American Journal of Obstetrics and Gynecology (2006) **194**, 954–60

ELSEVIER

American Journal of Obstetrics & Gynecology

www.ajog.org

Antepartum vaginal bleeding, fetal exposure to oral pathogens, and risk for preterm birth at <35 weeks of gestation

Kim A. Boggess, MD,[a] Kevin Moss,[b] Amy Murtha, MD,[c] Steven Offenbacher, DDS, PhD,[b] James D. Beck, PhD[b]

Department of Obstetrics and Gynecology,[a] and the Center for Oral and Systemic Diseases,[b] University of North Carolina at Chapel Hill, Chapel Hill, NC; Department of Obstetrics and Gynecology, Duke University Medical Center,[c] Durham, NC

Received for publication December 9, 2005; revised February 5, 2006; accepted February 15, 2006

KEY WORDS
Vaginal bleeding
Preterm birth

Objective: The purpose of this study was to determine the risks for fetal exposure to oral pathogens and the relationship between vaginal bleeding and fetal exposure in preterm birth risk.
Study design: An analysis of prospectively collected data for the longitudinal Oral Conditions and Pregnancy Study was conducted. Maternal factors that potentially were associated with fetal exposure to oral pathogens (defined as detection of umbilical cord serum Immunoglobulin M to 1 of 5 oral pathogens) were examined, and the role of vaginal bleeding and fetal exposure to oral pathogens in preterm birth risk was explored. Preterm birth was defined as delivery at <35 weeks of gestation. An adjusted relative risk (95% CI) for fetal exposure was calculated. Adjusted hazard ratios (95% CI) were calculated for preterm birth among women whose data were stratified by the presence/absence of bleeding and/or fetal exposure to oral pathogens.
Results: There were complete data for 661 women; 230 women (34.8%) with and 431 women (65.2%) without fetal exposure to oral pathogens. In multivariable analysis, first- or second-trimester bleeding and white race were associated significantly with fetal exposure to oral pathogens (adjusted relative risk, 1.8 [95% CI, 1.3-2.5] and 1.3 [95% CI, 1.1-1.7], respectively). The adjusted hazard ratio for preterm birth among women with first- or second-trimester bleeding and fetal exposure to oral pathogens was 6.4 (95% CI: 2.6-16.0).
Conclusion: Vaginal bleeding is associated with fetal exposure to oral pathogens, which increases preterm birth risk. Whether bleeding is the cause of or result of fetal exposure to oral pathogens remains to be determined.

Preterm birth continues to be a significant problem in modern obstetrics. Several maternal factors have been associated with preterm birth; however, successful preterm birth prevention remains elusive. We previously reported that maternal clinical periodontal disease, which is a chronic oral infection, is associated with

Supported by National Institutes of Health grants K08 HD043284, R01 DE12453 and the University of North Carolina General Clinical Research Center grant RR00046.
Presented at the 26th Annual Meeting of the Society for Maternal Fetal Medicine, January 30-February 4, 2006, Miami, Florida.
Reprints will not be available from the authors.

0002-9378/$ - see front matter © 2006 Mosby, Inc. All rights reserved.
doi:10.1016/j.ajog.2006.02.026

preterm birth[1-5]; there are preliminary data to suggest that antepartum treatment of periodontal disease reduces preterm birth risk.[6,7] We also have reported that fetal exposure to oral pathogens, which are detected by identification of oral pathogen-specific immunoglobulin M (IgM) in umbilical cord serum, is associated with preterm birth,[4] and this association may be mediated by a fetal inflammatory response.[8] The mechanism of fetal exposure to oral pathogens is unknown and may include systemic dissemination of pathogens from the mouth to the uterus, decidua, or placenta or the ascent of oral pathogens that are present in the vagina and subsequent colonization of amniotic membranes. It is unknown what maternal factors increase the risk for fetal exposure to oral pathogens and whether interruption of fetal exposure to oral pathogens is a potential avenue to pursue for preterm birth prevention.

Maternal antepartum vaginal bleeding has been associated with a modest increase in preterm birth risk.[9,10] Frequency, timing, and quantity of vaginal bleeding modifies this risk, such that women with >1 episode of vaginal bleeding in the second or third trimester or women with excessive bleeding have the highest risk for preterm birth.[11] Detailed understanding of the causes and consequences of vaginal bleeding are lacking, and it is unknown whether antepartum bleeding is the cause or a marker for some other maternal factor that predisposes to preterm birth.

We hypothesized that antepartum vaginal bleeding was associated with fetal exposure to oral pathogens, either by systemic dissemination of oral pathogens to the reproductive tract that causes local (decidual) inflammation and vaginal bleeding or, alternatively, disruption of the maternal-fetal interface could occur that results in vaginal bleeding, which serves as a conduit for maternal oral pathogens to gain access to the fetal compartment. Although it is difficult to distinguish between these 2 possibilities, we thought it important to identify maternal risk factors for fetal exposure to oral pathogens and to determine the interaction between vaginal bleeding as a risk factor and fetal exposure to oral pathogens on preterm birth risk.

The objective of this analysis was to investigate maternal factors that are associated with fetal exposure to oral pathogens. We hypothesized that antepartum vaginal bleeding would increase the risk for fetal exposure to oral pathogens and sought to determine the relationship between antepartum vaginal bleeding and fetal exposure to oral pathogens in mediating preterm birth risk.

Methods

This study was a planned secondary analysis of data that had been collected as part of the prospective, observational Oral Conditions and Pregnancy study, which was a study of the relationship between maternal periodontal disease and preterm birth. The sample size was determined by the primary analysis for the outcome of preterm birth.[12] Detailed methods of the Oral Conditions and Pregnancy study has been published previously.[13] Briefly, Institutional Review Board approval was granted to conduct the study, and written informed consent was obtained from all study participants. Eligible healthy women with a singleton pregnancy were enrolled at <26 weeks of gestation. Gestational age was assigned on the basis of the last menstrual period that was confirmed by a first- or second-trimester ultrasound examination. Demographic information, health behavior, and medical history data were obtained by patient questionnaire at the first visit and were reviewed by a physician at the first prenatal visit. Information on events of the pregnancy, labor and delivery, and health of the neonate were collected from the medical record, laboratory and pathologic data, and the infant's medical record and was entered in the Oral Conditions and Pregnancy study database (Microsoft Access, 1997 SR2; Microsoft Corporation, Redmond, WA). *Vaginal bleeding* was defined as present or absent and by first (<14 weeks of gestation) versus second (14-24 weeks of gestation) trimester and ascertained by a questionnaire that was administered by study personnel within 48 hours of delivery, at which time the women were asked about vaginal bleeding episodes during pregnancy. *Preterm birth* was defined as delivery at <35 weeks of gestation. Women with placenta previa, other clinical explanations for vaginal bleeding (rupture of membranes, labor), or intrauterine fetal death were excluded from this analysis.

Umbilical cord blood was collected at delivery and centrifuged, and the subsequent serum was stored at $-80°C$ for analysis for fetal IgM to 5 oral pathogens, *Prevotella micros, P nigrescens, Porphyromonas intermedia*, *Campylobacter rectus,* and *Fusobacterium nucleatum*.[4] Specimens were subjected to gel exclusion chromatography to separate fetal IgM from maternal IgG and analyzed with the use of checkerboard immunoblotting assay.[4,14] Briefly, for IgM separation, 100 μL of umbilical cord serum was loaded onto a chromatography column that was packed with separation gel and eluted with buffer into equal volume fractions. Elutes were followed spectrophometrically at optical density 280 and immunoblotted with anti-IgG and with anti-IgM to confirm the high molecular weight IgM that contained fraction and the separation from maternal IgG. For checkerboard analysis, bacterial antigens that were generated from sonicated suspensions of oral pathogens were deposited in parallel lanes on nitrocellulose membranes (Hybond ECL; Amersham, Arlington Heights, IL). To assay for oral pathogen-specific IgM, umbilical cord serum IgM fractions in 1:10 dilution were deposited in lanes that were perpendicular to the