OBSTETRICS

# Impact of interconception antibiotics on the endometrial microbial flora

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**OBJECTIVE:** The purpose of this study was to evaluate the impact of an interconception antibiotic regimen on endometrial microbial flora and histologic type.

**STUDY DESIGN:** This was a secondary analysis of a double-blind randomized placebo-controlled trial of prophylactic metronidazole plus azithromycin that was given to 241 women (antibiotics, 118 women; placebo, 123 women) with a previous preterm delivery to prevent recurrent preterm delivery. Endometrial cultures and histologic types were obtained at randomization and 2 weeks after treatment. The prevalence of either the new acquisition or the resolution of individual microbes, categories of microbes, and plasma cell endometritis were compared by chi-square or Fishers' exact tests.

**RESULTS:** Overall, antibiotics were associated with lower acquisition and higher resolution of microbes. Of women without *Gardnerella* at baseline, 14% of the women who received antibiotics vs 34% of the women who received placebo had positive endometrial culture for the organism after treatment ($P < .05$); of those women with *G vaginalis* at baseline, 57% of the women who received antibiotics vs 33% of the women who received placebo ($P < .05$) had a negative follow-up culture. Other gram-negative rods, especially aerobes in general, manifested similar patterns. The impact on anaerobes and plasma cell endometritis was not definitive, but there was a trend toward the increased resolution of the former (77% vs 55%) and reduced acquisition of the latter (28% vs 50%).

**CONCLUSION:** The antibiotic regimen prevented the acquisition and promoted the resolution, but not the eradication, of gram-negative rods such as *G vaginalis* and the aerobic subcategory.

**Key words:** endometrium, microbial flora, antibiotic

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There is an accumulating body of evidence that suggests that the human endometrium is not sterile but that it harbors a microbial flora.[1] Indeed, findings from a large observational study revealed that 82% of women had endometrial cultures that were positive for at least 1 microorganism approximately 3 months after delivery.[2] It is thought that microbial colonization of the upper genital tract that leads to infection of the chorioamnion and amniotic fluid might play a key role in the cause of spontaneous preterm birth.[3-9] Results of antibiotic intervention studies that have focused frequently on bacterial vaginosis for the prevention of preterm birth have been mixed and do not support the routine use of antibiotics.[10] However, in all of these studies, antibiotics were administered after the first trimester, and investigators have hypothesized that this may be too late in the disease process because preexisting chronic endometrial colonization may be a determinant.[2] In a recent double-blind randomized clinical trial, a 1-week course of interconception prophylactic antibiotics (metronidazole plus azithromycin) or placebo was administered every 4 months to 241 women who were at high risk for preterm delivery.[11] These women had endometrial cultures and histologic types that were evaluated at randomization and 2 weeks after the initial treatment. Antibiotics did not reduce the prevalence of subsequent preterm delivery among the 124 women who conceived; instead, there was a trend toward lower birth weight and gestational age. Overall among the 241 women who were assigned randomly to a group, 49% of women who were treated with antibiotics demonstrated a decrease in the number of individual culture-isolated microbial species, compared with only 31% of women who received placebo ($P = .01$). Only 22% vs 29% of women, respectively, demonstrated a resolution in plasma cell endometritis ($P = .4$). We hypothesized that the impact of this regimen on the endometrium may vary, depending on baseline demographic, microbial, or histologic characteristics of individual study women. Therefore, our primary objective was to evaluate com-

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prehensively the impact of the antibiotic regimen on the endometrial microbial flora and histologic type. As a secondary objective, we evaluated whether the impact of antibiotics was modified by individual baseline type.

## MATERIALS AND METHODS

We performed secondary analyses of data that were obtained as part of a double-blind placebo-controlled randomized clinical trial that was conducted at the Center for Women's Reproductive Health at the University of Alabama at Birmingham. The detailed methods have been described previously.[2,11] Briefly, the Center for Women's Reproductive Health research personnel recruited women with singleton pregnancies that ended in a spontaneous preterm birth or pregnancy loss between 16 and 34 weeks of gestation before discharge from the hospital. *Spontaneous preterm birth* was defined as birth after spontaneous preterm labor or premature rupture of membranes. Women who were not at risk of becoming pregnant (had a cesarean hysterectomy or sterilization or had levonorgestrel [Norplant] or intrauterine device placement) were excluded from the study, as were those women with a multiple gestation or fetal anomaly in the index pregnancy. The Institutional Review Board approved the study, and all participating women provided written informed consent. Enrollment occurred between January 1998 and August 2001.

At approximately 4 months after delivery, the study candidates were evaluated; after pregnancy was excluded, baseline endometrial specimens were obtained, and cultures were performed for aerobic and anaerobic bacteria and for *Ureaplasma urealyticum*, *Mycoplasma* species, *Neisseria gonorrhoeae*, group B *Streptococcus*, *Trichomonas vaginalis*, and a ligase chain reaction test for *Chlamydia trachomatis*. Histopathologic testing was also performed on the endometrial specimens. The methods for cultures, chlamydia ligase chain reaction, histologic type, and method of endometrial sampling have all been described previously.[2] During this initial visit, these nonpregnant women were assigned randomly in a double-blind fashion to an active-drug group or a placebo group, with the use of a computerized random number sequence with a block size of 4 and stratified according to gestational age at delivery of the index pregnancy ($<$23 or $\geq$23 weeks of gestation) and time from index delivery to randomization ($\leq$4 or $>$4 months). The active-drug group received 2 doses of azithromycin; 1.0 g was given 4 days apart plus sustained-release metronidazole 750 mg daily for 7 days. The control group received identical-appearing placebos that contained the same fillers and coatings as the active drug medications. The same treatment was repeated every 4 months until conception or until the study was terminated. Study women were reevaluated 2 weeks after randomization, at which time repeat endometrial cultures were performed and histopathologic types were determined. *Plasma cell endometritis* was defined as the presence of any plasma cells, which were seen with $\times$40 magnification.

Follow-up evaluations continued through March 2003, during which time 134 of 241 women conceived. Seven women electively terminated the pregnancy; there were no outcome data for 3 of the women. The outcomes of the remaining 124 pregnancies and the impact of antibiotics have been reported.[11] In the current study, we focused on the impact of antibiotics on the larger randomized population of 241 women.

The primary outcomes were the proportions of women who acquired a microbe, category of microbes, or plasma cell endometritis when these were not present at baseline (percentage of acquisition). Alternatively, if a microbe, category of microbes, or plasma cell endometritis was present at baseline, we determined the proportions of women with a corresponding change to a negative culture or histologic type at follow-up evaluation (percentage of resolution). The secondary outcomes involved proportions of acquisition and resolution stratified by the baseline characteristics.

Baseline demographic comparisons were performed with chi-square test or the Student *t* test for categoric and continuous variables, respectively. Chi-square or Fisher's exact tests were used, as appropriate, to compare the outcome measures (proportions of acquisition and resolution) for the women who received antibiotics vs the women who received the placebo. Chi-square test for homogeneity was used to test for effect modification by baseline characteristics. For all comparisons, a probability value of $<$.05 indicated statistical significance. Data management and statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc, Cary, NC).

## RESULTS

Of 736 women who were screened and the total of 241 women who were studied, 118 women were assigned randomly to receive antibiotics and 123 were assigned to receive placebo (Figure). Women in both arms of the study had similar baseline sociodemographic characteristics (Table 1), which included age, race, marital status, smoking, education, previous miscarriage, gestational age at previous delivery, and time from randomization to index delivery. Culture results were available for 95 women (80.5%) who received antibiotics vs 104 women (84.6%) who received placebo ($P$ = .41). Histologic results were available for 54 women (45.8%) who received antibiotics vs 55 women (44.7%) who received placebo ($P$ = .87). In addition, when the study was restricted to the subsamples of women with available culture results (n = 199) or women with histologic findings (n = 109), women in both treatment arms still maintained a similar sociodemographic profile (results not shown).

In Table 2, we present primary outcome results that correspond to the prevalence of acquisition or resolution of individual microbes and categories of microbes after treatment. In general, the acquisition of microbes was lower, and their resolution was higher among antibiotic-treated women, compared with women who received placebo. The acquisition of gram-negative rods, such as the aerobic subgroup and *Gardnerella*