their identity and to be enrolled, participants completed the baseline questionnaire and reported their civil registration number, a unique 10-digit personal identifier assigned to all Danish residents (17). In total, we enrolled 14,257 females, of whom we excluded 461 who participated more than once (multiple participation is possible), 753 with incomplete or implausible menstrual cycle data, and 216 who were pregnant at study entry. As prescription data were available only through 2020, we excluded participants who enrolled after November 29, 2020, thereby providing an exposure window corresponding to at least 1 menstrual cycle for all participants. Finally, we excluded 28 who withdrew their consent, and 3083 who had been trying to conceive for >6 cycles (1603) or 6 months (1480) at study entry, to limit reverse causation stemming from women altering their lifestyle and hesitating to take antibiotics in response to subfertility. Accordingly, the final cohort consisted of 9462 women (Supplemental Fig. 1, available online).

Assessment of Covariates

At study entry, participants reported on sociodemographic, lifestyle, and behavioral factors as well as reproductive and medical history. In the follow-up questionnaires, participants reported on pregnancy status and lifestyle-related exposures that are likely to change over time.

Assessment of Antibiotic Exposure

The Danish National Prescription Registry records individual-level data on all prescription medications dispensed at the Danish community pharmacies since 1995 (18). We defined antibiotic use by using filled prescriptions. We retrieved data on all antibiotic prescriptions issued in primary care to each of our study participants from the first cycle of observation to the end of observation (maximum 12 cycles of follow-up). We used the Anatomical Therapeutic Chemical (ATC) codes to identify all antibiotics used for systemic use: ATC group (J01). We categorized the medications according to the ATC subgroups of antibiotics: penicillin (J01C penicillin), sulfonamide including trimethoprim (J01E), macrolide including lincosamide and streptogramine (J01F), and other (J01A (tetracycline), J01B (amphenicol), J01D (cephalosporin and other β-lactam antibacterial), J01G (aminoglycoside), J01M (quinolone), J01R (combinations of antibacteria), J01X [other]). In addition, because urinary tract infections (UTI) are frequent among females of reproductive age, and UTI may be transmitted into the reproductive organs, penicillin exposure was categorized further according to typical indication, UTI (J01CA08 and J01CA11) vs. other indications (all other J01Cs). We used the civil registration number to link the registry and self-reported questionnaire data.

Assessment of Pregnancy and Cycles at Risk

On the baseline questionnaire, participants reported the number of menstrual cycles or months they had tried to conceive before enrollment, date of last menstrual period (LMP), menstrual cycle length (number of days), and cycle regularity (yes vs. no). On each follow-up questionnaire, participants reported their pregnancy status, including intervening pregnancy losses, initiation of fertility treatment, menstrual cycle length, cycle regularity, and date of the most recent LMP.

We constructed an algorithm to estimate intervening LMP dates not captured by the consecutive questionnaires, which were completed approximately every 8 weeks. The algorithm used reported LMP dates and data on cycle length (details described in supplemental text, available online). For participants who reported irregular cycles, we defined cycle length as 32 days (median cycle length assessed over follow-up for participants reporting irregular cycles at baseline). For participants reporting regular cycles, we used

reported cycle lengths where possible. If some cycle lengths were missing, we used participant-specific mean cycle length unless they were all missing, in which case we defined cycle length as 28 days.

A menstrual cycle at risk was defined by the time from an LMP date to the day before the next LMP date. The first cycle at risk started at the LMP date reported at baseline and the last cycle at risk ended at the LMP date from the most recent follow-up questionnaire plus cycle length or date of a censoring event. Time to pregnancy was estimated in discrete menstrual cycles as follows: (cycles of pregnancy attempts at baseline) + (number of cycles at risk from first cycle at risk to last cycle at risk).

Ethics

The study was registered with Aarhus University (record 2016-051-000001, number 431). Ethical approval was not required as the study only includes questionnaire and registry data.

Data Analysis

To compare antibiotic users and nonusers, we cross-tabulated the baseline characteristics of the participants with any antibiotic use from the first cycle of observation through follow-up, as well as any use of subtypes of antibiotics.

We used life-table methods to calculate the percentage of participants who conceived during follow-up, accounting for censoring events: start of fertility treatment, cessation of pregnancy attempt, loss to follow-up, and end of follow-up (12 menstrual cycles), or December 31, 2020 (the date after which registry data were not available), whichever came first.

To examine the association between time-varying (menstrual cycle-varying) antibiotic use and fecundability, we created an exposure variable that was updated for each menstrual cycle at risk. For example, a participant with a prescription in cycle 2 was considered exposed in cycle 2, but not in cycle 3 or the subsequent cycles unless she filled additional prescriptions. We used a proportional probabilities regression model to compute the fecundability ratios (FR) and 95% confidence intervals (CI). The FR represents the average per-cycle probability of conception comparing antibiotic users with the nonusers. Thus, an FR <1 indicates reduced fertility for exposed participants (19). We used menstrual cycles as the timescale, and each participant contributed discrete menstrual cycles at risk from the date of study entry until pregnancy or a censoring event, whichever came first. To account for left truncation, wherein some participants have been trying to conceive for several cycles (1–6) before enrolling, we used the Andersen-Gill data structure, with one observation per menstrual cycle (20, 21).

In the regression model, we adjusted for potential confounders, which we selected based on the literature and assessment of a causal graph. We included the following variables measured at baseline: female age (<25, 25–29, 30–34, ≥ 35 years), partner age (<25, 25–29, 30–34, 35–39, ≥ 40 years), body mass index (BMI, <20, 20–24, 25–29, ≥ 30 kg/m^2), timing of intercourse (yes vs. no), parity (nulliparous vs. parous), smoking (current/current occasional smoker vs. nonsmoker), cycle regularity (regular vs. irregular), vocational education (none, basic, <3 years, 3-–4 years, >4 years), folic acid supplementation (yes vs. no), and history of sexually transmitted infections (yes vs. no).

We stratified our analyses by age (<30 vs. ≥ 30 years) and BMI (<30 vs. ≥ 30 kg/m^2) to evaluate the effect measure modification, as a potential association between antibiotic use and fecundability may vary across these factors that are strongly related to fecundability (22, 23). Further, we stratified the analyses by calendar time of enrollment (2007–2013 vs. 2014–2020) because the indication for prescribing antibiotics potentially was more restrictive in recent years because of changes in Danish policies (24).

Because we do not have information on the duration of treatment, we assigned the antibiotic exposure to the menstrual cycle in which the participant filled the prescription. Thus, we may have misclassified the exposure for a cycle where the fertile window was already past. To address this, in a sensitivity analysis, we reclassified exposure for anyone with a prescription issued ≥ 18 days after the LMP date to the next menstrual cycle, for example, cycle 3 for anyone who had a prescription issued ≥ 18 days after the LMP date in cycle 2.

To evaluate the potential of reverse causation by altered lifestyle and willingness to take antibiotics, we conducted a sensitivity analysis, in which we restricted the study population to participants with an attempt time at study entry of ≤ 3 cycles (25).

To explore potential confounding by the underlying infection or fever, we conducted a sub-analysis among antibiotic users, in which we compared fecundability for sulfonamide and macrolide users with penicillin users. Finally, in a sensitivity analysis, we assessed the potential for unmeasured confounding stemming from the underlying disease by calculating the E-value (26).

The percentage of missing values for self-reported data ranged from 0.2% (partner age) to 6.5% (servings of dessert wine) except for diabetes, hypertension, and a few variables that were included in SG but not in SF or vice versa (Supplemental Table 1, available online). We used the fully conditional specification method to multiply impute missing covariate values (27, 28). To reduce the potential for selection bias because of differential loss to follow-up, we included participants who did not complete any follow-up questionnaires (13.6%), assigned them 1cycle of follow-up, and multiply imputed their outcome (pregnant: yes vs. no) (27, 28). We generated 20 imputed datasets, analyzed each separately, and combined effect estimates and standard errors across the imputed datasets to account for values between and within the imputation variation.

To evaluate the potential for selection bias further, we compared participants with complete follow-up (completers) with participants who stopped responding to questionnaires before the end of the study (noncompleters) according to baseline characteristics and registry-based antibiotic exposure during the first cycle of follow-up.

We used SAS 9.4 (SAS Institute, Cary, NC) for the statistical analyses. To comply with Danish regulations about data protection, we do not report numbers based on <5 persons.

RESULTS

The analytic cohort comprised 9462 participants, of whom 11.9% (1130) filled at least 1 antibiotic prescription at any time point during observation (baseline to end of follow-up): 2.8% (263) filled 1 prescription, 6.6% (625) filled 2 prescriptions, and 2.6% (242) filled ≥3 prescriptions. In total, 8.6% of the participants filled a prescription for penicillins, whereas 2.1% and 1.8% had a prescription for sulfonamides and macrolides, respectively (Table 1).

At study entry, the median age of antibiotic users and nonusers was 29 years. The distribution of baseline characteristics, such as partner age, cycle regularity, parity, alcohol consumption, and history of chronic disease, was similar in the 2 groups. Compared with nonusers, antibiotic users were slightly more likely to be obese, physically active, currently smoke, have a history of sexual transmitted infections, and have lower educational attainment.

Across the categories of antibiotics (penicillin, macrolide, or sulfonamide), the distributions of most baseline characteristics among participants were similar. However, compared with users of penicillin or macrolide, participants using sulfonamide were less likely to be parous, current smokers, and to time intercourse according to their fertile window.

The analytical sample included 9462 participants who provided 34,518 menstrual cycles over follow-up and 5846 (62%) who became pregnant. Median follow-up was 3 cycles (interquartile range 1–5 cycles). Using life-table methods, we calculated that 71.0% and 86.5% of the participants conceived within 6 and 12 cycles, respectively. In total, 773 (8.2%) did not conceive, 201 (2.1%) stopped trying to conceive, 503 (5.3%) initiated fertility treatment, and 2342 (24.7%) stopped responding to questionnaires before the end of the study period (12 cycles). Thus, the overall cohort retention was 75.3% (7120/9462).

Overall, the 7120 completers and 2342 noncompleters were similar according to baseline characteristics (female and partner age, cycle regularity, parity, frequency of intercourse, alcohol consumption, history of chronic disease, and history of sexually transmitted infection) (Supplemental Table 2, available online). However, noncompleters were more likely to be current smokers and have lower educational attainment than completers. In total, 4.5% of completers redeemed at least 1 prescription of antibiotics during the first cycle of follow-up compared with 4.8% among noncompleters.

Compared with nonusers of any antibiotic, the adjusted FR for antibiotic users (≥1 prescription) was 0.86 (95% CI, 0.76–0.99; Table 2). For users of penicillins, sulfonamides, or macrolides, the adjusted FRs were 0.97 (95% CI, 0.83–1.12), 0.68 (95% CI, 0.47–0.98), and 0.59 (95% CI, 0.37–0.93), respectively, compared with nonusers. Comparing no antibiotic use with the use of penicillin subtypes that were indicated for UTI or for non-UTI, the adjusted FRs were 1.21 (95% CI, 0.97–1.51) and 0.84 (95% CI, 0.69–1.02), respectively.

The association between any antibiotic use and fecundability was stronger among participants aged ≥30 years (adjusted FR, 0.81; 95% CI, 0.65–1.00) compared with those aged <30 years (adjusted FR, 0.90; 95% CI, 0.76–1.07), and among participants with a BMI ≥25 (adjusted FR, 0.67; 95% CI, 0.52–0.87) compared with participants with a BMI <25 (adjusted FR, 0.97; 95% CI, 0.83–1.12; Table 3). Likewise, the associations for fecundability and exposure to sulfonamides or macrolides were stronger among participants

TABLE 1

Baseline characteristics of 9462 participants by use off any antibiotic and separate types of antibiotics at any time point during observation (baseline to end of follow-up)

	Antibiotic use		Type of antibiotic use			
Characteristic	**Ever**	**Never**	**Penicillin**	**Sulfonamide**	**Macrolide**	**Other**
Number of women (%)	1,130 (11.9)	8,332 (88.1)	812 (8.6)	202 (2.1)	167 (1.8)	98 (1.0)
Age (y), median (IQR)	29 (26–32)	29 (27–32)	29 (26–32)	28 (26–31)	29 (26–32)	28 (25–31)
Partner's age (y), median (IQR)	30 (27–34)	31 (28–34)	30 (27–34)	30 (27–33)	30 (27–34)	30 (28–33)
Irregular cycles, yes (%)	281 (24.8)	2024 (24.3)	195 (24.0)	57 (28.2)	45 (27.0)	31 (31.6)
Parous, ever had live birth (%)	403 (35.7)	2859 (34.3)	304 (37.4)	55 (27.2)	87 (40.1)	23 (23.5)
Body mass index, ≥30 (%)	172 (15.2)	988 (11.9)	123 (15.2)	25 (12.2)	35 (21.0)	14 (14.3)
Physical activity, MET (≥40)	655 (58.0)	4456 (53.5)	464 (57.1)	118 (58.4)	99 (59.3)	65 (66.3)
Long vocational training, >4 y (%)	306 (27.1)	2741 (32.9)	218 (26.9)	52 (25.7)	39 (23.4)	28 (28.6)
Current smoking, yes (%)	229 (20.3)	1270 (15.2)	169 (20.1)	31 (15,4)	39 (23.4)	20 (20.4)
Folic acid supplements, yes (%)	772 (68.3)	5286 (63.4)	543(66.9)	145 (71.8)	108(64.7)	73 (74.5)
Alcohol consumption, ≥ 7 servings per wk (%)	62 (5.5)	500 (6.0)	40 (4.9)	16 (7.9)	12 (7,2)	5 (5.1)
Frequency of intercourse, ≥4 times per wk (%)	227 (20.1)	1359 (16.3)	162 (20.0)	50 (24.8)	25 (15.0)	22 (22.5)
Timing of intercourse, yes (%)	644 (57.0)	5261 (63.1)	472 (58.1)	101 (50.0)	96 (57.5)	53 (54.1)
History of sexually transmitted infection, yes (%)[a]	433 (38.3)	2669 (32.0)	305 (37.6)	73 (36.1)	68 (40.7)	37 (37.8)
Chronic disease, yes (%)[b]	144 (12.7)	1056 (12.7)	105 (12.9)	22 (10.9)	27 (16.2)	9 (9.2)

IQR = interquartile range; MET = total metabolic equivalents.
[a] Self-reported chlamydia, herpes, and gonorrhea.
[b] Self-reported asthma, diabetes, hypertension, and thyroid disease.

Mikkelsen. Antibiotic use and fecundability. Fertil Steril 2023.

TABLE 2

Fecundability by time-varying[a] antibiotic use (N = 9462 women)

			Full study, all cycles of observation	
				Unadjusted Adjusted[b,c]
Antibiotic use	**Pregnancies**	**Cycles**	**FR (95% CI)**	**FR (95% CI)**
None	5664	33,142	Ref.	Ref.
Antibiotic, any	204	1376	0.85 (0.75–0.97)	0.86 (0.76–0.99)
Penicillin	155	935	0.97 (0.83–1.13)	0.97 (0.83–1.12)
Sulfonamide	26	223	0.66 (0.46–0.95)	0.68 (0.47–0.98)
Macrolide	18	184	0.57 (0.36–0.91)	0.59 (0.37–0.93)
Other types	13	115	0.73 (0.44–1.21)	0.75 (0.45–1.25)

CI = confidence interval; FR = fecundability ratio.
[a] Time varying = menstrual cycle-varying antibiotic exposure.
[b] Adjusted for age, partner age, BMI, cycle regularity, timing of intercourse, parity, current smoking, length of education, history of sexual transmitted disease, and use of folic acid supplements.
[c] Models for subtypes of antibiotic also were mutually adjusted for the other antibiotics (e.g., penicillin analyses were adjusted for use of macrolides, sulfonamide, or other types of antibiotics).

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aged ≥30 years and those with a BMI ≥25. The numbers of participants exposed to sulfonamides or macrolides were small in the stratified analyses.

Stratifying by calendar time of enrollment, we observed a lower exposure to any antibiotic use in the more recent period, 15% (2007–2013) vs. 8% (2014–2020), and this pattern was similar across types of antibiotics. Further, compared with 2007–2013, the adjusted fecundability ratios were stronger for any antibiotic use (FR, 0.95; 95% CI, 0.82–1.09 vs. 0.65; 95% CI, 0.48–0.87) and penicillin use (FR, 1.05; 95% CI, 0.89–1.24 vs. 0.76; 95% CI, 0.56–1.05) in the more recent period 2014–2020.The association between any antibiotic exposure and fecundability did not change noticeably when the exposure was assigned to a subsequent menstrual cycle where the fertile window was not past (adjusted FR, 0.86; 95% CI, 0.75–0.99).

Restricting the cohort to participants with attempt times at study entry of ≤3 cycles did not substantially change the adjusted FRs for any use (0.87; 95% CI, 0.75–1.01) or for the subtypes, penicillins (0.99; 95% CI, 0.84–1.18), sulfonamides (0.69; 95% CI, 0.46–1.05), and macrolides (0.56; 95% CI, 0.31–0.98), compared with nonuse.

In a sub-analysis of the 1130 antibiotic users, the adjusted FRs were 0.73 (95% CI, 0.49–1.08) for women using sulfonamides and 0.59 (95% CI, 0.37–0.95) for users of macrolides compared with penicillin users (Table 4). The adjusted E-value for unmeasured confounding in the analysis of any antibiotic use was 1.58 (95% CI, 1.13–1.97).

DISCUSSION

In this prospective cohort study, using registry-based data on prescribed antibiotics and prospectively collected data on TTP, use of any antibiotic, specifically either a sulfonamide or macrolide, was associated with decreased fecundability compared with no use. The association appeared the strongest for older and heavier participants.

To our knowledge, only 3 studies have evaluated the association between antibiotic use and fertility (10–12). Methodological differences limit the ability to compare results across studies. Our observation of lower fecundability among antibiotic users agrees with a study of 4517 Danish pharmacy assistants, in which exposure was assessed as self-reported handling of unsealed antibiotics in pharmacies (production, dispensing, bottling, and packing) (10). The TTP was reported retrospectively in broad categories (0–6, 7–12, 13–24, 25–36 and >36 months) within the previous 7 years and compared with colleagues working in the administration. The odds ratio for a TTP >12 months was 1.34 (95% CI, 1.0–1.8) for the pharmacy assistants. However, the results in that study may reflect a heavier exposure originating from continued daily exposure during work hours than exposure during the few days of treatment. Further, the route of exposure for the pharmacy assistants likely is inhalation, whereas it is ingestion for our study participants. In contrast, 2 United States-based studies reported little association between antibiotic exposure and fertility (11, 12). In a case-control study of 1880 women with ovulatory disorders and 4023 controls, the estimated odds ratio between self-reported antibiotic use and infertility (defined as failure to conceive within 12 months) was 1.0 (95% CI, 0.7–1.4) (11). In a preconception cohort study of 9524 women trying to conceive, designed similarly to our study, adjusted FRs for time-varying use of any antibiotic (0.98; 95% CI, 0.89–1.07), penicillins (1.09; 95% CI, 0.94–1.28), sulfonamides (1.39; 95% CI, 0.90–2.15), and macrolides (0.70; 95% CI, 0.47–1.04) compared with nonuse (12). However, the investigators in that study relied on self-reported data on antibiotic use, and they measured antibiotic use within a 4-week window, rather than the date of prescription. The 2 North American studies seem more likely to have underestimated the association between antibiotic use and fecundability due to nondifferential misclassification of self-reported antibiotic exposure.

Macrolides, for example, clarithromycin and sulfonamides, including trimethoprim, have been associated with early miscarriage (29–31). Thus, the observed association between macrolides and sulfonamides and fecundability

TABLE 3

Fecundability by time-varying[a] antibiotic use stratified by age, BMI and TTP at study entry (N = 9462)

Antibiotic use	Pregnancies	Cycles	Unadjusted FR (95% CI)	Adjusted[b,c] FR (95% CI)	Pregnancies	Cycles	Unadjusted FR (95% CI)	Adjusted[b] FR (95% CI)
			< 30 y				≥30 y	
None	3382	20,039	ref.	ref.	2284	13,107	Ref.	Ref.
Antibiotic, any	127	850	0.88 (0.75–1.04)	0.90 (0.76–1.07)	77	526	0.81 (0.65–1.01)	0.81 (0.65–1.00)
Penicillin	95	567	1.03 (0.85–1.25)	1.04 (0.86–1.26)	60	368	0.87 (0.68–1.12)	0.87 (0.68–1.12)
Sulfonamide	19	148	0.75 (0.50–1.14)	0.78 (0.51–1.18)	7	75	0.51 (0.24–1.01)	0.52 (0.24–1.11)
Macrolide	11	111	0.62 (0.35–1.09)	0.63 (0.36–1.11)	7	73	0.52 (0.24–1.12)	0.54 (0.25–1.16)
Other types[d]	<5[4]	–	–	–	<5[4]	–	–	–
			BMI <25				BMI ≥ 25	
None	3945	22,383	ref.	ref.	1719	10,759	Ref.	Ref.
Antibiotic, any	148	865	0.96 (0.83–1.12)	0.97 (0.83–1.12)	56	508	0.66 (0.50–0.85)	0.67 (0.52–0.87)
Penicillin	111	589	1.05 (0.88–1.25)	1.03 (0.87–1.23)	47	346	0.83 (0.62–1.10)	0.85 (0.64–1.13)
Sulfonamide	22	152	0.81 (0.55–1.19)	0.84 (0.57–1.23)	<5[4]	–	–	–
Macrolide	12	105	0.67 (0.39–1.14)	0.68 (0.40–1.16)	6	79	0.45 (0.19–1.06)	0.45 (0.19–1.05)
Other types[d]	<5[4]	–	–	–	<5[4]	–	–	–
2007–2013							2014–2020	
None	3440	20,634	ref.	ref.	2224	12,508	Ref.	Ref.
Antibiotic, any	164	1,028	0.94 (0.81–1.09)	0.95 (0.82–1.09)	41	348	0.64 (0.47–0.87)	0.65 (0.48–0.87)
Penicillin	118	665	1.06 (0.89–1.25)	1.05 (0.89–1.24)	37	270	0.77 (0.56–1.06)	0.76 (0.56–1.05)
Sulfonamide	23	187	0.72 (0.49–1.05)	0.73 (0.50–1.07)	<5[4]	–	–	–
Macrolide	13	136	0.61 (0.36–1.03)	0.62 (0.37–1.05)	<5[4]	–	–	–
Other types[d]	13	104	0.81 (0.49–1.34)	0.83 (0.50–1.38)	<5[4]	–	–	–

CI = confidence interval; FR = fecundability ratio.

[a] Time varying = menstrual cycle-varying antibiotic exposure.

[b] Each of the analyses were mutually adjusted for age (excluded in age model), parity, BMI (excluded in BMI model), in addition to sexual transmitted disease, partner age, current smoking status, cycle regularity, timing of intercourse, length of education, and use of folic acid supplements.

[c] Models for subtypes of antibiotic were also mutually adjusted for the other antibiotics (*e.g.*, penicillin analyses were adjusted for use of macrolides, sulfonamides and other types of antibiotics).

[d] Numbers too small to report.

Mikkelsen. Antibiotic use and fecundability. Fertil Steril 2023.

TABLE 4

Fecundability by time-varying[a] antibiotic use among users (N = 1130)

			Full study, all cycles of observation	
			Unadjusted	Adjusted[b]
Antibiotic use	**Pregnancies**	**Cycles**	**FR (95% CI)**	**FR (95% CI)**
Penicillin	155	935	Ref.	Ref.
Sulfonamide	26	223	0.67 (0.45–0.99)	0.73 (0.49–1.08)
Macrolide	18	184	0.59 (0.36–0.95)	0.59 (0.37–0.95)
Other types	13	115	0.71 (0.42–1.20)	0.80 (0.47–1.36)

CI = confidence interval; FR = fecundability ratio.
[a] Time varying = menstrual cycle-varying antibiotic exposure.
[b] Adjusted for age, partner age, BMI, cycle regularity, timing of intercourse, parity, current smoking, length of education, history of sexual transmitted disease, use of folic acid supplements, and mutually adjustment the other antibiotics (e.g., sulfonamide was adjusted for use of macrolides, and other types).

Mikkelsen. Antibiotic use and fecundability. Fertil Steril 2023.

may be explained by early miscarriages leading to longer TTP. This seems likely as early miscarriages are more difficult to identify and are reported to occur in 13%–22% of all pregnancies before a clinical pregnancy is detected (32, 33).

Folate is essential in oocyte maturation, fertilization, and fetal growth (34, 35). Sulfonamide, including trimethoprim, acts as a folate antagonist and is prescribed typically for UTI; thus, it possible that the use of folate antagonist antibiotics may be associated reduced fecundability.

Consistent with the findings of Crowe et al. (12), we observed that the association between macrolide use and fecundability appeared strongest compared with the other types of antibiotics. Although the mechanisms of action are not understood fully, this variation in associations may be related to the bacteriostatic mechanism of macrolides inhibiting cell growth and the bactericidal mechanism of penicillins and sulfonamides in bacteria (36). In contrast to the study of Crowe et al. (12), we found that the association between any antibiotic use and fecundability was strongest for participants aged $\geq$30 years and women a BMI $\geq$25.

Some limitations of our study should be considered. Cohort retention to the end of follow-up was 75%; however, baseline characteristics and antibiotic exposure were similar for completers and noncompleters, indicating that differential loss to follow-up is unlikely to be a strong source of bias. In addition, exposure is assessed independently of the outcome, thus reducing the possibilities for selection bias and dependent misclassification (37).

Because the Danish National Prescription Registry covers all prescriptions redeemed at Danish pharmacies (18), we most likely have a complete exposure history for each participant. In our population, penicillin was the most used antibiotic, which is consistent with antibiotic use among Danish women (38). However, we may have overestimated antibiotic use as we assessed use by filled prescriptions in each menstrual cycle under observation, but we do not have information on whether the participants consumed the medication. Furthermore, we may have misclassified antibiotic exposure because we assigned exposure to a specific menstrual cycle based on LMP dates. When constructing consecutive LMP dates, we were unable to correct for delays in LMP due to recent breast feeding or giving birth. However, we expect this misclassification to be limited and nondifferential as two-thirds of the population is nulliparous, and LMP dates and antibiotic exposure were assessed independently.

Unfortunately, information on the indications or dosage of the prescribed antibiotics was not available in the registry data. For this reason, in addition to the small numbers, we are not able to explore in detail the extent to which the association between antibiotics and fecundability may be explained by confounding by indication. Ideally, we would compare the experience of women with the same condition treated by different antibiotics, but that option does not exist. Therefore, in a sub-analysis, we compared sulfonamide and macrolide use with penicillin use and found, similar to the overall results, that the association was strongest for macrolide use. Although some overlap exists, for example, penicillin and sulfonamide are used for UTI (penicillin primarily for acute UTI and sulfonamide primarily for recurrent UTI), and penicillin and macrolides are used for respiratory infections (macrolides primarily for patients allergic to penicillin), the indication for the subtypes of antibiotics differ (38, 39), and we cannot separate the effect of the antibiotics from the effect of the underlying infection.

The E-value indicates that the observed adjusted FR of 0.86 (95% CI, 0.76–0.99) for any antibiotic use could be explained away potentially by an unmeasured confounder (e.g., underlying disease) associated with antibiotic treatment and fecundability by a risk ratio of 1.58 (95% CI, 1.13–1.97). Meaning that participants with an infection should be 58% more likely to receive a prescription for antibiotics and 58% less likely to conceive, compared with participants with no infection, to explain away the reduction in fecundability (FR = 0.86), but weaker confounding could not do so. Considering the magnitude of the E-value and the fact that we observed a stronger association between antibiotic use and fecundability for the more recent years where the indication for a prescription presumably was more restrictive, confounding by indication remains possible.

Because UTI is a likely confounder, we stratified penicillin exposure according to the typical indication for UTI vs. non-UTI. In contrast to non-UTI-specific penicillin, UTI-specific penicillin was associated positively with fecundability, supporting the hypothesis that treatment for UTI could improve

fertility. Further, antibiotic treatment for severe infections may prevent damage to the reproductive organs, and if vital organs are infected, antibiotic treatment ultimately is life-saving. Usually, antibiotics are prescribed in a series of 1–10 days depending on the type (40). We used time-varying exposure, thus linking a prescription to the specific menstrual cycle. However, if any of the evaluated antibiotics primarily have an acute effect (e.g., on ovulation or implantation), we may have underestimated the effect, as we did not consider the exact timing of exposure during the fertile window. However, the results of the sensitivity analysis, in which we reassigned the antibiotic exposure to match the fertile window more precisely, did not support this hypothesis.

Although we adjusted for several potential confounders, unmeasured confounding is possible because we were unable to adjust for female fever episodes, which have been reported to adversely affect follicular development and ovarian estradiol production (41). In addition, we did not adjust for male partner infection or fever episodes. Partners are likely to share infections, and therefore, couple fecundability may be confounded by male fever episodes impairing semen quality (42). Finally, we were unable to adjust for acute lifestyle changes, for example, decreased intercourse frequency during the days of treatment.

Our population represents the full spectrum of fertility from highly fertile to less fertile individuals, and our recruitment procedure allowed residents across the country to participate without having any contact with the health care system.

In conclusion, Preconception use of antibiotics, particularly sulfonamides and macrolides, was associated with decreased fecundability compared with no use. The observed associations may be explained plausibly by confounding by indication. Because we lacked data on indications for the prescribed antibiotics, we cannot eliminate the possibility that the observed association stems from the effect of the underlying infection, rather than the effect of the medication.

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