American Journal of Obstetrics and Gynecology (2005) **192**, 887–91

American Journal of OBSTETRICS and GYNECOLOGY
www.ajog.org

Assessment of cervical antibody concentrations fails to enhance the value of cervical length as a predictor of preterm delivery

Rodney K. Edwards, MD, MS,[a] Ronald J. Ferguson, PhD,[a] Jonathan J. Shuster, PhD,[b] Douglas Theriaque, MS,[b] Susan Gentry, MSN, ARNP,[a] Patrick Duff, MD[a]

Departments of Obstetrics and Gynecology[a] and Statistics,[b] University of Florida College of Medicine, Gainesville, Fla

Received for publication July 6, 2004; revised September 11, 2004; accepted October 5, 2004

KEY WORDS
Antibody concentration
Bacterial vaginosis
Preterm delivery

Objective: The purpose of this study was to determine if cervical fluid antibody concentrations can enhance the value of cervical length in predicting risk of preterm delivery.
Study design: We obtained cervical fluid samples with preweighed cellulose wicks from a prospective cohort of women 23 to 32 weeks' gestation with signs and symptoms of preterm labor and intact membranes. Total immunoglobulin A and G (IgA and IgG) concentrations were determined by enzyme-linked immunosorbent assay. Bacterial vaginosis was diagnosed by Gram stain, and cervical length was measured with endovaginal ultrasound.
Results: For subjects with term (n = 77) and preterm (n = 24) deliveries, median IgA and IgG concentrations were 736 vs 643 μg/mL (P = .33) and 1528 vs 1769 μg/mL (P = .85). For subjects with normal flora (n = 71), intermediate flora (n = 14), and bacterial vaginosis (n = 16), median IgA and IgG concentrations were 717, 624, and 774 μg/mL (P = .90) and 1383, 1553, and 2731 μg/mL (P = .02). In a forward stepwise logistic regression model, cervical length was the only factor associated with preterm delivery ($P < .001$).
Conclusion: Measuring the concentrations of IgA and IgG in cervical fluid does not enhance the value of cervical length in predicting risk of preterm delivery.

More than 12% of all births occur before 37 weeks' gestation.[1] The complications of preterm birth cause more than 70% of the deaths of nonanomalous neonates, and are responsible for the majority of morbidity suffered by such infants.[2]

Bacterial vaginosis is a polymicrobial infection that is an established risk factor for preterm delivery.[3-6] Bacterial vaginosis is characterized by an increase in vaginal pH, an amine odor, and an increased bacterial count. In this disorder, there is a replacement of the naturally predominant bacteria in the vaginal flora (*Lactobacillus* sp.) by a complex mixture of anaerobic and facultative anaerobic bacteria.[7,8] The mechanism by which bacterial vaginosis causes preterm delivery is thought to be due to ascension of organisms through the cervix and into the uterus.[9]

Supported by the 2001-2003 Society for Maternal-Fetal Medicine Scholarship Award and NIH #M01-RR00082.
Reprints not available from the authors.

0002-9378/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ajog.2004.10.590

Bacteria colonize the vagina both in patients with normal vaginal flora and in those with bacterial vaginosis. Because the uterus normally is sterile, it seems logical that factors at the level of the cervix are involved in maintaining the sterility of the upper genital tract. The length of the cervix, measured with endovaginal ultrasound examination, is inversely related to the risk of preterm delivery.[10-12] However, the reason for this relationship is not well defined.

Cervical mucus displays antimicrobial properties in both nonpregnant and pregnant women.[13-15] Secretory antibodies, or immunoglobulins, function to perform immune exclusion at mucosal surfaces. Immunoglobulin A (IgA) and immunoglobulin G (IgG) are the predominant classes of antibody recoverable from the endocervix.[16] These antibodies act to exclude vaginal microorganisms from the upper genital tract. We speculated that the reason for the inverse relationship between cervical length and preterm delivery was that the cervix functions as an "immune tunnel" between the vagina and the uterine cavity. A shorter cervical canal and/or a lower concentration of antibodies within this cervical canal might allow organisms easier access to the uterine cavity. The objective of this study was to determine whether measuring the concentrations of antibodies in cervical fluid could further refine the risk of preterm delivery associated with cervical length.

Material and methods

We performed a prospective observational cohort study at Shands Hospital at the University of Florida. Subjects were enrolled from September 2001 to March 2003. Women were eligible for inclusion in this cohort if they presented to the labor and delivery unit for evaluation of uterine contractions, had a singleton gestation, and were between 23 and 32 weeks' gestation. Exclusion criteria included ruptured membranes, human immunodeficiency virus (HIV) infection, placenta previa or abruption, cervical dilation $\geq$3 cm, or administration of antibiotics within the preceding 2 weeks. In addition, women with clinical chorioamnionitis at the time of presentation were excluded. We defined clinical chorioamnionitis as a temperature $\geq$38.0°C and 1 or more of: maternal heart rate >100 beats per minute, fetal baseline heart rate >160 beats per minute, or uterine tenderness. The study was conducted in accordance with the guidelines established by the University of Florida Health Center Institutional Review Board.

After informed written and oral consent was obtained, and before any manipulation of the cervix, a sterile speculum examination was performed on each subject. Cervical fluid samples were obtained by placing a cellulose acetate wick (UniWick™, Whatman, Clifton, NJ) approximately 5 mm into the cervical os with a sterile forceps, taking care not to contact the vagina or vaginal fluid. The wick was left in place for approximately 60 seconds and removed. Wicks were stored in preweighed microcentrifuge tubes before sample collection. After sample collection, the wicks were returned to the same tube, taken immediately to the laboratory, postweighed, and stored at –80°C until fluid was eluted from the wicks.

At the time of the speculum examination, a swab from the vaginal fornix was obtained from each subject and used to prepare a slide for Gram stain for diagnosis of bacterial vaginosis using the method of Nugent et al.[17] The speculum then was removed, and the cervical length was measured with a real-time endovaginal ultrasound examination, according to the method described by Iams et al.[10]

For elution of fluid from the cervical wicks, 600 μL of elution buffer was added to each microcentrifuge tube containing a wick. This solution was composed of phosphate-buffered saline (PBS) with 2% Triton X-100, 0.2 mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride, 10 μmol/L leupeptin, 1 μg/mL aprotinin, and 3.25 μmol/L bestatin. Fluid was extracted on ice for 30 minutes, vortexing 3 to 4 times. Contents of the microcentrifuge tubes then were transferred into centrifuge filter tubes (VectaSpin 3™; Whatman, Maidstone, England) and centrifuged at 4°C for 12 minutes at 3000*g*. Supernatants were removed and used for analysis. Using known concentrations of antibodies absorbed by wicks (Cappel purified human secretory IgA and purified human IgG; ICN Pharmaceuticals, Aurora, Ohio), we determined that this elution process recovered >95% of antibody from the wicks (data not shown). We assumed that cervical fluid has a density equivalent to water (1 g/mL). Because we knew the difference in weight before and after sample collection and used a constant volume of elution buffer, true concentrations of antibodies in the cervical fluid could be calculated.

IgA and IgG enzyme-linked immunosorbent assays (ELISAs) were performed using modifications of the techniques described by Quesnel et al.[16] Briefly, for the IgA ELISA, Fisher*brand* high binding 96-well plates (Fisher Scientific, Pittsburgh, Pa) were coated with 2.5 μg/mL of Cappel goat affinity-purified antibody to human IgA (α-chain; ICN Pharmaceuticals) in carbonate buffer pH 9.6 as the primary antibody and a 1:2000 dilution of Cappel peroxidase-conjugated sheep affinity-purified antibody to human IgA (α-chain; ICN Pharmaceuticals) in PBS, pH 7.4, with 0.05% Tween 20 (PBS-T) as the secondary antibody solution. For the IgG ELISA, Fisher*brand* high binding 96-well plates were coated with 5 μg/mL of Cappel goat affinity-purified antibody to human IgG Fc (ICN Pharmaceuticals) in carbonate buffer pH 9.6 as the primary antibody, and a 1:8000 dilution of Cappel