A 3-dimensional power Doppler scan of 4 nuchal cord loops provided a clue to the etiology of nonplacental intrauterine growth restriction in an anatomically normal fetus, underscoring the value of 3DUS in fetal evaluation.

Page 320.

Sonogram courtesy of Dan V. Valsky, MD, and Simcha Yagel, MD, Obstetrics and Gynecology Ultrasound Center, Hadassah-Hebrew University Medical Centers, Mt. Scopus, Jerusalem, Israel.

Editorial

221 One size does not fit all
Robert Resnik
See related article, page 239

Reviews

223 Maternal obesity and risk of stillbirth: a metaanalysis
Susan Y. Chu; Shin Y. Kim; Joseph Lau; Christopher H. Schmid; Patricia M. Dietz; William M. Callaghan; Kathryn M. Curtis
This metaanalysis indicates an association between maternal obesity and the risk of stillbirth.

229 Prophylactic subcutaneous drainage for prevention of wound complications after cesarean delivery—a metaanalysis
Elizabeth K. Hellums; Monique G. Lin; Patrick S. Ramsey
Prophylactic use of subcutaneous drainage does not prevent significant wound complications after cesarean delivery.

Clinical Opinion

236 The intrapartum deceleration in center stage: a physiologic approach to the interpretation of fetal heart rate changes in labor
Jenny A. Westgate; Bert Wibbens; Laura Bennet; Guido Wassink; Julian T. Parer; Alistair J. Gunn
The mechanisms of intrapartum decelerations and associated changes in the fetal heart rate recording are reviewed in relation to their ability to predict fetal compromise in labor.
RESEARCH

OBSTETRICS

239 Small-for-gestational-age infants classified by customized or population birthweight centiles: impact of gestational age at delivery
Katie M. Groom; Katrina K. Poppe; Robyn A. North; Lesley M. E. McCowan
The use of customized birthweight centiles classify more preterm infants as small for gestational age, compared with population birthweight centiles.

EDITORS’ COMMENTARY: Every American obstetrician should read the full article by Groom et al and the accompanying editorial comment by Dr. Resnik. We have lost sight of the distinction between “small for gestational age” (SGA) and “intrauterine growth restriction” (IUGR) to the potential detriment of our patients. Dr. Groom and her colleagues remind us that an estimated fetal weight below the 10th percentile (SGA) is only a screening test that has imperfect sensitivity for truly compromised growth (IUGR). There are growth-restricted fetuses whose weight exceeds the 10th percentile, and an SGA fetus deserves additional evaluation before clinical decisions are made.

See related editorial, page 221

241 Complications of labor induction among multiparous women in a community-based hospital system
Leah Battista; Judith H. Chung; David C. Lagrew; Deborah A. Wing
Multiparous women are at increased risk for obstetric complications as the result of labor induction when compared to spontaneous labor.

See Journal Club, page 322

SELECTED PAPERS FROM THE 27TH ANNUAL SCIENTIFIC MEETING OF THE SOCIETY FOR MATERNAL–FETAL MEDICINE

244 Angiogenic factors for the prediction of preeclampsia in high-risk women
Tiffany A. Moore Simas; Sybil L. Crawford; Matthew J. Solitro; Sara C. Frost; Bruce A. Meyer; Sharon E. Maynard
Alterations in circulating angiogenic factors predict preeclampsia in high-risk women.

EDITOR’S COMMENTARY: Dr. Moore Simas and colleagues report a prospective evaluation of the performance of circulating angiogenic factors soluble fms-like tyrosine kinase-1 and placental growth factor as predictors of preeclampsia in women with common clinical risk factors for the disease. Although the sample size is small, the authors have brought recent research about alteration of angiogenic factors in preeclampsia to the clinical arena.

(continued on page 8A)
SELECTED PAPERS FROM THE 27TH ANNUAL SCIENTIFIC MEETING OF THE SOCIETY FOR MATERNAL–FETAL MEDICINE (continued)

247 The global network: a prospective study of stillbirths in developing countries
Elizabeth M. McClure; Linda L. Wright; Robert L. Goldenberg; Shivaprasad S. Goudar; Sailajanandan N. Parida; Imtiaz Jehan; Antoinette Tshefu; Elwyn Chomba; Fernando Alhabe; Ana Garces; Hillary Harris; Richard J. Derman; Pinaki Panigrahi; Cyril Engmann; Pierre Buekens; Michael Hambidge; Waldemar A. Carlo; the NICHD FIRST BREATH Study Group
In a multisite, multicountry, population-based study of stillbirths in developing countries with a mean stillbirth rate of 24 per 1000 deliveries, stillbirths were associated significantly with lower skilled providers, out-of-hospital births, and low cesarean section rates.

EDITOR’S COMMENTARY: McClure and colleagues in the Global Health Network reviewed the causes of stillbirth in developing nations and found significant opportunities for intervention.

250 Signature pathways identified from gene expression profiles in the human uterine cervix before and after spontaneous term parturition
Sonia S. Hassan; Roberto Romero; Adi L. Tarca; Sorin Draghici; Beth Pineles; Andrej Bugrim; Nahla Khalek; Natalia Camacho; Pooja Mittal; Bo Hyun Yoon; Jimmy Espinoza; Chong Jai Kim; Yoram Sorokin; John Malone Jr
Cervical remodeling after term labor is associated with the gene pathways of chemokines and adhesion, extracellular matrix remodeling, vascular endothelial growth factor–family signaling, and unsuspected pathways, such as plasmin signaling.

EDITOR’S COMMENTARY: Studies that are aimed at improved understanding of the onset and process of labor in humans have been difficult with conventional biochemical methods. Hassan and coworkers report work that is aimed at identifying “signature networks” by applying network and pathway analysis to understand the gene expression changes in the cervix. These investigations hold great promise for the understanding of cervical change, which is a central event in human reproduction.

253 Are women who have had a preterm twin delivery at greater risk of preterm birth in a subsequent singleton pregnancy?
Francesca L. Facco; Kate Nash; William A. Grobman
Idiopathic preterm birth of twins is associated with an increased risk of preterm birth in a subsequent singleton pregnancy.

EDITOR’S COMMENTARY: Facco et al studied the risk of preterm birth in singleton pregnancies in women with a previous preterm birth of twins and found a 3-fold increased risk, compared with women with a previous twin pregnancy who were delivered at term. This finding may be the result of a particularly low risk of preterm birth in the reference group, an increased risk in the study group, or both. More than a decade ago, Menard et al (Menard MK, Newman RB, Keenan A, Ebeling M. Prognostic significance of prior preterm twin delivery on a subsequent singleton pregnancy. Am J Obstet Gynecol 1996;174:1429-32), in a similar study, found a gestational age effect in which the rate of preterm birth in a singleton pregnancy increased as the gestational age of the previous twin delivery declined. In women whose previous twin pregnancy was delivered after 37 weeks of gestation, the rate of preterm birth at <37 weeks of gestation was 6.9%. The preterm birth rate increased to 9.8%, 25%, and 42% when the previous twins were born at 34-36, 30-33, and <30 weeks of gestation, respectively. At Ohio State University, we consider women with a previous twin pregnancy who were delivered spontaneously at <34 weeks of gestation to be at increased risk of preterm birth in a subsequent singleton gestation.

(continued on page 10A)
Reduced third-trimester levels of soluble human leukocyte antigen G protein in severe preeclampsia

Rinat Hackmon; Arie Koifman; Hirohito Hyobo; Hagit Glickman; Eyal Sheiner; Daniel E. Geraghty

A reduced level of maternal human leukocyte antigen (HLA)-G protein is associated with severe preeclampsia during the third-trimester; maternal HLA-G may have a diagnostic and/or etiologic role in preeclampsia.

Stillbirths in an urban community in Pakistan

Imtiaz Jehan; Elizabeth M. McClure; Sohail Salat; Sameera Rizvi; Omrana Pasha; Hillary Harris; Nancy Moss; Robert L. Goldenberg

In an urban area of a developing country, stillbirth is a common adverse pregnancy outcome that accounts for >3 of all deliveries, despite reasonable access to prenatal and obstetric care.

Prophylaxis with 17 alpha-hydroxyprogesterone caproate for prevention of recurrent preterm delivery: does gestational age at initiation of treatment matter?

Helen Y. How; John R. Barton; Niki B. Istwan; Debbie J. Rhea; Gary J. Stanziano

Initiation of 17alpha-hydroxyprogesterone caproate prophylaxis at 21-26.9 weeks, is as effective as initiation at 16-20.9 weeks.

EDITOR’S COMMENTARY: How et al compared the effect of prophylactic treatment with 17 alpha-hydroxyprogesterone caproate (17 OH-PC) on the risk of recurrent preterm birth in 906 women according to whether prophylaxis was initiated at 16-20 weeks versus 21-26 weeks. Rates of recurrent preterm birth before 37 weeks were similar to those reported by Meis et al in 2003, and did not differ according to early versus late initiation of prophylactic 17 OH-PC.

Clinical characteristics of women prescribed 17 alpha-hydroxyprogesterone caproate in the community setting

Charles Rittenberg; Scott Sullivan; Niki Istwan; Debbie Rhea; Gary Stanziano; Roger Newman

Use of 17 alpha-hydroxyprogesterone caproate in a community setting met National Institute of Child Health and Human Development study criteria including initiation at 16 to 20.9 weeks’ gestation in only 36 of cases.

A short interpregnancy interval is a risk factor for preterm birth and its recurrence

Emily A. DeFranco; David M. Stamilio; Sarah E. Boslaugh; Gilad A. Gross; Louis J. Muglia

A short interval between pregnancies is an important risk factor for preterm birth, recurrence of preterm birth, and delivery at early extremes of gestational age.

Understanding the mechanism of learning enhancement: NMDA and GABA receptor expression

Laura Tosso; Andrea Johnson; Stephanie Bissell; Robin Roberson; Daniel Abebe; Catherine Y. Spong

NAPVSIPQQ + SALLRSIPA postnatal treatment resulted in learning enhancement in aged mice that is not mediated through alterations in the N-methyl-d-aspartate and gamma-aminobutyric acid receptor subunits.
SELECTED PAPERS FROM THE 27TH ANNUAL SCIENTIFIC MEETING OF THE SOCIETY FOR MATERNAL–FETAL MEDICINE (continued)

269 Effects of acute alcohol intoxication in the second trimester of pregnancy on development of the murine fetal lung
Xiangyuan Wang; Prasra Gomutputra; Debra J. Wolgemuth; Laxmi Baxi
Acute high exposure of alcohol during midgestation disrupts the development and differentiation of fetal lungs as seen by morphological alterations and aberrant expression of Hoxb5.

271 Vascular endothelial growth factor gene +936 C/T polymorphism is associated with preeclampsia in Korean women
Jae-Yoon Shim; Jong Kwan Jun; Bok-Kyung Jung; Sung Hoon Kim; Hye-Sung Won; Pil Ryang Lee; Ahm Kim
Carriage of the +936 T allele of the vascular endothelial growth factor gene may be associated with an increased susceptibility to the development of preeclampsia.

273 Chronic hypertension and risk of placental abruption: is the association modified by ischemic placental disease?
Cande V. Ananth; Morgan R. Peltier; Wendy L. Kinzler; John C. Smulian; Anthony M. Vintzileos
The strong association between chronic hypertension and abruption appears modified by pregnancy-induced hypertension and fetal growth abnormalities.

275 The influence of maternal cigarette smoking on placental pathology in pregnancies complicated by abruption
Lilian M. Kaminsky; Cande V. Ananth; Vinay Prasad; Carl Nath; Anthony M. Vintzileos; New Jersey Placental Abruption Study Investigators
Increased intervillous thrombi and decreased placental infarcts were observed among women with abruption who smoked versus nonsmokers.

278 Antibodies to the 70 kDa heat shock protein in midtrimester amniotic fluid and intraamniotic immunity
Shari E. Gelber; Ann Marie Bongiovanni; Claudel Jean-Pierre; Iara M. Linhares; Daniel W. Skupski; Steven S. Witkin
The intraamniotic concentration of IgG antibodies to the 70 kDa heat shock protein correlates with intraamniotic levels of tumor necrosis factor-α, secretory leukocyte protease inhibitor, and interferon-α.

279 Effect of a previous pregnancy on vascular function in endothelial nitric oxide synthase 3 knockout mice
Labib M. Ghulmiyyah; Esther Tamayo; Shannon M. Clark; Gary D. V. Hankins; Garland D. Anderson; George R. Saade; Monica Longo
Vascular reactivity is improved in a subsequent pregnancy in mice that lack functional endothelial nitric oxide synthase.

(continued on page 12A)
The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network Beneficial Effects of Antenatal Repeated Steroids study: impact of repeated doses of antenatal corticosteroids on placental growth and histologic findings
Joram Sawady; Brian M. Mercer; Ronald J. Wapner; Yuan Zhao; Yoram Sorokin; Frances Johnson; Donald J. Dudley; Catherine Y. Spong; Alan M. Peaceman; Kenneth J. Leveno; Margaret Harper; Steve N. Caritis; Menachem Miodovnik; John M. Thorp; Susan Ramin; Marshall W. Carpenter; Dwight J. Rouse for the National Institute of Child Health and Human Development Maternal Fetal Medicine Units Network
Repeated weekly antenatal steroid administration reduces placental weight but does not alter the frequency of significant histopathologic placental abnormalities.

Betamethasone vs dexamethasone for the prevention of morbidity in very-low-birthweight neonates
Deborah M. Feldman; Jeannine Carbone; Laura Belden; Adam F. Borgida; Victor Herson
Betamethasone is associated with less respiratory morbidity than dexamethasone in very-low-birthweight infants.

Nucleated red blood cell counts in the first week of life: a critical appraisal of relationships with perinatal outcome in preterm growth-restricted neonates
Ahmet A. Baschat; Sadettin Gunor; Michelle L. Kush; Christoph Berg; Ulrich Gembruch; Christopher R. Harman
Failure to clear elevated nucleated red blood cell counts by day 4 of life is associated with increased perinatal morbidity in preterm growth-restricted neonates.

Differential expression of microRNAs with progression of gestation and inflammation in the human chorioamniotic membranes
Daniel Montenegro; Roberto Romero; Beth L. Pineles; Adi L. Tarca; Yeon Mee Kim; Sorin Drăghici; Juan Pedro Kusanovic; Jung-Sun Kim; Offer Erez; Shali Mazaki-Tovi; Sonia Hassan; Jimmy Espinoza; Chong Iai Kim
Human chorioamnion membranes display differential expression of a subset of microRNAs with advancing gestation and inflammation.

A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes
Kun Woo Kim; Roberto Romero; Hyun Soo Park; Chan-Wook Park; Soon-Sup Shim; Jong Kwan Jun; Bo Hyun Yoon
A rapid matrix metalloproteinase-8 bedside test is a simple and sensitive bedside test to detect intraamniotic infection/inflammation and to predict adverse outcome in preterm premature rupture of membranes.
294 The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity
Si Eun Lee; Roberto Romero; Hanna Jung; Chan-Wook Park; Joong Shin Park; Bo Hyun Yoon
The intensity of the systemic fetal inflammatory response is greater in intraamniotic inflammation that is associated with microbiologically proven infection than in cases with sterile amniotic fluid.

296 Toll-like receptors in the uterus, cervix, and placenta: is pregnancy an immunosuppressed state?
Juan M. Gonzalez; Hua Xu; Ella Ofori; Michal A. Elovitz
The innate immune system is a dynamic system during gestation. Immunosuppression during pregnancy appears to be valid only in the placenta in regards to TLR expression.

299 A French randomized controlled trial of ST-segment analysis in a population with abnormal cardiotocograms during labor
Christophe Vayssière; Eric David; Nicolas Meyer; Renaud Haberstich; Valérie Sebahoun; Emmanuel Roth; Romain Favre; Israël Nisand; Bruno Langer
In a population with abnormal cardiotocography and liberal use of scalp pH, ST-segment analysis did not reduce the operative delivery rate for nonreassuring fetal status.

301 Intrapartum electronic fetal heart rate monitoring and the identification of metabolic acidosis and hypoxic-ischemic encephalopathy
Joel D. Larma; Anadir M. Silva; Cynthia J. Holcroft; Richard E. Thompson; Pamela K. Donohue; Ernest M. Graham
Although fetuses who experience hypoxic-ischemic encephalopathy exhibit more bradycardia, decreased variability, and nonreactivity, these abnormalities have poor predictive value in the identification of these infants.

303 Predictors of umbilical artery acidosis in preterm delivery
Marianna Andreani; Anna Locatelli; Francesca Assi; Sara Consonni; Silvia Malguzzi; Giuseppe Paterlini; Alessandro Ghidini
In preterm infants, umbilical artery acidosis is significantly more common in the presence of placental abruption, fetal distress, and histologic evidence of placental vascular disease.

306 Cesarean delivery outcomes after a prolonged second stage of labor
Joyce F. Sung; Kay I. Daniels; Laura Brodzinsky; Yasser Y. El-Sayed; Aaron B. Caughey; Deirdre J. Lyell
Prolonged second stage of labor is associated with an increase in the frequency of unintentional hysterotomy extensions at cesarean delivery and with the length of surgery.

308 Predictors of failed operative vaginal delivery: a single-center experience
Avi Ben-Haroush; Nir Melamed; Boris Kaplan; Yariv Yoge
Failure of operative delivery occurs more often with vacuum extraction than with forceps and is more common in cases of fetal macrosomia and fetal head in the occiput posterior position and in the absence of analgesia.
SELECTED PAPERS FROM THE 27TH ANNUAL SCIENTIFIC MEETING OF THE SOCIETY FOR MATERNAL–FETAL MEDICINE (continued)

310 Risk factors for sonographic internal anal sphincter gaps 6-12 months after delivery complicated by anal sphincter tear
Catherine S. Bradley; Holly E. Richter; Robert E. Gutman; Morton B. Brown; William E. Whitehead; Paul M. Fine; Christiane Hakim; Frank Harford; Anne M. Weber; Pelvic Floor Disorders Network
The presence of sonographic internal anal sphincter gaps 6-12 months after delivery complicated by sphincter laceration was associated with laceration extent, episiotomy, and race.

312 Stepwise sequential screening for fetal aneuploidy
Peter A. Benn; Winston A. Campbell; Carolyn M. Zelop; Charles Ingardia; James F. X. Egan
Sequential provision of first- and second-trimester screening for fetal aneuploidy can be introduced successfully and reduces the number of invasive tests.

315 Cervical length ≤25 mm in low-risk women: a case control study of cerclage with rest versus rest alone
Maddalena Incerti; Alessandro Ghidini; Anna Locatelli; Sarah H. Poggi; John C. Pezzullo
Cerclage placement does not improve pregnancy outcome in low-risk women with incidental detection of cervical length ≤25 mm in the early second trimester.

317 Transabdominal cerclage after comprehensive evaluation of women with previous unsuccessful transvaginal cerclage
Robert H. Debbs; Guillermo A. DeLa Vega; Stephanie Pearson; Harish Sehdev; Dominic Marchiano; Jack Ludmir
Transabdominal cerclage has a low risk of fetal loss and delivery before 24 weeks of gestation in women with previous unsuccessful transvaginal cerclage procedures.

319 Histologic evidence of inflammation and risk of placental abruption
Carl A. Nath; Candie V. Ananth; John C. Smulian; Susan Shen-Schwarz; Lillian Kaminsky; New Jersey–Placental Abruption Study Investigators
Severe histologic chorioamnionitis is associated with abruption in both preterm and term gestations, implicating inflammation as a potential contributor to causal pathways.

JOURNAL CLUB

322 Discussion: ‘Complications of labor induction among multiparous women’ by Battista et al
William A. Grobman; George A. Macones
See related article, page 241, and full discussion, page e1
Can you explain the difference between internal and external validity? This study offered a good opportunity to ponder these important statistical concepts.

IMAGES IN OBSTETRICS

324 Overburdened and undernourished
Angelika Bord; Simcha Yagel; Dan V. Valsky
Doppler ultrasound identified the source of severe, symmetric intrauterine growth restriction in a fetus at 30 weeks’ gestation. Delivery occurred 1 week later.

(continued on page 17A)
LETTERS TO THE EDITORS

325 Comment on single-dose methotrexate regimen in the treatment of low-risk gestational trophoblastic neoplasia
Niraj N. Mahajan; Rajani N. Soni; Kshitija N. Mahajan

325 Reply
Karen K. L. Chan; Hextan Y. S. Ngan

325 Randomized, double-blind, placebo-controlled trial of transdermal nitroglycerin for preterm labor
Anwar H. Nassar; Ihab M. Usta

326 Reply
Graeme N. Smith; Mark C. Walker; On behalf of the Canadian Preterm Labour Nitroglycerin Trial Queen’s University

327 Faulty interpretation of observed racial disparity in recurrent preterm birth
Jay S. Kaufman; Arline T. Geronimus; Sherman A. James

327 Reply
Michael R. DeBaun; Louis J. Muglia

328 Racial disparity in preterm birth: role of social determinants
Aubrey L. Spriggs

329 Psychosocial contributors to preterm birth must be considered
Stephanie Z. Moultrie

329 Reply
Louis J. Muglia; Michael R. DeBaun

330 Commentary on microarray analysis for gynecologists and obstetricians
Ching-Ming Liu

331 Reply
Roberto Romero; Gerard Tromp

CORRECTIONS

332 Robotic hysterectomy: technique and initial outcomes
Kho et al

332 Maternal herpes simplex virus antibody avidity and risk of neonatal herpes
Brown et al

333 Administration of cefazolin prior to skin incision is superior to cefazolin at cord clamping in preventing post-cesarean infectious morbidity: a randomized controlled trial
Sullivan et al

333 Teaching and practice of pelvic floor muscle exercises in primiparous women during pregnancy and the postpartum period
Fine et al

Worth 1000 words

The editors invite submissions to new sections of the Journal that focus on visually arresting clinical materials.

Images in Obstetrics, Images in Gynecology
Have you encountered a remarkable clinical image—a clinical photograph or results of a diagnostic test such as an ultrasound scan, MRI film, slide, photomicrograph, DNA blot, or similar—that elucidates some aspect of obstetrics or gynecology in a way that words alone could never achieve?

Share it with AJOG readers in one of these new sections, published in alternating issues.

A short version of the text, with 1 image, appears in print; a longer version, typically with more images, online.

Surgeon’s Corner
This quarterly offering describes a new surgical technique or instrument or a new application of a standard one. Step-by-step photos are welcome. AJOG’s print edition includes a sample image and synopsis, referring readers to the full-length version online. Authors are encouraged to provide a video clip, with discussion, to be posted online with the article.

For manuscript length and other requirements, please see the Information for Authors at www.AJOG.org.


**Journal Club Roundtable**

**e1** Discussion: ‘Complications of labor induction among multiparous women’ by Battista et al
Moderator: William A. Grobman; discussants: Susan E. Gerber; Svena D. Julien; Emily J. Su; LaTasha D. Nelson; Francesca L. Facco; Deborah S. Lindner; Melissa A. Dugan

Induction of labor has become rather common. Journal Club members thought efforts to investigate the procedure’s outcome in multiparous women were both well-planned and informative.

**Case Report**

**e5** Successful twin pregnancy after vaginal radical trachelectomy using transabdominal cervicoisthmic cerclage
Keun-Young Lee; Hyun-Ah Jun; Ju-Won Roh; Ji-Eun Song

We report the first successful twin pregnancy after vaginal radical trachelectomy using transabdominal cervicoisthmic cerclage.

**e7** An unusual case of acute hyperkalemia during pregnancy
Amir Said Alizadeh Naderi; Biff F. Palmer

We report a rare case of acute hyperkalemia in the setting of maternal chronic kidney disease, intrauterine fetal demise, and uterine rupture.
MANUSCRIPT SUBMISSIONS

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One size does not fit all

Robert Resnik, MD

In 1963 Lubchenco et al\(^1\) published their classic paper that showed that neonatal mortality risk increased at every gestational age when the birthweight fell below the 10th centile. These findings served to bring the concept of fetal intrauterine growth restriction (IUGR) into sharp focus and led to radical changes in the way in which obstetricians diagnosed and managed such pregnancies as well as the care provided for the newborns. The Denver fetal growth curves became widely utilized, and many more were published, taking advantage of much larger populations of pregnant women.

These population-based growth curves took gestational age and sex into consideration and set the normal range for fetal weight in the United States to be between 2 SD from the mean or between the 10th and 90th centiles at any given gestational age. The term small for gestational age (SGA) was frequently used to describe newborns whose weights fell below the 10th centile and was often used interchangeably with IUGR.

We have come to recognize, however, that there are many newborns whose weights are around or below the 10th centile, who are constitutionally small but normal, and have none of the complications associated with suboptimal fetal growth. When their growth and function is evaluated by ultrasound across gestation, they exhibit normal growth velocity and amniotic fluid volume and have normal umbilical artery flow as measured by Doppler. Conversely, some newborns have birth weights well within the normal range and would not be classified as SGA but have many of the metabolic, hematologic, and neurologic alterations seen in growth-restricted infants whose weight are less than the 10th centile.

These observations formed the basis for the development of customized rather than population-based fetal growth standards. In contrast to population-based growth standards, customized fetal growth standards utilize optimal birth weight as the endpoint of a growth curve and are based on the ability of any individual fetus to achieve its growth potential determined prospectively, independent of maternal pathology, and with consideration of variables such as maternal ethnicity, parity, height, and weight in early pregnancy as well as fetal sex.

All of these variables have been shown to be of significant importance in determining fetal growth potential. Drooger et al\(^2\) demonstrated the effects of ethnic differences on prenatal growth among immigrant women in Holland, compared with native Dutch women, and similar findings have been reported elsewhere in Europe\(^3\) and Australia.\(^4\) Fetal growth velocities prior to 32 weeks’ gestation have also been reported to differ between French and Chinese infants.\(^5\) These studies collectively and clearly show that there may be significant differences in fetal growth not only between populations of women but also within the same population group, thus emphasizing the need for individualizing expectations for optimal fetal weight based upon individual potential.

In the current study by Groom,\(^6\) the outcomes of 17,855 New Zealand women of various ethnic groups were studied, comparing customized to population birth weight centiles, and it was shown that using customized centiles, more preterm infants were identified as SGA, whereas at term more were classified as SGA using population centiles. Perinatal deaths among preterm infants occurred only in those classified as SGA by customized standards, an important observation in that prematurity and true fetal growth restriction are frequent co-morbidities. In contrast, there were no perinatal deaths and low rates of preterm birth for SGA infants classified by population percentiles, a finding reported earlier by Ego et al.\(^7\) Other investigators have also reported that customized birthweight standards more accurately predict stillbirths, neonatal deaths, and neurologic morbidity.\(^8\)\(^,\)\(^9\)

Interestingly, as one peruses the references of this carefully conducted and provocative study, it is notable that with a few exceptions, almost all the research in this area has been conducted outside the United States and published in outstanding journals from other countries. It seems clear from the Groom study, as well as those by other investigators, that customized fetal growth standards have a sound physiologic and epidemiologic rationale and are more likely to discriminate between the fetus that is constitutionally small but normal from 1 with true IUGR. The population standards that have been used in this country have been useful and have served us well but do not adequately take into account the many variables that influence fetal growth. One size does not fit all, and it would seem to be an appropriate time for American obstetricians to adopt the use of customized fetal growth standards.

REFERENCES


OBSTETRICS

Maternal obesity and risk of stillbirth: a metaanalysis

Susan Y. Chu, PhD, MSPH; Shin Y. Kim, MPH; Joseph Lau, MD; Christopher H. Schmid, PhD; Patricia M. Dietz, DrPH; William M. Callaghan, MD, MPH; Kathryn M. Curtis, PhD

The rate of stillbirths in the United States has declined substantially since the 1950s, primarily because of improvements in medical care. But they are still not rare events, with nearly 7 stillbirths occurring per 1000 deliveries (live and stillbirths); in the year 2000, there were 27,003 of these deaths. A number of risk factors for stillbirths have been identified, which include fetal abnormalities, intrauterine growth restriction, abruptio placentae, infection, older maternal age, and smoking during pregnancy, yet the causes of almost one-half of all stillbirths are unknown. Several epidemiologic studies have reported an increased risk of stillbirth among women who are obese, compared with normal-weight women, but the magnitude of that association remains uncertain. Because of the high and increasing prevalence of obesity in the United States (nearly 1 in 3 women is obese), this relationship is important to define because even a modest effect of obesity could have substantial population impact. We performed a systematic review and metaanalysis to summarize the available epidemiologic evidence on the relationship between maternal overweight and obesity and the risk of stillbirth.

We conducted this metaanalysis to summarize the available epidemiologic evidence on the relationship between maternal overweight and obesity and the risk of stillbirth. We identified studies from 3 sources: (1) a PubMed search of relevant articles that were published between January 1980 and September 2005, (2) reference lists of publications that were selected from the PubMed search, and (3) reference lists of review articles on obesity and maternal outcomes that were published between 2000 and 2005. We used a Bayesian random effects model to perform the metaanalysis and metaregression. Nine studies were included in the metaanalysis. The unadjusted odds ratios of a stillbirth were 1.47 (95% CI, 1.08-1.94) and 2.07 (95% CI, 1.59-2.74) among overweight and obese pregnant women, respectively, compared with normal-weight pregnant women. The metaregression analysis found no evidence that these estimates were affected by selected study characteristics. Maternal obesity is associated with an increased risk of stillbirth, although the mechanisms to explain this association are not clear.

Key words: fetal death, maternal obesity, metaanalysis, stillbirth

Materials and Methods

Search process

Using recommendations from the Meta-analysis of Observational Studies in Epidemiology guidelines, we identified studies for possible inclusion in this analysis using 3 sources. First, we searched PubMed from January 1980 to September 2005 using the following criteria: overweight or obesity or body mass index (BMI) or weight gain AND pregnancy or prepregnancy AND risks or effects or complications. From this search, we retrieved the full text for abstracts that mentioned a relationship between maternal obesity and pregnancy complications from a case-control or cohort study. Studies that reported fetal death, neonatal death, stillbirth, or perinatal death as an outcome were included for consideration. Studies that did not have full text in English were translated for review.

Next, we manually reviewed the reference lists of the publications that had been retrieved previously and obtained the entire text of studies that potentially could be included in the metaanalysis. Finally, we obtained review articles on obesity and maternal outcomes that were published between 2000 and 2005 and searched through their reference lists for additional potential studies. If there were multiple articles on stillbirth from the same study population, we included only the most current publication. We did not attempt to locate any unpublished studies.

Study selection

Studies that were considered to be eligible potentially by this 3-step process were then evaluated for inclusion in the metaanalysis if they met the following criteria: (1) the study reported obesity measures (maternal weight, percent over ideal weight, body mass BMI) that reflected status preceding any significant pregnancy weight gain (ie, measured or reported prepregnancy or during the first trimester or first prenatal visit). We did not include the small number of studies that examined pregnancy weight gain and risk of stillbirth because outcomes that are related to prepregnancy weight and pregnancy weight gain often differ; (2) the study had a comparison group of normal-weight women; (3) if the study focused on perinatal deaths,
the study reported a separate risk estimate for stillbirths (the causes of stillbirths and early neonatal deaths, which are the 2 components of perinatal deaths, have notable differences7); (4) the study reported on all stillbirths, not just on a subgroup (eg, unexplained stillbirths only); and (5) the study presented data in tables, figures, or text that allowed for a quantitative measurement of obesity and risk of stillbirth.

Data abstraction
All articles were read and abstracted by 2 reviewers (S.Y.C. and S.Y.K.) who used the same structured data form. A final abstraction form was compiled from the 2 forms after correction or resolution of any differences between reviewers. Abstracted information included study design, setting, location, time period, number and characteristics of study subjects, the source and categorization of obesity measures, the sources of the outcome (eg, birth certificates, medical records), and statistical methods that included adjustment factors.

Statistical analysis
For each study, we constructed separate 2 × 2 tables to calculate the odds ratios (ORs) and 95% CIs of stillbirth for each BMI/weight category that was analyzed (ie, overweight and obese vs normal BMI, respectively). Because not all studies provided adjusted ORs, we used crude ORs only. BMI categories varied among the studies. In general, we used the BMI categories as defined in each study (Table 1); in 1 study, narrow intervals were collapsed into groupings that more appropriately fit overweight, obese, and severely obese categories. Sources for information on type of delivery, prepregnancy BMI, and other variables varied among studies but most frequently were medical records or perinatal/obstetric clinical databases (Table 1).

Metaanalyses that combined the odds ratios across studies were conducted with both the DerSimonian-Laird and Bayesian random effects models.8,9 Both models incorporate within and between study variances. In addition, the Bayesian model incorporates uncertainty in the between-study variance, which gives slightly wider CIs. Because the point estimates of the 2 models were similar, we chose to use the more conservative Bayesian estimates.

The Bayesian model assumes that the counts in the exposed and unexposed groups follow binomial distributions with different mean probabilities. These means are modeled on the logit scale so that their difference represents the log odds ratio. The model is therefore a hierarchic logistic regression. The mean and variance of the log odds ratio are random variables in the Bayesian model. To represent our lack of previous knowledge about the value of these hyperparameters, we used diffuse priors that en-

<table>
<thead>
<tr>
<th>Study</th>
<th>Country/study period</th>
<th>Type and source of cohort</th>
<th>Cohort size</th>
<th>BMI category (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Nohr et al (2005)17</td>
<td>Denmark/1998-2001</td>
<td>Retrospective cohort of population-based birth registry (Danish National Birth Cohort) and telephone interviews</td>
<td>54,505</td>
<td>18.5-24.9</td>
</tr>
</tbody>
</table>

NA, not available.
compassed a wide range of possible values and were essentially uniform distributions. For mathematic convenience, we used normal distributions with mean 0 and extremely large variance $10^7$ for means and regression coefficients and flat inverse gamma (1.0, 0.1) distributions for the variance parameters. The choice of inverse gamma parameters allows a diffuse prior without putting too much weight on very large values of the between-study variance.\footnote{To compute the Bayesian estimates, we used a Markov chain Monte Carlo algorithm, running 3 parallel chains and monitoring convergence with the Gelman-Rubin diagnostic.\footnote{On convergence, which generally occurred within 1000 runs, we saved 15,000 samples from each chain to estimate posterior distributions of model parameters. The Markov chain Monte Carlo algorithm that was used is described in greater detail by Schmid et al.\footnote{9}}} To compute the Bayesian estimates, we used a Markov chain Monte Carlo algorithm, running 3 parallel chains and monitoring convergence with the Gelman-Rubin diagnostic.\footnote{10} On convergence, which generally occurred within 1000 runs, we saved 15,000 samples from each chain to estimate posterior distributions of model parameters. The Markov chain Monte Carlo algorithm that was used is described in greater detail by Schmid et al.\footnote{9}

**RESULTS**

Figure 1 shows the flow diagram of the literature search results. The PubMed search identified 7112 studies; 127 abstracts reported a finding on the relationship between maternal obesity and pregnancy complications from a case-control or cohort study; these articles were retrieved for detailed examination. Of the retrieved articles, 15 studies mentioned stillbirth, fetal death, or perinatal death as an outcome. After reviewing the reference lists of the 127 studies that were retrieved, we identified another 8 studies; however, after a review of the entire text of these 8 studies, only 1 study could be considered for possible inclusion. No additional studies were identified from our examination of recent review article reference lists. Of the total 16 studies that were screened for final inclusion in the metaanalysis, 7 studies were excluded because the BMI or weight measure did not reflect prepregnancy status ($n = 2$), because no normal-weight comparison group or overweight and obese groups were combined ($n = 2$), only data for perinatal deaths overall were given ($n = 2$), or data were not presented in a way to allow the construction of appropriate 2 \times 2 tables ($n = 1$). Two studies were translated to English (1 from French, 1 from Spanish).

Therefore, this metaanalysis included 9 studies, 6 and 3 with cohort and case-control designs, respectively (Tables 1 and 2).\footnote{11-19} Two studies were conducted in the United States; the remainder of the studies were from Sweden, Norway, Benin, Denmark, United Arab Emirates, Denmark, and the United Kingdom. Five studies were population-based (2 cohort, all the case-control); the remaining 4 cohort studies were based in hospi-

![Flow diagram of the study selection process](http://example.com/flow_diagram.png)
or clinic populations. All studies but 1 restricted their study population to nulliparous women; in the 1 study that included multiparous women, adjustment for parity did not affect results significantly.13,16 For the cohort studies, the rates of stillbirth for normal-weight women ranged from 2 in 1000 to 7 in 1000, except for 1 study that had been conducted in a developing African country that reported a stillbirth rate of 8 in 100 in normal-weight women.12

Based on our metaanalysis, the odds of a stillbirth were 1.47 (95% CI, 1.08-1.94) and 2.07 (95% CI, 1.59-2.74) higher among overweight and obese pregnant women, respectively, compared with normal-weight pregnant women (Table 3). When we repeated the metaanalysis using only cohort studies, the magnitudes of the odds did not change substantially (Table 4). None of the covariates in the metaregression analysis (study years <2000, 2000-2003, 2004-2005; study design [prospective, retrospective]; geographic location [United States, not the United States]; or rate of stillbirth in normal-weight women in each study) were significant.

**COMMENT**

Based on metaanalysis of the literature, we estimate that the risk of stillbirth is almost twice as high among obese pregnant women compared with normal-weight pregnant women. Because obesity is a modifiable risk with a substantial prevalence in the United States and other developed countries, the impact of reducing that exposure can be considerable.

A biologic pathway for the increased risk of stillbirth related to obesity has not been established, but several mechanisms have been proposed. Obesity during pregnancy increases the risk of gestational diabetes mellitus and hypertensive disorders, both of which are established risk factors for stillbirth. However, al-

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**TABLE 2**

Characteristics of case-control studies that examined the relation between body mass index and stillbirth

<table>
<thead>
<tr>
<th>Study</th>
<th>Country/study period</th>
<th>Type and source of cases/control subjects</th>
<th>Case/control (n)</th>
<th>BMI category (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overweight: 25-29.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Obese: &gt;30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severely obese: NA</td>
</tr>
<tr>
<td>Little and Weinberg (1993)16</td>
<td>United States/1980</td>
<td>Representative sample of married women from birth or death certificates: case, fetal death of at least 28 wks gestation or 1000 g; control, live birth of at least 28 wks gestation or 1000g</td>
<td>1590/1565</td>
<td>Normal: 18.1-22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overweight: 22.1-30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Obese: &gt;30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overweight: 25-29.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Obese: ≥30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severely obese: NA</td>
</tr>
</tbody>
</table>

NA, not available.

**TABLE 3**

Pooled estimates of the effect of prepregnancy weight on the odds of stillbirth

<table>
<thead>
<tr>
<th>Comparison group</th>
<th>Studies (n)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight vs normal</td>
<td>7</td>
<td>1.47</td>
<td>1.08-1.94</td>
</tr>
<tr>
<td>Obese vs normal</td>
<td>8</td>
<td>2.07</td>
<td>1.59-2.74</td>
</tr>
</tbody>
</table>

**TABLE 4**

Pooled estimates of the effect of prepregnancy weight on the odds of stillbirth (cohort studies only)

<table>
<thead>
<tr>
<th>Comparison group</th>
<th>Studies (n)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight vs normal</td>
<td>4</td>
<td>1.35</td>
<td>0.71-2.30</td>
</tr>
<tr>
<td>Obese vs normal</td>
<td>5</td>
<td>2.04</td>
<td>1.30-3.17</td>
</tr>
</tbody>
</table>
though pathologic examination results are typically not available and a substantial proportion of cases remain unexplained,3 most studies have suggested that the relationship between obesity and stillbirth is not explained fully by increases in gestational diabetes mellitus or hypertensive disorders.2,3,15,18-22 Some unexplained stillbirths, however, may be related to undiagnosed diabetes mellitus or glucose intolerance3 or other unrecognized factors that are associated with obesity during pregnancy.23

Other investigators have suggested that obesity during pregnancy increases the risk of hyperlipidemia, which reduces prostacyclin secretion and enhances peroxidase production and results in vasoconstriction and platelet aggregation.24 It also has been proposed that thinner women may be better able to perceive decreased fetal movements than heavier women,2 which would enable more timely medical treatment. One study suggested that obese pregnant women have more extended periods of snoring, have more apnea-hypoxia events, and have more episodes of oxygen desaturation during sleep than nonobese women, which can reduce blood flow to the fetus, which is a factor that increases the risk of a stillbirth.25

Several sources of error should be considered. First, because we accepted different definitions for BMI categories, there would be some misclassification of the exposure; if significant, the findings would be biased, or there could be significant heterogeneity in the metaanalysis model. The fairly consistent results among studies (Figures 2 and 3) suggest a minimal effect on our findings, although our pooled estimates would be more affected by the much larger sample sizes of 2 studies rather than the consistency among studies. Second, differences in the definition or classification of the outcome also could have affected our findings. However, the definition for stillbirth was quite consistent, at least among the studies that provided this information. Among the 9 studies that were included, 3 studies did not define stillbirth; 1 study defined stillbirth as a fetal death at ≥28 completed weeks of gestation. One study reported a notably higher stillbirth rate than the other included studies and did not report a positive relationship between BMI and risk of stillbirth.12 This study was conducted in a developing country in Africa where infant mortality rates are high36; when we conducted a separate metaanalysis that excluded this study, there was little change in the summary odds ratio (without this study; overweight vs normal: OR, 1.55 [95% CI, 1.17-2.07]; obese vs normal: OR, 2.10 [95% CI, 1.60-2.88]).

Third, because not all studies had adjusted ORs and adjustment factors varied among those that did, we used only crude study estimates in our metaanalysis. If there were strong effects from confounding factors (eg, maternal age is associated with both increased body weight and risk of stillbirth), the estimates that were included in the metaanalysis might be biased. However, when we did a separate metaanalysis pooling studies that provided adjusted ORs (n = 6), there was little change in the summary odds ratio (OR overweight vs normal-weight, 1.37 [95% CI, 0.74-2.53]; OR obese vs normal-weight, 2.08 [95% CI, 1.30-3.63]), which suggests minimal bias. Finally, our findings may be biased because published studies do not represent

---

**FIGURE 2**

**Association of stillbirth with maternal overweight versus normal BMI**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Djololo</td>
<td>2002</td>
<td>232</td>
</tr>
<tr>
<td>Froen</td>
<td>2001</td>
<td>498</td>
</tr>
<tr>
<td>Kristensen</td>
<td>2005</td>
<td>21742</td>
</tr>
<tr>
<td>Little</td>
<td>1993</td>
<td>3203</td>
</tr>
<tr>
<td>Nohr</td>
<td>2005</td>
<td>43231</td>
</tr>
<tr>
<td>Sebire</td>
<td>2001</td>
<td>255937</td>
</tr>
<tr>
<td>Stephansson</td>
<td>2001</td>
<td>1036</td>
</tr>
<tr>
<td>OVERALL</td>
<td></td>
<td>325879</td>
</tr>
</tbody>
</table>


---

**FIGURE 3**

**Association of stillbirth with maternal obese versus normal BMI**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedergren</td>
<td>2004</td>
<td>595181</td>
</tr>
<tr>
<td>Djololo</td>
<td>2002</td>
<td>191</td>
</tr>
<tr>
<td>Froen</td>
<td>2001</td>
<td>449</td>
</tr>
<tr>
<td>Kristensen</td>
<td>2005</td>
<td>20120</td>
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<tr>
<td>Little</td>
<td>1993</td>
<td>3154</td>
</tr>
<tr>
<td>Nohr</td>
<td>2005</td>
<td>37354</td>
</tr>
<tr>
<td>Sebire</td>
<td>2001</td>
<td>208199</td>
</tr>
<tr>
<td>Stephansson</td>
<td>2001</td>
<td>876</td>
</tr>
<tr>
<td>OVERALL</td>
<td></td>
<td>865524</td>
</tr>
</tbody>
</table>

sent all studies that were ever done on a particular subject, and there is generally a greater likelihood that statistically significant results are more likely to be submitted and published than nonsignificant and null results.27 If study publication bias was strong, we would overestimate the risk of stillbirth with increasing BMI.

Our findings suggest that maternal obesity increases the risk of stillbirth, although it remains unclear whether this adverse outcome occurs as an independent effect of obesity or through comorbidities that are associated with obesity in pregnancy. Given the numerous other benefits of weight reduction, obese women should be encouraged to undertake a weight reduction program before attempting pregnancy. The idea of a pregnancy and planning for children may give younger obese women additional incentive to lose weight.

ACKNOWLEDGMENT
We thank Mark Klebanoff, MD, National Institute of Child Health and Human Development, National Institutes of Health, for his thoughtful review and helpful comments.

REFERENCES
Prophylactic subcutaneous drainage for prevention of wound complications after cesarean delivery—a metaanalysis

Elizabeth K. Hellums, MD; Monique G. Lin, MD; Patrick S. Ramsey, MD, MSPH

A systematic literature review and meta-analysis of published data evaluating the effectiveness of prophylactic subcutaneous drainage to prevent wound complications in women undergoing cesarean delivery was performed. We identified 6 randomized trials of prophylactic subcutaneous drainage after cesarean delivery. Meta-analysis was performed and Peto odds ratios were calculated for each study outcome. The use of prophylactic subcutaneous drainage was not associated with a reduction in the rate of wound disruption (odds ratio 0.74, 95% CI: 0.39-1.42, P = .36, infection (odds ratio 1.15, 95% CI: 0.70-1.90, P = .58), hematoma (odds ratio 1.05, 95% CI: 0.33-3.30, P = .94), or seroma (odds ratio 0.44, 95% CI: 0.14-1.43, P = .17) when compared with women who were not receiving subcutaneous drainage. Prophylactic use of subcutaneous drainage does not prevent significant wound complications after cesarean delivery.

Key words: cesarean, subcutaneous drainage, wound complications

In an era where the rate of cesarean delivery and obesity are on the rise, delineation of optimal surgical techniques to minimize complications from cesarean delivery is of great clinical importance.1 The rate of cesarean delivery in the United States has increased significantly over the past decade with the current rate of cesarean delivery in the United States 29.1%.1 Depending on the population studied, approximately 3-30% of women undergoing cesarean delivery had wound complications develop postoperatively.2 Risk factors for wound complications include maternal obesity, diabetes, prolonged labor with multiple vaginal examinations, the use of internal monitors, and infections, such as chorioamnionitis.3

In particular, obese gravid women are at very high risk for wound complications.

From the Center for Women’s Reproductive Health, Division of Maternal-Fetal Medicine, Department of Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL. Received Feb. 5, 2007; revised May 10, 2007; accepted May 18, 2007. Reprints not available from the authors.

The rate of diabetes and cesarean delivery are increased in this population.4,5 Moreover, increased subcutaneous thickness has been shown to be an independent risk factor for development of postcesarean wound complications.6 Thus, as the incidence of obesity rises, not only does it contribute to the increase in cesarean delivery, but postcesarean wound complications, including infections, seromas, dehiscence, and hematomas are becoming more prevalent.3,6-8

Many techniques have been investigated to decrease wound complications, including perioperative antibiotic prophylaxis, skin preparation techniques, subcutaneous suture closure, and subcutaneous drainage.3 The premise behind these techniques is to reduce the presence of bacteria and decrease the amount of subcutaneous tissue dead space. This potential space can be a focal point for collection of serous fluid or blood, which can become infected and ultimately culminate in wound disruption.

Surgical drains are often used therapeutically in the presence of gross purulence, bleeding, or excessive lymph drainage.3,9 The use of prophylactic drain placement to prevent wound complication, however, is controversial and investigations that have evaluated its efficacy in this setting have reported conflicting results.10-20 To address the clinical uncertainty related to the use of prophylactic subcutaneous drainage in women undergoing cesarean delivery, we conducted a systematic review of the literature and meta-analysis of data from available randomized clinical trials.

Materials and Methods

For the studies that presented data in abstract form and for those studies in which the data sought were unpublished, a written request for additional data was sent to the primary study authors.

Randomized controlled trials (RCTs) evaluating the prophylactic use of subcutaneous drains in women undergoing cesarean delivery with published results, as either an abstract or complete article, were identified (Figure 1). Investigations that used concurrent subfascial drains in the study design were excluded (Figure 1). All RCTs meeting the above criteria were included in our analysis (Tables 1 and 2). All 3 study investigators (E.H., M.L., P.R.) reviewed the identified publications for study design attributes, inclusion/exclusion criteria, and outcomes. Any disagreement was resolved by consensus.

The primary outcomes for the meta-analysis were as follows: (1) wound separation or disruption, (2) wound infection, (3) wound hematoma, or (4) wound seroma. Specific outcome definitions for the studies included in our analysis are shown in Table 3.

Meta-analysis was conducted in accordance with the QUORUM guidelines by using the Comprehensive Meta-Analysis software package - Version 2.2.020 (Biostat, Englewood, NJ, 2005; www.meta-analysis.com) with a Mantel-Haenszel fixed effects model and formal tests of heterogeneity.21 A Q χ² test of heterogeneity was used for the formal test of heterogeneity in this investigation. When significant heterogeneity (P < .05) was noted, a random effects method for pooling the data was used.22 Statistical significance was defined as a P < .05.

RESULTS
Systematic review of the literature identified 21 potential publications for consideration (Figure 1). Of these, a total of 9 RCTs were identified for further evaluation.10-14,23-26 Two studies were excluded because of the combined use of subfascial and subcutaneous drains.23,24 One study, which used a subfascial drain alone, was also excluded.13 The remaining 6 randomized trials were included in our meta-analysis (Figure 1) and are described in Tables 1 and 2.10-12,14,25 Three of 6 of the studies were performed in the United States10,11,25 and 5 of 6 were performed in training hospitals with resident house staff.10-12,14,25 All investigations were performed within the last 20 years (1986-2004). Four of the 6 studies were defined as intent to treat analysis.10,11,25 All investigations had similar inclusion/exclusion criteria (Table 1).

Information on the drainage systems and antibiotics used in the studies included in the meta-analysis are summarized in Table 2. The types of subcutane-

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Systematic review of the literature identified 21 potential publications for consideration (Figure 1). Of these, a total of 9 RCTs were identified for further evaluation.10-14,23-26 Two studies were excluded because of the combined use of subfascial and subcutaneous drains.23,24 One study, which used a subfascial drain alone, was also excluded.13 The remaining 6 randomized trials were included in our meta-analysis (Figure 1) and are described in Tables 1 and 2.10-12,14,25 Three of 6 of the studies were performed in the United States10,11,25 and 5 of 6 were performed in training hospitals with resident house staff.10-12,14,25 All investigations were performed within the last 20 years (1986-2004). Four of the 6 studies were defined as intent to treat analysis.10,11,25 All investigations had similar inclusion/exclusion criteria (Table 1).

Information on the drainage systems and antibiotics used in the studies included in the meta-analysis are summarized in Table 2. The types of subcutane-
ous drains used were similar across the investigations. All but 1 study used preoperative antibiotics.\textsuperscript{12} The subcutaneous drains were left in place for varying number of hours, from 6-72 hours postoperatively. All but 1 study required a subcutaneous tissue thickness of 2 cm or greater for patient inclusion.\textsuperscript{10,11,14,25,26} For 5 of the 6 studies, subcutaneous drainage alone was compared with a no-drain/no-subcutaneous reapproximation group.\textsuperscript{10-12,14,26} The sixth study,\textsuperscript{25} which evaluated women with 4 cm or greater of subcutaneous tissue, compared suture closure with drainage to subcutaneous suture closure alone. We included this study\textsuperscript{25} in our analysis given the findings of a recent meta-analysis by Chelmow et al.,\textsuperscript{27} which demonstrated that the use of prophylactic subcutaneous suture placement in women undergoing cesarean delivery decreased the odds of wound seroma and disruption and that this trial\textsuperscript{25} evaluated a

---

### TABLE 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Drain type</th>
<th>Drain location</th>
<th>Duration of drain</th>
<th>Preoperative antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allaire et al\textsuperscript{10}</td>
<td>7-mm Jackson Pratt</td>
<td>Subcutaneous</td>
<td>72 hrs after surgery or until drainage &lt;30 mL/24 h</td>
<td>Cefazolin</td>
</tr>
<tr>
<td>Al-Inany et al\textsuperscript{14}</td>
<td>Redivac</td>
<td>Subcutaneous</td>
<td>24 h or &lt;50 mL</td>
<td>Cephobid every 8 h</td>
</tr>
<tr>
<td>Loong et al\textsuperscript{12}</td>
<td>2-cm corrugated drain</td>
<td>Subcutaneous</td>
<td>6-36 h after surgery</td>
<td>No</td>
</tr>
<tr>
<td>Magann et al\textsuperscript{11}</td>
<td>Jackson Pratt</td>
<td>Subcutaneous</td>
<td>Unknown</td>
<td>Cephalosporin at cord clamp</td>
</tr>
<tr>
<td>Ramsey et al\textsuperscript{25}</td>
<td>10-mm Jackson Pratt</td>
<td>Subcutaneous</td>
<td>72 h after surgery or until drainage &lt;30 mL/24 h</td>
<td>Yes, Cefazolin and Azithromycin at cord clamp</td>
</tr>
<tr>
<td>Kumar\textsuperscript{26}</td>
<td>10-French closed suction drain</td>
<td>Subcutaneous</td>
<td>72 h after surgery or until drainage &lt;50 mL/24 h</td>
<td>Cefazolin</td>
</tr>
</tbody>
</table>

BMI: body mass index; C/S: cesarean delivery; PROM: premature rupture of membranes; RCT: randomized controlled trial.
TABLE 3
Summary of outcomes and definitions from individual RCTs

<table>
<thead>
<tr>
<th>Study</th>
<th>Wound outcome</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allaire et al</td>
<td>Disruption</td>
<td>A ≥1 cm separation that required packing and healing by secondary intention.</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>Collection of blood in absence of infection.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Wound that had 2 of the following: drainage of purulent material, erythema,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tenderness, induration, fever, if it required opening and drainage, or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>antibiotic use.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>Presence of serous fluid in absence of infection.</td>
</tr>
<tr>
<td>Al-Inany et al</td>
<td>Disruption</td>
<td>The presence or absence of infection with separation of incision above fascia.</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>Not explicitly defined by the authors.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Wound with purulent or serous drainage with tissue warmth, erythema, and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>increasing tenderness.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>Outcome not reported by the authors nor explicitly defined.</td>
</tr>
<tr>
<td>Loong et al</td>
<td>Disruption</td>
<td>Separation of the incision above the fascia.</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>Outcome not reported by the authors nor explicitly defined.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Presence of wound edge hyperemia, induration, purulent discharge, or actual</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wound dehiscence.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>Outcome not reported by the authors nor explicitly defined.</td>
</tr>
<tr>
<td>Magann et al</td>
<td>Disruption</td>
<td>Seroma, hematoma, infection that required incision to be opened, evacuated,</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>Demonstrable blood clot between the fascia and the skin.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Wound with induration and erythema that contained purulent material.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>A collection of serous fluid in the wound without evidence of infection.</td>
</tr>
<tr>
<td>Ramsey et al</td>
<td>Disruption</td>
<td>Greater than 1 cm separation of subcutaneous tissue.</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>Demonstrable blood clot between the fascia and the skin.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Wound with induration and erythema that contained purulent material.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>A collection of serous fluid in the wound without evidence of infection.</td>
</tr>
<tr>
<td>Kumar</td>
<td>Disruption</td>
<td>Separation of &gt; 1 cm in the incision that required drainage, packing, and</td>
</tr>
<tr>
<td></td>
<td>Hematoma</td>
<td>healing by secondary intention.</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>Presence of blood collection in the absence of infection.</td>
</tr>
<tr>
<td></td>
<td>Seroma</td>
<td>Presence of serous fluid in the absence of infection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Wound outcome</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Inany (2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allaire (2000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loong (1988)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magann (2000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kumar (2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summation of studies reporting the effect of prophylactic subcutaneous drains used at the time of cesarean delivery on postoperative wound disruption. \( \chi^2 \) test of heterogeneity \( P = .037 \).


uniquely high-risk population with 4 cm or greater of subcutaneous thickness.

Results of the meta-analysis for the 4 main study outcomes are shown in Figures 2-5. All 6 studies included data for the outcomes of wound disruption and infection. Only 4 of the 6 studies reported results for the outcomes of hematoma and seroma.\( ^{11,14,25} \) No significant differences were noted between the subcutaneous drainage group and the control group for the outcomes of wound disruption (Figure 2: odds ratio [OR] 0.74, 95% CI: 0.39-1.42, \( P = .36 \), infection (Figure 3: OR 1.15, 95% CI: 0.70-1.90, \( P = .58 \)), hematoma (Figure 4: OR
1.05, 95% CI: 0.33-3.30, \(P = .94\), or seroma (Figure 5: OR 0.44, 95% CI: 0.14-1.43, \(P = .17\)). A random effects model was used to calculate the Peto OR for wound disruption and seroma, as the assumption of homogeneity was rejected (\(P < .05\)).

A subgroup analysis eliminating the Ramsey et al article, demonstrated that prophylactic subcutaneous drainage, when compared to a no treatment group, decreased the odds of seroma formation (OR 0.28, 95% CI: 0.11-0.74, \(P = .01\)). However, the analysis failed to show a difference in the rate of actual wound disruption (OR 0.69, 95% CI: 0.43-1.12, \(P = .13\)), wound infection (OR 1.03, 95% CI: 0.61-1.75, \(P = .91\)), or hematoma formation (0.94, 95% CI: 0.19-4.76, \(P = .94\)).

**Comment**

With the increasing rates of obesity and cesarean delivery, identification of optimal surgical techniques to minimize wound complications is of utmost importance. The use of prophylactic perioperative antibiotics and subcutaneous tissue reaproximation has been shown to improve outcomes and reduce wound complication rates in women undergoing cesarean delivery. 

The use of prophylactic subcutaneous drainage would theoretically seem advantageous. A subcutaneous drain should reduce the potential subcutaneous deadspace and remove residual fluid and blood from the wound that can serve as a medium for bacterial growth. The theoretical benefits of subcutaneous drainage have not been clearly borne out in the literature. Through our systematic literature review, we identified 6 randomized trials that evaluated the independent use of subcutaneous drains to prevent wound complications in women undergoing cesarean delivery. Results of our meta-analysis of these studies demonstrate that the use of prophylactic subcutaneous drains in women undergoing cesarean delivery does not reduce the odds of seroma, hematoma, wound infection, or separation when compared with a no-drain group.

Current available mechanical modalities to prevent wound complications after cesarean delivery are limited to subcutaneous drainage and subcutaneous suture placement. Chelmow et al demonstrated the superiority of suture (when compared with no treatment) in the prevention of seroma formation and wound disruption. Interestingly, in our meta-analysis, a subgroup analysis eliminating the Ramsey et al article, which compared suture closure with suture plus subcutaneous drainage in high risk women with 4 cm or greater subcutaneous thickness, demonstrated that prophylactic subcutaneous drainage, when compared with a no treatment group, decreased the odds of seroma formation. However, this protective effect did not translate to a decrease in the incidence of actual wound disruption. Moreover, in the presence of a subcutaneous stitch, drainage offers no additional benefit in the reduction of any of the outcomes studied, including seroma formation.

Several other investigations from the general surgical literature have similarly reported that the use of subcutaneous wound drainage is not efficacious, and in some instances, may increase risk for wound complications. Cruse et al noted, in a prospective study of 23,649 surgical patients who had a wound drain placed and brought out through the wound, a higher rate of wound infection (4.0%) compared with those with no drain (1.5%). The rate of wound infection was decreased when the drain was brought through the skin via a separate stab wound (2.4%) but was still increased compared to patients with no drain.

There are several postulated mechanisms by which subcutaneous drains

---

### Figure 3

**Wound infection**

<table>
<thead>
<tr>
<th>Model</th>
<th>Citation</th>
<th>Peto odds ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>p-Value</th>
<th>Events / Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drain</td>
<td>No Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Isa (2002)</td>
<td>0.26</td>
<td>0.06</td>
<td>1.16</td>
<td>0.08</td>
<td>3/78</td>
<td>5/40</td>
</tr>
<tr>
<td>Aliare (2000)</td>
<td>0.15</td>
<td>0.00</td>
<td>7.39</td>
<td>0.34</td>
<td>0/24</td>
<td>1/20</td>
</tr>
<tr>
<td>Loong (1998)</td>
<td>2.12</td>
<td>0.82</td>
<td>5.46</td>
<td>0.12</td>
<td>13/66</td>
<td>7/60</td>
</tr>
<tr>
<td>Magann (2003)</td>
<td>1.06</td>
<td>0.49</td>
<td>2.29</td>
<td>0.88</td>
<td>14/104</td>
<td>14/205</td>
</tr>
<tr>
<td>Ramsey (2000)</td>
<td>3.98</td>
<td>0.67</td>
<td>23.31</td>
<td>0.13</td>
<td>4/124</td>
<td>1/144</td>
</tr>
<tr>
<td>Kumar (2004)</td>
<td>0.72</td>
<td>0.12</td>
<td>4.31</td>
<td>0.72</td>
<td>0/24</td>
<td>3/50</td>
</tr>
<tr>
<td>Fixed</td>
<td>1.15</td>
<td>0.70</td>
<td>1.90</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summation of studies reporting the effect of prophylactic subcutaneous drains used at the time of cesarean delivery on postoperative wound infection. \(\chi^2\) test of heterogeneity \(P = .13\).


---

### Figure 4

**Wound hematoma**

<table>
<thead>
<tr>
<th>Model</th>
<th>Citation</th>
<th>Peto odds ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>p-Value</th>
<th>Events / Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drain</td>
<td>No Drain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Isa (2002)</td>
<td>0.05</td>
<td>0.00</td>
<td>3.29</td>
<td>0.16</td>
<td>0/78</td>
<td>1/40</td>
</tr>
<tr>
<td>Magann (2002)</td>
<td>2.90</td>
<td>0.40</td>
<td>20.74</td>
<td>0.29</td>
<td>3/194</td>
<td>1/205</td>
</tr>
<tr>
<td>Ramsey (2005)</td>
<td>1.17</td>
<td>0.23</td>
<td>5.89</td>
<td>0.85</td>
<td>3/124</td>
<td>3/144</td>
</tr>
<tr>
<td>Kumar (2004)</td>
<td>0.15</td>
<td>0.00</td>
<td>7.41</td>
<td>0.34</td>
<td>0/46</td>
<td>1/50</td>
</tr>
<tr>
<td>Fixed</td>
<td>1.05</td>
<td>0.33</td>
<td>3.30</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summation of studies reporting the effect of prophylactic subcutaneous drains used at the time of cesarean delivery on postoperative wound hematoma.* \(\chi^2\) test of heterogeneity \(P = .26\). *Allaire et al reported hematoma outcomes: Drain (0/24) vs No Drain (0/26). Data not shown as Peto odds ratio could not be calculated.

may contribute to wound complications. First, drains may provide a route by which bacteria can gain access to de-vitalized tissues within the wound, thus inciting inflammation and setting the stage for infection. Second, drains are foreign bodies, which may serve as a reservoir for bacteria in clean contaminated or contaminated surgical cases that may promote wound infection and inflammation. The culmination of these effects may negate any potential benefit that wound drainage may have related to reduction in wound dead space or removal of residual blood or serous fluid.

Magee et al.22 evaluated the above mechanisms through an elegant series of experiments using a guinea pig animal model to evaluate the role of subcutaneous drainage in the potentiation of wound infection. By using this animal model, fresh subcutaneous wounds were seeded with a subinfective dose of Staphylococcus aureus and closed with and without subcutaneous drain placement.32 They observed that wounds in animals with a drain had a significantly greater incidence of gross wound infection as well as degree of induration and bacterial colonization compared with animals without a subcutaneous drain.32 Interestingly, these differences were also noted even when the drain was placed entirely within the wound.32

In a recent systematic review of the literature, by Berghella et al.,20 to derive an evidence-based surgical approach to cesarean delivery, the use of prophylactic drainage in women with a subcutaneous tissue thickness of 2 cm or greater was advocated after the evaluation of only 2 studies.10,11 In our meta-analysis, we have included 6 studies, that when appropriately combined, demonstrate no benefit associated with the use of prophylactic subcutaneous drainage in this subpopulation of women. Thus, with these observations, it is difficult to justify the prophylactic use of subcutaneous drains in women undergoing cesarean delivery.

A recent meta-analysis33 published in the Cochrane Library (2005) examined the effects of prophylactic subcutaneous drainage after cesarean delivery and concluded that prophylactic wound drainage did not offer any benefit to women undergoing cesarean delivery. The outcomes evaluated in the Cochrane meta-analysis included wound infection, wound complication (broadly defined), febrile morbidity, endometritis, blood loss, duration of surgery, and duration of postpartum hospital stay. Importantly, 3 of the 7 randomized trials included in their analyses concurrently used both subfascial and subcutaneous drainage. In our meta-analysis, only trials evaluating subcutaneous drains were used. Two additional studies were included in our meta-analysis, compared with the Cochrane analysis, and our focus was on the clearly defined outcomes of importance to practicing obstetrician/gynecologists, namely, the specific wound complications of seroma, disruption, hematoma, and infection (Table 3).

One limitation of this meta-analysis is the potential for publication bias. To minimize this concern, we conducted a thorough review of the literature, including cross-referencing citations to identify all material presented in either manuscript or abstract form. Where data were lacking in published citations, the primary study authors were contacted to seek additional data or study information. We also were highly selective in the inclusion of results only from RCTs, which generally are carried through to publication in some form, in contrast to less rigorous study designs such as case-control, case-series, and cohort studies.

Study heterogeneity is also a problem often encountered with meta-analyses. The randomized trials included in out meta-analysis had relatively similar inclusion/exclusion criteria and subcutaneous drainage techniques. Also, by eliminating studies that used subfascial drains from our analysis, we were able to solely evaluate the efficacy of the use of subcutaneous drains alone to prevent wound complications. Formal heterogeneity testing was also used for the statistical analyses conducted.

Although there are limitations of a meta-analysis approach, this type of investigation provides the advantage of increased sample size and statistical power to evaluate study outcomes of interest. On the basis of the findings from our systematic literature review and meta-analysis, the use of prophylactic subcutaneous drainage is ineffective for the prevention of significant postcesarean wound complications. As wound complications represent a serious surgical morbidity that dramatically increases health resource, identification of methods to further reduce wound complications are of utmost importance.

REFERENCES
The intrapartum deceleration in center stage: a physiologic approach to the interpretation of fetal heart rate changes in labor

Jenny A. Westgate, MBChB, MD; Bert Wibbens, MD; Laura Bennet, PhD; Guido Wassink, MSc; Julian T. Parer, MD, PhD; Alistair J. Gunn, MBChB, PhD

Routine electronic fetal heart rate (FHR) monitoring has been associated with a significant reduction in fetal mortality rates and early onset neonatal seizures. However, the positive predictive value of changes in the FHR pattern is very low, and there has been a disproportionate increase in the rates of operative intervention in labor relative to the very modest reductions in neonatal encephalopathy. Recent studies have demonstrated that postasphyxial encephalopathy in term babies now derives almost entirely from low- and medium-risk pregnancies. This strongly suggests that the current model of obstetric care is very effective in high-risk populations but must be improved for wider application. Thus, an important part of ongoing efforts to improve fetal surveillance in labor must be to improve our understanding of how different aspects of changes in FHR relate to fetal condition.

From the Departments of Obstetrics and Gynaecology (Drs Westgate, Bennet and Gunn) and Physiology (Drs Wibbens, Bennet, and Gunn and Mr Wassink), Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ, and the Department of Obstetrics, Gynaecology, and Reproductive Sciences, University of California, San Francisco, School of Medicine, San Francisco, CA (Dr Parer).

Reprints not available from the authors.

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One of the most distinctive features of fetal heart rate recordings in labor is the deceleration. In clinical practice, there has been much confusion about the types of decelerations and their significance. In this review, we will describe the pathophysiologic condition of decelerations and explain some of the reasons underlying confusion about terminology. We will dissect recent studies that systematically have examined various aspects of decelerations in relation to fetal condition and associated changes in FHR recording that may help to identify infants who are being compromised in labor. This review will not address the prolonged bradycardia that accompanies an acute catastrophic event (such as abruption or prolapse of the umbilical cord). Such extreme events account for approximately 25%-30% of cases of moderate-to-severe postasphyxial encephalopathy, are not difficult to detect, and are seldom predictable or even potentially preventable.

Terminology

Confusion about terminology used to describe FHR patterns has been a longstanding problem. This was addressed by a workshop in 1997 that agreed on definitions of key FHR parameters. These definitions have been widely supported. In our experience, the terminology most resistant to change and most likely to cause major errors is that relating to decelerations. Therefore, it is relevant to review the history behind this.

Three types of decelerations (early, variable, and late) were described by Hon and Quilligan, on the basis of both shape and timing of decelerations relative to the contractions. Caldeyro-Barcia et al described type 1 dips that occur...
with the contraction and type 2 dips that occur after the contraction. The brief shallow decelerations originally described by Hon and Quilligan as “early” decelerations have mystified some clinicians who claim that early decelerations occur very infrequently, if at all. We believe the reason for this discrepancy is in the paper speed at which the FHR is recorded. In the United States, paper speed is 3 cm/min; in most of Europe, the United Kingdom, and Australasia, 1 cm/min is used. The shallow early decelerations described by Hon and Quilligan with a paper speed of 3 cm/min look like mild variable decelerations at 1 cm/min. Whatever the terminology used, brief and shallow decelerations per se are not associated with fetal acidosis. Unfortunately, it is often assumed mistakenly that any deceleration that is synchronous with a contraction is an early deceleration and therefore is innocuous, irrespective of its severity, with the potential for tragic results. In practice, most decelerations that are related to labor are variable, with an abrupt fall in FHR from the baseline, and frequently vary in shape, depth, and duration. As we discuss in depth later, there is good clinical and experimental evidence that frequent, deep variable decelerations can lead to serious fetal compromise.

We therefore propose that, because of this unhelpful confusion terms such as early, vagal, reflex, and hypoxic deceleration should be abandoned. The reasons for this recommendation are discussed later; however, it is likely that effective clinical fetal assessment will focus on assessing the key features of the deceleration (such as depth, duration, and frequency) and associated intercontraction FHR changes (such as variability) that reflect, however imperfectly, the severity of the fetal insult.

Placental perfusion in labor

FHR decelerations are not seen in most antenatal recordings of the FHR. When they occur more than sporadically, they indicate that further assessment of fetal condition is urgently required. However, during labor, decelerations are common, particularly in second stage, and in most cases are mild and require no special action or intervention. Most intrapartum decelerations occur as a direct consequence of uterine contractions and consequent reductions in uterine or fetal placental blood flow and fetal oxygenation. Doppler studies have shown that uterine contractions are associated with increased intrauterine pressure and a nearly linear fall in maternal uterine artery blood flow. Indeed, even physiologic prelabor contractions are associated with a marked increase in maternal uterine vascular resistance. The impact of contractions on umbilical blood flow in humans is not fully described and is likely to be more complex than changes in uterine artery blood flow. However, experimentally, fetal hypoxia is associated with reduced umbilical venous blood flow.

The acute fall in FHR (ie, deceleration) FHR decelerations

The acute fall in FHR (ie, deceleration) during hypoxia is a key fetal adaptation that is generally believed to help reduce myocardial work and oxygen requirements. This initial fall in FHR is mediated by the fetal chemoreflex and can be prevented by parasympathetic blockade (Figure 1). Thus, the observation of a brief deceleration in labor tells us that the fetus has responded to an hypoxic stimulus with a vagally mediated bradycardia. There are older data that indicate that pressure on the fetal head in second stage can also cause bradycardia. Dural stimulation may be involved, but the most likely mechanism for this is increased intracranial pressure and reduced cerebral perfusion. If the degree of hypoxia is severe and prolonged (typically ≥3 minutes), the initial vagal bradycardia is sustained by myocardial hypoxia. Fortunately, most episodes of hypoxia during labor are brief and last for ≤1 minute and are associated with only brief decelerations. Thus, decelerations because of true myocardial hypoxia are extremely uncommon and occur only in the context of pathologically prolonged bradycardia.

The conflicting terminology and the common emphasis on distinguishing between reflex and hypoxic bradycardia are unhelpful in understanding decelerations.
tions. The chemoreflex, which mediates the first few minutes of the FHR deceleration, is not only a significant component of fetal adaptation but also is a highly sensitive indicator of the presence of fetal hypoxemia. The depth to which FHR falls is related broadly to the severity of the hypoxia, in that shallow decelerations indicate a modest reduction in uteroplacental blood flow, and a deep deceleration indicates near-total or total reduction. Thus, by definition, a shallow deceleration in labor indicates a correspondingly mild fall in fetal oxygen tensions. The fetus can fully maintain normal oxygen delivery to vital organs during such mild-to-moderate hypoxemia, essentially indefinitely.

In contrast, although most intrapartum decelerations are reflex in origin, this term should not be used to imply that they are somehow benign in their effect on the fetus. Each deep deceleration reflects profound, albeit transient, hypoxemia. Not surprisingly, prolonged deep decelerations in the fetal sheep are associated with a profound decrease in the availability of oxygen to the brain and can trigger neuronal injury after approximately 10 min in experimental studies of healthy term fetuses. In contrast, as discussed later, it is clear that healthy term fetuses can adapt to brief decelerations without injury for surprisingly long periods of time. Whether the repeated hypoxia that is associated with typical short FHR decelerations is benign depends critically on the fetal condition and adequacy of its prelabor placental reserve and the duration and frequency of the decelerations.

Summary. The central clinical diagnostic issue for fetal monitoring in labor is how to determine whether the fetus is or is not able to adapt to repeated brief decelerations. As will be discussed later, once deep decelerations are established, the subsequent changes in the pattern of changes in FHR are informative.

Experimental Studies of Brief Repeated Asphyxia
Most experimental studies of FHR responses have been performed in the chronically instrumented fetal sheep in utero. Sheep are a highly precocial species, with neural development that approximates that of the term human at approximately 0.8-0.85 of gestation. Thus, most studies have been performed at that age. The reader should note that the baseline heart rate of the fetal sheep is approximately 20 beats per minute higher than that of the human fetus.

Brief repeated asphyxia has been produced in the fetal sheep by repeated complete occlusion of the umbilical cord at frequencies that were chosen to represent different stages of labor. This allows us to examine not only FHR and blood gas changes but also the accompanying blood pressure changes and the effects on cerebral perfusion, which is information that is not available clinically. Recent studies compared the effect of 1 minute of umbilical cord occlusion repeated every 5 minutes (1:5 group) with that of 1-minute occlusions repeated every 2.5 minutes (1:2.5 group). The former frequency of decelerations every 5 minutes is consistent with early labor; the latter with decelerations every 2.5 minutes is consistent with late first stage and second stage labor. The FHR and blood pressure changes were monitored continuously (Figure 2), and occlusions were continued for 4 hours or until fetal hypotension (mean arterial blood pressure [MAP], <20 mm Hg) developed.

The 1:5 occlusion series
The onset of each occlusion was accompanied by a variable FHR deceleration, with rapid return to baseline levels between occlusions. Fetal MAP rose at the onset of each occlusion and never fell below baseline levels during the occlusions. There was a sustained elevation in baseline MAP between occlusions. A small fall in pH and a rise in BD and lactate occurred in the first 30 minutes of occlusions (pH, 7.34 ± 0.07; BD, 1.3 ± 3.9 mmol/L; lactate, 4.5 ± 1.3 mmol/L). Subsequently these values remained stable, despite a further 3.5 hours of occlusions. This experiment demonstrated the remarkable capacity of the healthy fe-
tus to adapt fully to a low frequency of repeated episodes of severe hypoxia.

The 1:2.5 occlusion series
Although this paradigm was also associated with a series of variable decelerations, the outcome in this group was substantially different. The rapid occlusion frequency provided only a brief period of recovery between occlusions, which was insufficient to allow full recovery of fetal cellular metabolism and replenishment of glycogen stores. The following 3 distinctive phases of the fetal response to occlusions were observed in this 1:2.5 occlusion series:

First 30-minute period. During the first 3 occlusions, there was a sustained rise in MAP during occlusions, followed by recovery to baseline once the occlusion ended. After the third occlusion, all fetuses experienced a biphasic blood pressure response to successive occlusions, with initial hypertension followed by a fall in MAP that reached a nadir a few seconds after the release of the occluder. However, minimum MAP did not fall below baseline values. Over this initial 30 minutes, pH fell from 7.40 ± 0.01 to 7.25 ± 0.02; BD rose from -2.6 ± 0.6 to 3.3 ± 1.1 mmol/L, and lactate rose from 0.9 ± 0.1 to 3.9 ± 0.6 mmol/L.

Middle 30-minute period. In the middle 30 minutes, minimum FHR during occlusions fell, and interocclusion baseline rose, compared with the first 30 minutes. Although the minimum MAP did fall over the course of this phase, it never fell below baseline levels. Despite a stable blood pressure response, without hypotension, the metabolic acidosis slowly worsened: pH fell from 7.14 ± 0.03 to 7.09 ± 0.03; BD rose from 11.8 ± 1.1 to 13.6 ± 1.2 mmol/L, and lactate rose from 8.2 ± 0.8 to 9.9 ± 0.7 mmol/L.

Final 30-minute period. Finally, in the last 30 minutes, before terminal hypotension developed, minimum FHR during decelerations continued to fall, compared with the mid 30 minutes; there was no further rise in interocclusion baseline FHR. Although minimum MAP fell below baseline levels and the degree of hypotension became greater with successive occlusions. All animals had a severe metabolic acidosis, with pH 6.92 ± 0.03; BD, 19.2 ± 1.5 mmol/L, and lactate 14.6 ± 0.8 mmol/L by the end of the occlusions. Studies were stopped after a mean of 183 ± 43 minutes (range, 140-235 minutes).

The key difference in outcome between these protocols was that frequent occlusions (1 minute every 2.5 minutes) were associated with focal neuronal damage in the parasagittal cortex, the thalamus, and the cerebellum, whereas no damage was seen after less frequent occlusions (1 minute every 5 minutes). These findings are highly consistent with clinical evidence that fetal intracerebral oxygenation is impaired during short contraction intervals (<2.3 minutes) in labor.

Summary. These experimental studies demonstrate that a prolonged series of brief variable decelerations can lead ult-
mately to severe repeated hypotension and profound metabolic acidosis, even in healthy singleton fetuses, if they are repeated sufficiently frequently. The changes in the pattern of the FHR that is associated with this deterioration develop progressively and surprisingly slowly, even during frequent occlusions.

**Useful Features of the Deceleration From Experimental Studies**

The experiments described earlier have been used to evaluate features of the interocclusion FHR and the shape of decelerations that are suggested to help distinguish the state of fetal compensation. These include the slope and timing of the decelerations, the presence or absence of overshoot tachycardia after the deceleration, and changes in interocclusion FHR and variability.

**Slope of the FHR deceleration**

Several studies in near-term fetal sheep have suggested that, during repeated variable decelerations, there is a progressive slowing of the initial fall in FHR.52-54 However, whereas Akagi et al52 found that reduced slope during complete occlusions corresponded closely with the development of fetal acidosis and hypotension, others have reported a similar attenuation with repeated partial or complete cord occlusions without significant metabolic deterioration, which suggested that this phenomenon might reflect attenuation of the chemoreflex.53,54 This apparent finding (that repeated episodes of hypoxia seemed to blunt the chemoreflex response) is in many ways counterintuitive. If, as other evidence suggests, the chemoreflex is central to fetal adaptation to severe hypoxia,32,38,55,56 then we might anticipate that its attenuation would compromise adaptation to labor.55 Indeed, other chemoreflex responses, including cardiovascular centralization of combined ven- tricular output, are reported to be enhanced in chronically hypoxic fetal sheep, compared with normoxic fetuses.57,58

In view of these issues, a recent study in near-term fetal sheep examined whether repeated complete short umbilical cord occlusions alone or fetal compromise, as shown by the development of hypotension and acidosis, led to attenuation of the initial slope of fetal variable decelerations. This study found that the rate of initial fall in FHR actually increased during an occlusion series that was associated with severe developing acidosis, which indicated sensitization, not attenuation, of the vagally mediated chemoreflex.56 Late recovery from the variable decelerations was seen only in a few fetuses at the time of developing profound hypotension51; there is some evidence that this may have been related to reversible subendocardial injury that led to cardiac dysfunction.59 Further, there was a significant correlation in this group between the rate of initial fall of the FHR and the severity of evolving hypotension during the episodes of umbilical cord occlusion.56 These findings strongly suggest that attenuation of the chemoreflex occurs only during episodes of relatively mild hypoxia to which the fetus has been able to adapt wholly.52-54

Conceptually, it is important to consider that “attenuation” should not necessarily be interpreted as meaning “im- pairment.” In the present context, attenuation of the chemoreflex response seems to reflect better compensation for the hypoxic stress, such that there is maintenance of adequate homeostasis and the fetal responses do not need to be as rapid or sustained.

**Summary.** The magnitude of the chemoreflex during repeated hypoxia adapts dynamically to the severity of fetal stress, such that, during repeated brief decelerations, developing acidosis is associated with a steeper, not slower rate of fall of the FHR.

**Timing of the deceleration**

The shallow late decelerations originally described by Hon and Quilligan8 and van Geijn HP et al60 are relatively uncommon in active labor, occurring almost exclusively in antenatal or early labor recordings. In these circumstances, the shallow late decelerations usually are accompanied by fetal tachycardia and reduced or absent variability, often with a history of reduced fetal movements or chronic intrauterine growth restriction. This combination of findings is almost always accompanied by chronic fetal hypoxia. Because of this association, it has been assumed that all late decelerations must indicate direct myocardial hypoxia. This is not the case. Late decelerations can occur for 90-100 minutes during labor before acidosis develops61 and are associated with soft markers of fetal distress (low 1-minute Apgar scores or metabolic acidosis) in only 12%-50% of cases.62,63

During labor it is possible to observe decelerations that occur late in timing with respect to the onset and peak of the uterine contraction. Those decelerations with a rapid fall to the nadir of the FHR (<30 seconds) are classified as variable decelerations and may be further subclassified as mild, moderate, severe, or complicated.64 Late decelerations are those with both a gradual fall to nadir (defined as >30 seconds)5 and with the nadir occurring after the peak of the contraction.64 This definition does not specify any further classification on the basis of the amplitude or duration or any associated features of the late deceleration.

The specific mechanisms that lead to variable decelerations that are late in timing and to late decelerations themselves remain unclear. In 1 experimental study, the maternal aorta was occluded up-stream from the fetus and resulted in a time lag between occlusion and the reduction in fetal oxygenation and, consequently, the deceleration.36 Another study associated late decelerations with background (ie, preexisting) hypoxia and acidosis during induced labor in the rhesus monkey.65 In both studies, however, the deceleration was associated directly in time with the fall in fetal arterial saturation. Given that repeated partial (50%) reduction of umbilical cord blood flow is associated with a relatively slow onset of bradycardia,35 we speculate that late decelerations may occur in a fetus with limited reserves, who is exposed to modest reductions in uterine blood flow that would not cause bradycardia in a healthy fetus. In a study of 5522 low-risk pregnancies, the positive predictive value for low arterial pH (<7.1) rose
from approximately 12% of 99 patients with recurrent late decelerations to >50% (9/16 patients) in the small subset of patients with recurrent late decelerations plus loss of FHR variability. It is striking that these infants had a high rate of reduced variability on admission, which strongly suggests an element of antenatal hypoxia, preceding labor. As yet we have no hypothesis to explain the occurrence of variable decelerations with a late fall to nadir, nor do we know whether such decelerations cause or indicate a greater or lesser degree of fetal hypoxia compared with late decelerations.

**Summary.** Overall, the available evidence suggests that most cases of late decelerations reflect reduced fetal reserve rather than myocardial hypoxia or acidosis. The incidence is low, and it is likely that they are of most value in the identification of fetuses who are at risk of hypoxia when they are accompanied by additional features such as reduced variability. Further research is needed.

**Features of the Interdeceleration FHR**

**Interdeceleration FHR and variability**

In the fetal sheep experiments described earlier (Figure 2), the decelerations progressively became deeper as the umbilical cord occlusion was continued. This change was partly due to a fall in the nadir but also to the development of interocclusion fetal tachycardia. This tachycardia is due to increased catecholamine activity and is not seen in less frequent well-compensated occlusions (Figure 2A). In addition to the absolute FHR, the FHR variability in the intercontraction period is 1 of the classic indices of fetal well-being. As recently reviewed, there is good clinical evidence that moderate levels of FHR variability are a strong indicator that the fetus is coping well with labor and is unlikely to have significant acidosis (umbilical pH, <7.15) or a low Apgar score. A reduction in FHR variability, particularly when it is combined with other FHR abnormalities, is reported to be an important indicator of fetal hypoxia and developing acidemia both in the term and preterm fetus. Overall, a systematic review has suggested that undetectable or minimal FHR variability in the presence of late or variable decelerations is the most consistent predictor of newborn infant acidemia, although the association was relatively low (only 23%).

Perhaps surprisingly, however, some clinical studies have suggested that there is either a weak or no relationship between FHR variability and Apgar scores or cord acid-base measures during labor. Indeed, the initial response to acute experimental hypoxemia or repeated asphyxia in the term fetus is an increase in FHR variability rather than a decrease (Figures 3 and 4). Typically FHR variability then becomes suppressed if the insult is chronic or repeated. Consistent with these data, during repeated brief umbilical cord occlusions in term-equivalent fetal sheep, FHR variability increased with the onset of occlusions. After this transient increase, the onset of severe acidosis and hypotension during repeated umbilical cord occlusions was associated with a fall in FHR variation in two-thirds of fetuses but by a terminal increase in the remaining one-third. The significance of this finding of terminal increase in FHR variability remains unclear; however, this feature may well be related to the presence of overshoot instability (ie, to a pattern of tachycardia that is followed by a secondary fall in FHR between decelerations). Clinical examples of this phenomenon can be seen commonly (Figure 4); however, further studies, perhaps of existing databases of fetal FHR surveillance,
will be required to demonstrate their significance.

**Summary.** The combination of progressive intercurrent tachycardia and loss of variability between recurrent deep decelerations suggests that the fetal ability to continue to compensate for repeated hypoxia may be limited.

**FHR overshoot**

It is not unusual to see FHR accelerations or “shoulders” immediately before or after a variable deceleration, perhaps because of different degrees or rate of occlusion in the cord vein compared with the arteries. A variable FHR deceleration that has a transient shoulder only after the deceleration is referred to as an *overshoot deceleration pattern*. This pattern was described soon after the introduction of clinical FHR recording and was described soon after the introduction of clinical FHR recording.78 and was noted to follow umbilical cord occlusion in both the preterm human79 and animal experiments.80 Several authors have attempted to ascribe clinical significance to this pattern. Goodlin and Lowe79 reported that a deceleration-overshoot pattern was associated with newborn infants who required resuscitation and suggested that the pattern may be caused by an acute fetal hypoxic insult. Schifrin et al81 described overshoot after a deceleration as 1 component of a chronic fetal distress pattern that was associated with subsequent cerebral palsy. They suggested that the combination of a normal baseline heart rate, but absent variability and mild variable decelerations with overshoot, was due to attenuation of vagal control of heart rate, possibly caused by previous cerebral ischemia in the fetus.

In the present discussions of FHR overshoot, we will refer only to the occurrence of a FHR shoulder after a variable deceleration. In experimental studies in fetal sheep, the overshoot pattern has been related to the development of fetal acidosis and a fall in cerebral glucose metabolic rate during recurrent umbilical cord occlusions.82,83 However, there is currently no definitive evidence for the prognostic importance of the overshoot FHR pattern in human labor.84

The relationship between the appearance of overshoot FHR after decelerations and the duration of individual umbilical cord occlusions and the development of fetal compromise with hypotension and acidosis has been examined during brief repeated occlusions in fetal sheep. Overshoot accelerations after the decelerations were seen only in longer occlusions (2-minute duration of occlusions; Figure 3) or in association with developing fetal acidosis and hypotension during 1-minute occlusions in normoxic fetuses.48 The overshoot pattern occurred after the very first occlusion in a group that was exposed to one 2-minute occlusion repeated every 5 minutes, when by definition the fetuses were neither acidotic nor hypotensive. In contrast, overshoot was never seen during the initial stages of 1-minute occlusion series groups. When 1-minute occlusions were repeated every 5 minutes, overshoot never appeared, despite continuing occlusions for 4 hours. The fetuses remained normotensive, with minimal acidosis throughout. When the frequency of occlusions was increased to 1 every 2.5 minutes, progressive hypotension and acidosis developed. Under these conditions, overshoot appeared in all fetuses in association with worsening acidosis and a variable degree of hypotension. In these experiments, the mean pH associated with the onset of overshoot was 7.17, which is consistent with the pH of approximately 7.15 reported by Saito et al82 in 4 fetal sheep that were subjected to 1-minute cord occlusions.

The mechanisms involved are unclear but may include 2 factors: reduced vagal stimulation during the occlusion and beta-adrenergic myocardial stimulation immediately after the occlusion ends.81 The initial component of a FHR deceleration caused by umbilical cord occlusion is vagal, mediated by the carotid chemoreflex. However, as the occlusion is continued, decelerations are maintained by direct hypoxic myocardial depression.35 Consistent with a role of reduced myocardial vagal tone in the overshoot pattern, atropine produces overshoot tachycardia both in the human7,85 and the fetal sheep.36 Catecholamine stimulation must also be required, because the FHR overshoot induced by atropine can be abolished by concurrent administration of propranolol.82 This suggests that overshoot is caused by beta-adrenergic stimulation that is unopposed because vagal tone has become relatively attenuated during decelerations.

We propose that 2-minute periods of occlusion are sufficient to trigger FHR...
overshoot from the beginning, because the insult is long enough to result in complete loss of vagal tone by the end of the first occlusion. In contrast, after only 1 minute of occlusion, there is likely to be some persisting vagal stimulation, combined with markedly less catecholamine release compared with 2-minute occlusions, preventing subsequent tachycardia. The later development of overshoot with 1-minute occlusions is likely to reflect greater catecholamine release because of worsening systemic compromise. In conclusion, these data suggest that, although it is a reflex mechanism, FHR overshoot has been underappreciated as a potential marker of fetal compromise; further experimental data are essential to elucidate how its appearance is modulated by other factors, such as fetal condition. It was striking that the overshoot events that are associated with profound hypoxia and acidosis were followed by marked instability of the FHR between occlusion, which strongly suggests that, in this very specific setting, it may reflect near end-stage fetal decompensation.

**Summary.** The clinical significance of the FHR deceleration-overshoot pattern is not yet established and requires further research; in some situations, it may be a useful marker of developing fetal acidosis and hypotension.

### The Impact of Fetal Condition

The studies that have been addressed so far have examined the responses of healthy, well-oxygenated fetuses to hypoxia. Naturally, the prelabor condition of the fetus, which is seldom easy to ascertain, must have a considerable impact. Cordocentesis has shown that antenatal hypoxia (eg, because of growth retardation and multiple pregnancy) is associated with an increased incidence of stillbirth, metabolic acidosis during labor, and subsequent abnormal neurodevelopment. Although this clinical experience strongly suggests that such infants are likely to be compromised by otherwise well-tolerated labor, intriguingly, experimental studies seem to suggest improved or greater cardiovascular adaptation to moderate induced hypoxemia. When chronically hypoxic fetal sheep were exposed to a further episode of acute hypoxia, they exhibited more pronounced centralization of circulation, with enhanced femoral vasoconstriction. This was associated with greater increases in plasma noradrenaline and vasopressin. It is important to note, however, that these studies tested the response to mild-to-moderate hypoxia only rather than to labor-like or profound hypoxic insults. Thus, it may be speculated that these greater reflex responses reflect reduced fetal reserve that would be expected during a more severe insult.

We tested the response of chronically hypoxic fetuses from multiple pregnancies to 1-minute occlusions of the umbilical cord repeated every 5 minutes, which is a rate that is well-tolerated by normoxic fetuses. Strikingly, whereas the normoxic fetuses were able to tolerate this occlusion series for 4 hours, the fetuses with preexisting hypoxia experienced severe, progressive metabolic acidosis (pH, 7.07 ± 0.14 vs 7.34 ± 0.07) and hypotension (nadir, 24 ± 2 mm Hg vs 45.5 ± 3 mm Hg after 4 hours of occlusion). In experimental studies, the presence and severity of hypotension are major factors that are associated with neural injury. These data support the clinical concept that fetuses with preexisting hypoxia are vulnerable even to relatively infrequent periods of additional hypoxia in early labor.

### The Effect of Previous Neural Injury

The viability of intrapartum FHR monitoring to improve outcomes is largely based on the concept that fetuses have not experienced prolonged antenatal hypoxia and consequent neuronal damage before labor. Although this is correct for most fetuses, there is strong evidence that severe hypoxia is not uncommon before labor and that consequent neurologic injury can lead to abnormal FHR patterns both in the short and long term. Several studies have reported that near-term or preterm fetal lambs that are exposed to a prolonged asphyxial insult had epileptiform brain activity that was accompanied by abnormal fetal breathing movements and rapid fluctuations in fetal blood pressure and FHR shortly after the insult. These rapid fluctuations in FHR caused an apparent increase in FHR variability. Clinically, Cruikshank has described a similar FHR pattern with frequent small accelerations, the so-called “checkmark” pattern. A subsequent case report associated this pattern with regular repetitive movements that are seen on ultrasound scans. Because the movements continued for more than just the diaphragm and the chest wall, it was speculated that they represented fetal seizure activity.

In contrast, by 24-72 hours after severe hypoxia, FHR variability was dramatically suppressed in both term and preterm fetal lambs that had severe injury. This is consistent with the clinical association of absent FHR variability with severe antenatal neural injury. Postnatal studies in infants with congenital brain lesions suggest that the mechanism of the long-term loss of variability is damage to the medulla oblongata and midbrain.

**Summary.** Fetal neural injury is associated with significant acute and chronic changes in FHR pattern. Although it is tempting to speculate that such injury may compromise fetal responses to further hypoxia, there is no direct evidence of this at present.

### Overall Summary of FHR Changes in Labor

Our understanding of the pathophysiologic mechanisms that are involved in labor has been derived almost entirely from studies in the near-term fetal sheep. Many predictions from this work (for example, FHR variation changes during repeated decelerations) have been supported by findings in humans. It would be premature to propose specific criteria for intervention; for example, further research is essential on the usefulness of overshoot. However, many key principles are clear.
The central unique aspect of labor is the repetitive hypoxia that is associated with contractions. As highlighted in this review, in 1 manner or another, typical short decelerations are mediated by the fetal chemoreflex, in most cases in response to falls in systemic fetal oxygen tensions. Shallow decelerations reflect a corresponding mild fall in oxygen tension; however, if they are seen in early labor, they should raise concerns about the fetus’s ability to tolerate labor, especially second stage. Once deep decelerations are present, however, no deceleration pattern is necessarily benign. If the fetus is healthy, with a normal placental reserve, it may be able to stably adapt to even deep brief decelerations for prolonged periods, indeed essentially indefinitely at rates consistent with early labor. In contrast, fetuses with limited placental reserve (such as twins or growth-retarded fetuses) may decompensate rapidly even in early labor.

Critically, experimental evidence suggests that, when it occurs, the progressive development of metabolic acidosis and impaired blood pressure responses that indicate deterioration during repeated deep decelerations are accompanied by changes in baseline rate and variability and perhaps overshoot. As the fetus deteriorates, the sequence of events includes increasing amplitude of decelerations, more rapid rate of initial deceleration, a rising baseline, an initial increase and then loss of baseline variability, and finally brief overshoot immediately after the deceleration. Given the progressive evolving nature of these FHR changes, continuous monitoring is important to allow comprehensive assessment of fetal progress, compared with the limited data that are provided by a single snapshot in time.

**Conclusion**

This review illustrates that, although our understanding of the pathophysiologic mechanism of fetal responses to hypoxia is incomplete, considerable useful information is already available to support the assessment of fetal well-being in labor. Given the consistent confusion over many decades about the terminology of FHR decelerations, the authors believe that simplifying this terminology is vital. We propose that, rather than focus on descriptive labels, clinicians should be trained to understand the physiologic mechanisms of FHR decelerations and the patterns of FHR change that indicate progressive loss of fetal compensation.

There is already some evidence that significant improvements in the quality of intrapartum fetal assessment can be made by the more effective use of existing knowledge. For example, a prospective study showed that most of a group of 17 experts did agree when they assessed cardiotocographic patterns and could accurately identify most of the cases that did and did not require intervention. More recently, a retrospective assessment of a compulsory fetal monitoring education program at a single institution suggested that education was associated with an approximate halving of the incidence of babies who are born with low Apgar scores and with neonatal encephalopathy. Thus, these data support the potential for a simplified physiologic approach to improve intrapartum fetal monitoring.

**REFERENCES**


Small-for-gestational-age infants classified by customized or population birthweight centiles: impact of gestational age at delivery

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Objective: Evidence of placental disease and poor perinatal outcome is more common in infants who are small by customized centiles, compared with population centiles. Because preterm births are more likely to be associated with placental pathology, a greater proportion of preterm births are likely to be small for gestational age (SGA) by customized centiles, compared with population centiles. Our objective was to compare the proportion of infants classified as SGA by customized and population birthweight centiles at different gestational ages at delivery.

Study design: This was a retrospective observational study of 17,855 nulliparous women delivering between 1992 and 1999 at National Women’s Hospital, Auckland, New Zealand. The proportion of SGA infants (birthweight less than the 10th centile) classified by customized and population birthweight centiles delivering at less than 34 weeks, 34-36+6 weeks, and 37 weeks or longer were compared.

Results: A total of 1847 infants (10.3%) were customized SGA, compared with 2111 (11.8%) who were population SGA (relative risk [RR] 0.9, 95% confidence interval [CI] 0.8 to 0.9). Of preterm deliveries less than 34 weeks (n = 392), 29.1% were customized SGA and 17.1% were population SGA (RR 1.7, 95% CI 1.3 to 2.2). Of deliveries at 34-36+6 weeks (n = 946), 18.0% were customized SGA and 13.7% were population SGA (RR 1.3, 95% CI 1.1 to 1.6). The converse was observed at term (n = 16,517), 9.5% classified as customized SGA and 11.5% as population SGA (RR 0.82, 95% CI 0.77 to 0.87). Of all early preterm perinatal deaths (less than 34 weeks) 31 of 72 infants (43%) were customized SGA and 23 of 72 infants (32%) were population SGA. There were no perinatal deaths or deliveries less than 34 weeks in infants who were classified as SGA by population criteria only.

Conclusion: Customized centiles classified more infants as SGA, compared with population centiles, in preterm births but not for term births in nulliparous women.

Key words: customized birthweight centiles, gestational age, intrauterine growth restriction, population birthweight centiles, small for gestational age


Editors’ Choice

The terms small for gestational age (SGA) and intrauterine growth restriction (IUGR) are often used interchangeably by obstetric and pediatric clinicians and in the obstetric and pediatric literature. Although there is considerable overlap between these conditions, these terms are not synonymous.1 SGA has been defined in many ways but most commonly is used to describe a birthweight below the 10th centile for gestational age. This definition can be applied only after birth and will include some small but normal babies. The term IUGR suggests a fetus failing to reach its full growth potential because of placental dysfunction. These growth-restricted babies are often also small for gestational age and if they are diagnosed antenatally are likely to have abnormal umbilical and/or uterine artery Doppler waveforms.2 Because failure to reach full fetal growth potential is difficult to measure and define, the term SGA is often used as an alternative. However, this can be misleading because many infants are small but not growth restricted and therefore not at risk of morbidity or mortality, and some infants are not conventionally SGA but are growth restricted.

Population birthweight centiles, which account for fetal sex and gestational age at delivery, are typically used to classify size at birth. However, customized birthweight centiles, which in addition adjust newborn size at birth for the maternal variables parity, ethnicity, height, and weight,3 may provide a better estimate of infants that are SGA as well as growth restricted. Customized centiles identify a subgroup of infants born to bigger mothers, who are SGA by customized but not population centiles and have high rates of morbidity and mortality.2 Conversely, approximately 30% of infants who are SGA by population centiles and born...
Comparisons of customized and population birthweight centiles have demonstrated that infants classified as SGA by customized centiles have substantially higher rates of perinatal morbidity and mortality than those classified as SGA by population centiles only.²,⁴,⁵ Prospective studies in which SGA was diagnosed before birth have shown that approximately two thirds of pregnancies with babies that are SGA by customized centiles have abnormal umbilical and/or uterine artery Doppler studies antenatally,² suggesting that these pregnancies are associated with placental disease and that these small babies are likely to be growth restricted.

Infants born preterm have a higher incidence of SGA than infants born at term.⁶,⁷,⁸ Approximately one third of all preterm deliveries are iatrogenic, and this is most commonly indicated for fetal or maternal disease related to placental insufficiency. High rates of SGA would be expected and have been demonstrated in this subgroup of iatrogenic preterm births.⁵,⁶ It has also been shown that preterm delivery following spontaneous onset of labor or preterm prelabor rupture of membranes also has a higher rate of SGA than is present in term births.⁷,⁹ It is speculated that placental insufficiency may be a factor triggering the initiation of labor in these cases.⁷,⁹

Because both iatrogenic and spontaneous preterm births are thought to be linked with placental disease and because customized centiles are associated with abnormal umbilical and uterine Doppler waveforms as well as poor perinatal outcome, it may be expected that customized centiles will classify more preterm infants as SGA than population centiles. Although it has been demonstrated that customized SGA is more common preterm than at term, there are only very few reports in which comparing customized centiles were referred to as “customized SGA only,” and infants who were SGA by population centiles but not customized centiles were referred to as “population SGA.” Because of the nature of this study, antenatal Doppler data were not available.

Statistical analysis
In women with data for maternal height but no weight (n = 728) or weight but no height (n = 9945), the corresponding missing value was imputed based on a multivariate normal distribution within each appropriate ethnic group. For each individual, the average value of 30 imputations was calculated and used as the final value for subsequent analyses.

All continuous data were normally distributed and expressed as mean values (SD). Comparisons were made between the proportion of SGA infants delivered using each method to calculate birthweight centiles. Comparisons among three gestational age at delivery categories were made: less than 34 weeks (reflecting severe and moderate prematurity), 34-36 weeks (reflecting mild prematurity), and 37 weeks or longer. Relative risk (RR) and associated 95% confidence intervals (CIs) were calculated. Pearson’s χ² test was used to compare categorical data with Fisher’s exact test substituted when more than 1 cell within the comparison had a value less
than 5. Statistical significance has been determined when $P < .05$.

**Results**

A total of 17,855 nulliparous women were studied. Ethnicity comprised 9624 European (53.9%), 1575 Maori (8.8%), 1648 Chinese (9.2%), 1433 Samoan (8%), 797 Indian (4.4%), 712 Tongan (4%), and 2066 of other ethnic origins (11.6%). The mean maternal age was 27.3 years (range 13-46), and the mean body mass index was 25.1 (range 12.8 to 61.0). The overall rate of preterm delivery was 7.5% ($n = 1338$) with 2.2% ($n = 392$) delivering less than 34 weeks and 5.3% ($n = 946$) at 34-36+6 weeks. Hypertensive disease occurred in 1881 women (10.5%), 520 women (2.9%) had preeclampsia, and 1361 (7.6%) had GH.

Of the total population, 1847 (10.3%) infants were customized SGA and 2111 (11.8%) were population SGA (RR 0.9, 95% CI 0.8 to 0.9). A total of 1523 infants (8.5%) were SGA by both customized and population centiles, 324 infants (1.8%) were SGA by customized centiles alone, and 588 infants (3.3%) were SGA by population centiles alone.

The rates of SGA infants were higher by both criteria for preterm births, compared with term births. For customized birthweight centiles, 29.1% of women delivering at less than 34 weeks (RR 3.1, 95% CI 2.6 to 3.6) and 18.0% delivering at 34-36+6 weeks had an SGA infant (RR 1.9, 95% CI 1.6 to 2.2), compared with 9.5% delivering at term (RR 1.0). For population birthweight centiles, 17.1% of women delivering at less than 34 weeks (RR 1.5, 95% CI 1.2 to 1.8) and 13.7% at 34-36+6 weeks had an SGA infant (RR 1.2, 95% CI 1.0 to 1.4), compared with 11.5% at term (RR 1.0). Even though the large majority of SGA infants by either criteria was delivered at 37 weeks or longer, a greater proportion of all population SGA infants were delivered at term, compared with customized SGA infants, 90.6% and 84.6%, respectively ($P < .0001$) (see Figure 1).

The use of customized birthweight centiles increased the proportion of preterm infants classified as SGA, compared with those classified as SGA by population birthweight centiles. The converse was observed at term (Table 1). No infants delivered less than 34 weeks were classified as population SGA only, and only 5 at 34-36+6 weeks (0.5%) were population SGA only (Table 2). Of all early preterm perinatal deaths (n = 72 at less than 34 weeks), 31 (43%) were classified as customized SGA and 23 (32%) were classified as population SGA ($P = .23$). At 34-36+6 weeks and 37 weeks or longer, all perinatal deaths of SGA infants were SGA by both customized and population criteria. There were no perinatal deaths at any gestation in infants who were classified as population SGA only.

Women delivering preterm with preeclampsia or GH were more likely to have infants classified as SGA by customized rather than population birthweight centiles (Figure 2). Of all births to women with preeclampsia or GH, at less than 34 weeks (n = 110), 52 infants (47.3%) were customized SGA, compared with 38 infants (34.5%) who were population SGA (RR 1.4, 95% CI 1.0 to 1.9), and at 34-36+6 weeks (n = 215), 67 infants (31.2%) were customized SGA and 44 infants (20.5%) were population SGA (RR 1.5, 95% CI 1.1 to 2.1). There was little difference in the incidence of SGA by customized or population centiles in women with preeclampsia or GH delivering at term (n = 1556), 208 customized SGA (13.4%), and 219 population SGA (14.1%) (RR 1.0, 95% CI 0.8 to 1.1).

**Comment**

This is the first study in a large population to assess the rates of customized SGA with population SGA in relation to gestational age at delivery. Preterm infants were more likely to be customized SGA than population SGA, and the converse was true for infants born at term. Previous population-based studies comparing customized and population SGA have provided very limited data for rates of SGA by each criteria in relation to gestational age at delivery. A single small study of 217 high-risk pregnancies reported rates of SGA infants by customized and population criteria in 26 women who delivered preterm. Of these, 54% delivered customized SGA infants, compared with 27% who delivered population SGA infants ($P < .05$).

The higher rate of customized SGA infants, compared with population SGA infants, in preterm births may reflect a closer association among customized SGA, placental disease, and true growth restriction. Further evidence to support this association is our finding of higher rates of customized SGA infants, compared with population SGA infants, in women with preeclampsia or GH delivered at term.

**Table 1**

<table>
<thead>
<tr>
<th>Gestation at delivery (wks)</th>
<th>Customized SGA (%)</th>
<th>Population SGA (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 34 (n = 392)</td>
<td>114 (29.1)</td>
<td>67 (17.1)</td>
<td>1.7 (1.3 to 2.2)</td>
</tr>
<tr>
<td>34-36+6 (n = 946)</td>
<td>170 (18.0)</td>
<td>130 (13.7)</td>
<td>1.3 (1.1 to 1.6)</td>
</tr>
<tr>
<td>37 or longer (n = 1517)</td>
<td>1563 (9.5)</td>
<td>1914 (11.5)</td>
<td>0.8 (0.8 to 0.9)</td>
</tr>
<tr>
<td>All gestations (n = 17,855)</td>
<td>1847 (10.3)</td>
<td>2111 (11.8)</td>
<td>0.9 (0.8 to 0.9)</td>
</tr>
</tbody>
</table>

*Figure 1* Proportion of all SGA infants by gestational age at delivery. Asterisk indicates $P < .05$ for comparison of proportion of all customized SGA vs population SGA in each gestational age at delivery group.
The methods used to generate population and customized centiles differ and may partly explain the large difference seen in the prevalence of SGA by each criterion in preterm deliveries. The sex-specific population centiles used in this study were calculated from birthweight data for all live births including preterm deliveries in a predominantly Caucasian population. The number of infants at very preterm gestations were small, and the mean birthweights will have been influenced by the high proportion of pathological pregnancies and accompanying growth-restricted infants, which are delivered at preterm gestations. Population centiles are therefore likely to underestimate SGA at early gestations.

Customized centiles are gestation and fetal sex specific but also adjusted for maternal variables of ethnicity, parity, height, and weight. The centiles are calculated from an adjusted birthweight range expected at 40 weeks and extrapolated back using a standard, longitudinal, ultrasound-derived curve of intrauterine weight gain. This method therefore estimates normal expected weights at premature gestations from subjects with ongoing pregnancies and results in higher mean weight at preterm gestations. Infants born prematurely are more likely to have associated pathology including placental disease and customized centiles are therefore likely to estimate a more accurate incidence of growth restriction at preterm gestations.

At term the use of customized birthweight centiles reduced the number of infants who were classified as SGA, compared with those classified as SGA by population centiles. Previous studies have already demonstrated that up to 30% of population SGA infants are reclassified as normally grown after application of customized centiles. This subgroup of small babies are likely to be small but normal infants because they have a very low incidence of abnormal umbilical and uterine Doppler waveforms and very low rates of morbidity, suggesting they are not growth-restricted infants. The lower incidence of SGA infants at term using customized birthweight centiles is probably due to the reclassification of these constitutionally small normal babies once ethnicity, parity, and maternal size have been accounted for.

There were no perinatal deaths and very low rates of preterm birth (0.9%) in population SGA only infants. These findings are reassuring and are consistent with previous studies that have shown population SGA only infants have low rates of morbidity and mortality with perinatal outcomes similar to non-SGA infants and again provides reassurance that they are not growth restricted.

Our results again highlight the important contribution of maternal factors to fetal size. Therefore, when considering a diagnosis of growth restriction, either antenatally or after birth, these maternal factors must be considered. To illustrate this point, a male infant born at 40 weeks with a birthweight of 3200 g to a Caucasian mother of small stature (weight 50 kg, height 155 cm) has a customized

---

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>SGA both (n = 1523)</th>
<th>Customized SGA only (n = 324)</th>
<th>Population SGA only (n = 588)</th>
<th>Non-SGA both (n = 15,420)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery less than 34 wks (n = 392)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number (%)</td>
<td>67 (4.4)</td>
<td>47 (14.5)</td>
<td>0</td>
<td>278 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Perinatal death (per 1000)</td>
<td>23 (343)</td>
<td>8 (170)</td>
<td>-</td>
<td>41 (147)</td>
<td>.001</td>
</tr>
<tr>
<td>Delivery 34-36+6 weeks (n = 946)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number (%)</td>
<td>125 (8.2)</td>
<td>45 (13.9)</td>
<td>5 (0.9)</td>
<td>771 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Perinatal death (per 1000)</td>
<td>3 (24)</td>
<td>0</td>
<td>0</td>
<td>5 (6.5)</td>
<td>.16</td>
</tr>
<tr>
<td>Delivery 37 wks or longer (n = 16,517)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number (%)</td>
<td>1331 (87.4)</td>
<td>232 (71.6)</td>
<td>583 (99.1)</td>
<td>14,371 (93.2)</td>
<td></td>
</tr>
<tr>
<td>Perinatal death (per 1000)</td>
<td>9 (6.8)</td>
<td>0</td>
<td>0</td>
<td>24 (1.7)</td>
<td>.007</td>
</tr>
</tbody>
</table>

See text for relative risks comparing customized and population SGA for each gestational age at delivery group.

SGA both, SGA by customised and population centiles; customized SGA only, SGA by customised but not population centiles; population SGA only, SGA by population but not customized centiles; non-SGA both, not SGA by either customized or population centiles.
birthweight centile of 39; however, a male infant born at 40 weeks to a larger Caucasian mother (weight 98 kg, height 180 cm) has a customized birthweight centile of 7, is SGA, and is likely to be growth restricted. Population growth standards would not have identified the second baby as being growth restricted.

Although the use of customized centiles may identify more growth-restricted infants than population centiles, they can not be applied until after birth and hence can not be used for clinical management antenatally. However, customized antenatal growth charts that incorporate the same maternal factors as those used in customized birthweight centiles are available (GROW, software available as free download, www.gestation.net). GROW generates an individualized fetal weight standard for each week of gestation from 24 weeks. A chart is generated for each woman with fundal height on 1 axis and estimated fetal weight on the other. Fundal height is plotted serially, and if an ultrasound is performed, estimated fetal weight is recorded on the graph. Use of GROW has been shown to increase antenatal detection of SGA babies, and its use in clinical practice is recommended. Use of GROW has the potential to improve clinical decision making by both reducing the number of ultrasound scans required for small women with appropriately sized babies and prompting further investigation with Doppler studies for those with suspected growth restriction.

Conclusions

The use of customized birthweight centiles significantly increases the number of infants delivering preterm who are classified as SGA, compared with those classified SGA after application of population centiles. This may reflect the association of preterm birth with placental disease and growth restriction. The reduction in the number of infants classified as SGA by customized centiles at term is likely to be due to the exclusion of small but normal non-growth-restricted infants. Our findings reinforce the importance of using customized birthweight centiles when classifying infants as SGA, in particular when considering preterm births, because they are more likely to represent truly growth-restricted infants.
Complications of labor induction among multiparous women in a community-based hospital system

Leah Battista, MD; Judith H. Chung, MD; David C. Lagrew, MD; Deborah A. Wing, MD

OBJECTIVE: The purpose of this study was to examine complications of labor induction compared to spontaneous labor in multiparas.

STUDY DESIGN: This was a retrospective cohort study of multiparous women with live, singleton pregnancies at term, who had no contraindications to labor or labor induction. Cesarean delivery was the primary outcome.

RESULTS: Of the study subjects, 7208 experienced spontaneous labor, 2190 underwent labor induction with oxytocin, and 239 underwent labor induction requiring cervical ripening agents. Oxytocin-induced multiparas were 37% more likely to require cesarean compared to those with spontaneous labor (OR, 1.37; 95% CI, 1.10-1.71) and nearly 3 times more likely to undergo cesarean when cervical ripening agents were used (OR, 2.82; 95% CI, 1.84-4.53). Women requiring cervical ripening were also 10 times more likely to spend more than 12 hours in labor than those with spontaneous labor.

CONCLUSION: Multiparas undergoing labor induction are at increased risk for obstetric complications compared to spontaneous labor.

Key words: cesarean, labor induction, multipara


Induction of labor is one of the most commonly performed obstetrical procedures in the United States, and its frequency has doubled from 10% in 1990 to 20% in 1998. In some institutions, the labor induction rate is as high as 40%. One known adverse consequence of induced labor is an increased risk of cesarean delivery, which has been most clearly delineated in nulliparous patients. Other complications, such as prolonged labor, chorioamnionitis, assisted vaginal delivery, vaginal lacerations, postpartum hemorrhage, admission to the neonatal intensive care unit (NICU), and prolonged postpartum stays, have also been reported with greater frequency in nulliparas undergoing labor induction. While studies appear to show clear risk in nulliparas, the data in parous women undergoing labor induction have revealed conflicting results. Some investigators fail to demonstrate differences in cesarean rates and other complications. Other reports findings similar to nulliparous patients. These conflicting results may be related to the inclusion of women in the spontaneous labor groups who would not normally undergo a trial of labor or the inadequate control of confounding clinical factors between the groups. Therefore, the purpose of this investigation was to determine the risk of obstetric complications among multiparas in the MemorialCare System who experienced spontaneous labor compared to those who underwent labor induction.

MATERIALS AND METHODS

This is a retrospective cohort study using a quality assurance database of prospectively collected data. This database contains antenatal and birth information on all pregnant women delivering within the MemorialCare System, a community-based 5-hospital network, with 4 delivery services, that serves a diverse patient population in Southern California. Two of the 4 hospitals (hospitals B and D) provide both 24-hour perinatal consultative services and regional neonatal intensive care services. One of the 4 hospitals (hospital B) has a resident service, supervised by members of the division of perinatology. All 4 hospitals have 24-hour obstetric anesthesia availability. For this analysis, data from a 2-year period (January 1, 2003-December 31, 2004) were used. This time period was chosen because information regarding the use of a cervical ripening agent was added to the database at the beginning of 2003.

Before study initiation, institutional review board approval was obtained from Long Beach Memorial Medical Center. Subjects with live, singleton pregnancies at term (37-42 weeks’ gestation) who had no contraindication to a trial of labor, such as breech presentation, placenta previa, or previous myomectomy, and those who underwent either indicated or elective induction of labor were included in the study. Subjects with previous cesarean deliveries were excluded.
Intercooled Stata version 8.0 (Stata Corp, College Station, TX) was used for data management and statistical analyses. Demographic data were evaluated as a function of labor induction with oxytocin, labor induction requiring cervical ripening agents (often before the subsequent administration of oxytocin), or spontaneous labor. The χ² test was used for the analyses of categorical data, and analysis of variance (ANOVA) was used for continuous data. Unconditional logistic regression (crude and adjusted) was then used to determine the odds ratio (OR) and 95% confidence interval (95% CI) for the primary outcome of cesarean delivery, as a function of labor induction with oxytocin, labor induction requiring a cervical ripening agent, or spontaneous labor. The spontaneous labor group was designated as the reference group. Covariates in the adjusted model were identified a priori and included ethnicity, parity, maternal age, epidural anesthesia, birthweight, diabetes mellitus, and maternal weight gain. Because the incidence of cesarean delivery was relatively uncommon in the study population, the odds of cesarean delivery were assumed to approximate risk.

As the propensity to induce labor and perform cesarean deliveries may be affected by the managing or delivering physician, random effects regression modeling was employed to account for this level of clustering. Such regression modeling is imperative for the correct calculation of standard errors, which can be underestimated if clustering is unaccounted. Random effects regression modeling was employed over fixed effects, as the results of the analysis were intended to be generalizable, the physician effects were not thought to be correlated with the other covariates in the model, and the random effects model yielded consistent estimates of the βs, as determined by the Hausman–Wu test statistic.

Secondary outcomes that were investigated included the following maternal and neonatal complications: operative vaginal delivery, vaginal/perineal laceration, estimated blood loss >500 mL among women having a vaginal delivery, blood loss >1000 mL among women having a cesarean delivery, hysterectomy, postpartum curettage, length of stay on labor and delivery unit >12 hours, Apgar scores <3 at 1 minute and <7 at 5 minutes, the need for neonatal ventilation, and admission to the NICU. Although the specific methods of cervical ripening were not recorded, the most common methods used during the study period were intravaginally administered misoprostol and intracervical Foley catheter placement.

RESULTS
During the study period, there were 25,251 deliveries, with 15,332 occurring in multiparous women. Of the multiparous deliveries, 12,971 were singleton gestations between 37-42 weeks and in vertex presentation. After further excluding women undergoing elective repeat cesarean deliveries and those who had medical and/or obstetric contraindications to a trial of labor, 10,356 remained in the study population. Those with a previous cesarean delivery who were undergoing a trial of labor were also excluded (664), as were those with premature rupture of membranes (55). We decided to exclude the latter subjects because we could not definitively discern whether labor induction or labor augmentation had occurred from the information in our database. The above exclusions left 9637 women for data analyses.

Of the 9637 subjects in the study population, 25.2% underwent induction of labor. The greatest number of deliveries occurred in hospital B (44.9%). Labor induction was most frequently performed in hospital D (31.8%), with hospital-specific labor induction rates ranging from 19.8-31.8%. Hospital B had the highest cesarean delivery rate among multiparous women (5.73%), with a range from 4.09-5.73%. The most common indication for labor induction was elective (47.6%), with the second most common indication being postdated pregnancy (17.4%), defined as a gestational age ≥41 weeks. Other indications for labor induction included suspicious antepartum fetal heart rate testing (4.5%), diabetes mellitus (3.0%), chronic hypertension or gestational hypertension (4.0%), intrauterine growth restriction (1.4%), suspected macrosomia (6.4%), other indications (6.9%), and unknown indications (8.8%). Of the subjects undergoing labor induction, 9.8% (239) required cervical ripening agents.

With respect to the delivering physician, each woman was cared for by 1 of 241 different physician or physician groups, who performed between 1 and 298 deliveries during the study period. Among the physician/physician groups, the median cesarean delivery rate in this multiparous study population was 1.52%, with an interquartile range of 0.6-5.9%. The median induction rate was 20.6%, with an interquartile range of 8.3-33.3%.

The maternal demographics and peripartum outcomes of the induced groups (induction with oxytocin alone and induction requiring a cervical ripening agent) and spontaneous labor group are presented in Tables 1 and 2. Those that underwent induction of labor were more likely to be of advanced maternal age and white. They were also more likely to have gained >30 pounds during the course of the pregnancy and more likely to have had epidural anesthesia in labor. Those requiring cervical ripening agents were more likely to have gestational hypertension, chronic hypertension, preexisting diabetes mellitus, or oligohydramnios. With respect to unadjusted peripartum outcomes, induction of labor with a cervical ripening agent was associated with the highest cesarean delivery rate. These same subjects also experienced the most prolonged labors. The incidence of an estimated blood loss of >500 mL in subjects undergoing vaginal delivery was similar among the 3 groups, as was the incidence of an estimated blood loss of >1000 mL in subjects requiring cesarean delivery. Operative vaginal delivery and perineal laceration rates were also relatively similar among the 3 groups. Only 8 postpartum dilatation and curettages and 2 hysterectomies were performed in the study population. Small numbers of subjects requiring cesarean delivery with an estimated blood loss >1000 mL, sub-
jects requiring postpartum dilation and curettage, and subjects requiring hysterectomy precluded further analysis.

In Table 3, the crude and adjusted odds and 95% CIs for the primary and secondary maternal outcomes are presented. Those women undergoing induction of labor with oxytocin were 37% more likely to undergo cesarean delivery compared to those with spontaneous labor (OR, 2.82; 95% CI, 1.84-4.53). Women with oxytocin-induced labors were more than twice as likely to remain on labor and delivery for >12 hours (OR, 2.32; 95% CI, 2.02-2.67). If cervical ripening agents were used, the odds were 10-fold higher, as compared to spontaneously labored multiparas (OR, 10.51; 95% CI, 7.80-14.1). Women induced with oxytocin and who underwent vaginal delivery had a 62% higher odds of excessive blood loss, defined as >500 mL, whereas women induced with cervical ripening agents did not appear to have any excess risk. In addition, there were no statistically significant differences in the odds of perineal lacerations or operative vaginal deliveries among those undergoing vaginal delivery.

In the subgroup of women undergoing elective induction, oxytocin and cervical ripening agents were consistent with a 28% and 91% increased odds of cesarean delivery, respectively, as compared to women with spontaneous labor, although the CIs for these estimates crossed 1 (Table 3). With respect to
length of time on labor and delivery, oxytocin-induced elective inductions were associated with a 63% increased odds of spending >12 hours on labor and delivery as compared to those with spontaneous labor (OR, 1.63; 95% CI, 1.34-1.98). Women who required cervical ripening for their elective induction were nearly 7 times more likely to spend >12 hours on labor and delivery as compared to spontaneously laboring women (OR, 6.84; 95% CI, 4.12-11.2). Additional secondary outcomes in this subgroup of women, including estimated blood loss >500 mL in women undergoing vaginal delivery, perineal lacerations, and operative vaginal delivery, were not statistically significant (data not shown).

Neonatal demographics and outcomes as a function of induction with oxytocin alone, induction requiring a cervical ripening agent, and spontaneous labor are presented in Table 4. Although the average birthweights in the 3 groups were relatively similar, neonates with birthweights greater than 4000 g were more common in the induction groups. There were no differences in the rates of low 1- and 5-minute Apgar scores defined as <3 and <7, respectively. Intrapartum meconium passage was more common among neonates who underwent spontaneous labor, whereas admission to the NICU occurred most frequently among neonates who underwent induction with cervical ripening agents.

**TABLE 2**

<table>
<thead>
<tr>
<th>Peripartum outcomes</th>
<th>Induced labor oxytocin (N = 2190)</th>
<th>Induced labor cervical ripening (N = 239)</th>
<th>Spontaneous labor (N = 7208)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal delivery</td>
<td>2051 (93.7%)</td>
<td>205 (85.8%)</td>
<td>6906 (95.8%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Operative vaginal delivery</td>
<td>145 (6.6%)</td>
<td>20 (8.4%)</td>
<td>395 (5.5%)</td>
<td>.031</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>139 (6.3%)</td>
<td>34 (14.2%)</td>
<td>302 (4.2%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time to delivery &gt;12 hr</td>
<td>472 (21.9%) (N = 2152)</td>
<td>123 (51.9%) (N = 237)</td>
<td>700 (10.0%) (N = 7018)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intrapartum meconium passage</td>
<td>419 (20.2%)</td>
<td>40 (18.1%)</td>
<td>1636 (24.2%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Estimated blood loss &gt;500 mL among those undergoing vaginal delivery</td>
<td>32 (1.77%) (N = 1810)</td>
<td>4 (20.6%) (N = 194)</td>
<td>63 (1.05%) (N = 6008)</td>
<td>.03</td>
</tr>
<tr>
<td>Estimated blood loss &gt;1000 mL among those undergoing cesarean delivery</td>
<td>7 (6.42%) (N = 109)</td>
<td>1 (3.57%) (N = 28)</td>
<td>10 (4.41%) (N = 227)</td>
<td>.68</td>
</tr>
<tr>
<td>3rd or 4th degree perineal laceration*</td>
<td>25 (1.2%) (N = 2051)</td>
<td>4 (2.0%) (N = 205)</td>
<td>110 (1.6%) (N = 6906)</td>
<td>.42</td>
</tr>
<tr>
<td>Postpartum dilatation and curettage</td>
<td>1 (0.05%) (N = 2190)</td>
<td>0 (0%) (N = 239)</td>
<td>7 (0.1%) (N = 7208)</td>
<td>.69</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (0.03%)</td>
<td>.71</td>
</tr>
</tbody>
</table>

* Among those undergoing vaginal delivery.

**COMMENT**

This investigation was undertaken to help characterize the risks of labor induction among multiparous women, because induction of labor is becoming increasingly more common. The existing literature on labor induction in multiparous women is somewhat conflicting. Yeast et al. concluded that labor induction in multiparas increased the chance of cesarean delivery by 1.3-3.5 times compared to spontaneously laboring patients. Maslow and Sweeney had similar results and found a 2-fold increase in cesarean deliveries among parous women who underwent labor induction. However, Coonrod et al. and Macer et al. found no difference in cesarean delivery rates for multiparous women undergoing labor induction compared to those who experienced spontaneous labor. These discrepancies may be caused by dissimilar study populations and/or methodologic approaches. In addition, some of these results may be biased, because crude or unadjusted cesarean delivery rates appear to have been reported, without employing risk adjustment methods to account for potential differences with respect to clinical characteristics and medical providers. It is also unclear as to whether women undergoing cesarean delivery before labor and women with previous cesarean deliveries undergoing trials of labor were excluded. The potential inclusion of these subjects may further bias these studies’ conclusions.

Our results suggest that labor induction of multiparous women is associated with increased odds of cesarean delivery, and women who require cervical ripening are at particular risk. When the subgroup of electively induced women was compared to women with spontaneous labor, similar results were seen, although they did not reach statistical significance.
In comparison to previously published studies evaluating the risks of multiparous induction, we have attempted to more accurately define the true population of interest and have adjusted for potential confounding and for the effects of the delivering physician. Therefore, our results may represent more accurate risk estimates than previously reported, within the limitations of observational data.

Our results also show that multiparas undergoing labor induction were > twice as likely to spend >12 hours in labor if oxytocin was used, and 10 times as likely to have labors lasting more than 12 hours if cervical ripening agents were needed. Similar results were seen when the subgroup of electively induced women were compared to those that were spontaneously laboring. These

| TABLE 3 | Crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for the primary and secondary outcomes |
|-----------------|-------------------------------------------------|-------------------------------------------------|
| **Cesarean delivery among all multiparous women undergoing induction of labor as compared to women with spontaneous labor (N = 9637)** | **Crude OR (95% CI)** | **Adjusted OR (95% CI)** |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 1.55 (1.26-1.91) | 1.37 (1.10-1.71)* |
| Induced labor with cervical ripening | 3.79 (2.59-5.55) | 2.82 (1.84-4.53)* |
| **Cesarean delivery among those undergoing elective induction as compared to those with spontaneous labor (N = 8363)** | | |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 1.30 (0.97-1.73) | 1.28 (0.94-1.74)* |
| Induced labor with cervical ripening | 2.31 (1.05-5.09) | 1.91 (0.83-4.41)* |
| **Estimated blood loss > 500 mL among those undergoing vaginal delivery (N = 8012)** | | |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 1.70 (1.06-2.61) | 1.62 (1.02-2.55)† |
| Induced labor with cervical ripening | 1.99 (0.72-5.51) | 1.14 (0.38-3.4)† |
| **Perineal laceration among those undergoing vaginal delivery (N = 9162)** | | |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 1.04 (0.94-1.15) | 1.00 (0.90-1.11)‡ |
| Induced labor with cervical ripening | 1.11 (0.84-1.47) | 1.00 (0.75-1.34)‡ |
| **Operative vaginal delivery among those who had a successful vaginal delivery (N = 8996)** | | |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 1.25 (1.03-1.53) | 1.16 (0.94-1.43)‖ |
| Induced labor with cervical ripening | 1.78 (1.11-2.86) | 1.30 (0.77-2.20)‖ |
| **Length of time on labor and delivery >12 hours (N = 9407)** | | |
| Spontaneous labor | 1.0 | 1.0 |
| Induced labor with oxytocin | 2.53 (2.23-2.88) | 2.32 (2.02-2.67)§ |
| Induced labor with cervical ripening | 9.74 (7.46-12.7) | 10.51 (7.80-14.1)§ |

* Adjusted for maternal age, ethnicity, parity, diabetes mellitus, gestational hypertension, chronic hypertension, oligohydramnios, maternal weight gain, epidural anesthesia, delivering physician, birth weight.
† Adjusted for length of labor, mode of delivery, delivering physician, birth weight.
‡ Adjusted for ethnicity, diabetes mellitus, delivering physician, birth weight.
‖ Adjusted for gestational hypertension, chronic hypertension, diabetes mellitus, epidural anesthesia, delivering physician.
§ Adjusted for birth weight, length of labor, delivering physician.
findings are consistent with previous reports of nulliparous women, where elective labor induction was associated with prolonged labors, more time spent on labor and delivery units with 1-to-1 nursing care, and longer postpartum stays, which translated into total hospital costs for elective induction that were 17.4% higher than that for spontaneous labor. In another investigation from a single institution, it was estimated that an additional 63,882 was spent on patients who underwent elective labor induction compared to those who entered spontaneous labor. These additional health care costs associated with labor induction are likely attributable in part to the added resources needed per patient, such as nursing care, labor room occupation, medications, and other equipment. Although our investigation was not designed to evaluate the economic impact of labor induction, it stands to reason that the cost of induced labor in our study population is also higher than that of spontaneous labor.

With respect to other perinatal outcomes and to neonatal outcomes, there were minimal-to-no differences seen. One interesting observation of our study was that there were more infants with meconium-stained fluid in the spontaneously laboring group as compared to the 2 induction groups. The significance of this finding is unclear. Further study is necessary to discern whether this difference is of true clinical significance or whether this difference was caused by statistical chance.

The strengths of this analysis include the large sample size, the diverse patient population representing 4 different community-based hospital delivery services, and the employment of risk adjustment for clinical characteristics and medical provider. Adjustment for case mix that may affect health care outcomes such as cesarean rates has long been known to be important in the estimation of true risk. In addition, the contribution of physician decision-making in the propensity to perform medical procedures has become increasingly recognized.

One limitation of this study is that cervical status at the initiation of labor induction was not known. However, we specifically examined the use of cervical ripening agents as a proxy for cervical status and acknowledge that discrete data regarding the cervical examinations of our subjects are lacking. We also acknowledge that the methods of cervical ripening were poorly defined in our dataset, although the method of cervical ripening and its associated risk of cesarean delivery were not the outcomes of interest for this particular study.

From a clinical relevance standpoint, our study does not definitively answer the fundamental question of whether or not to proceed with induction of labor in a term, parous patient. Certainly in those with a clear indication for labor induction, the potential for more cesarean deliveries and prolonged labors may be outweighed by the risks of ongoing pregnancy. Therefore, this risk–benefit ratio should be carefully pondered. For patients and/or their physicians who are contemplating elective induction, it is not entirely clear as to whether an increase in cesarean delivery exists with this practice. The answer to this dilemma can be best determined by a carefully performed, adequately powered, prospective, randomized controlled trial—the gold standard for determining causal effect. Nonetheless, our study does suggest that cervical status should probably be considered when making the decision to proceed.

In conclusion, multiparas who are offered induction of labor, especially when a cervical ripening agent is used, are at greater risk for prolonged labors and cesarean deliveries. Patients should therefore be informed that, although the overall risk of cesarean delivery in this multiparous population is relatively low, induction of labor may result in an unnecessary increase in the number of cesarean deliveries performed. In addition, they should be aware that induction of labor may require a longer hospitalization and may be more costly than if they were to await spontaneous labor. The results of this study may be particularly useful in the counseling of patients undergoing labor induction, especially among those with unfavorable cervixes and those who are undergoing elective induction. Future studies should include a well-designed, prospective, randomized controlled trial to determine the true impact of elective induction. Studies may also be directed towards a more in depth investigation as to which particular cervical rip-
ening agent(s), if any, are associated with the greatest risk of cesarean delivery among multiparous women. In addition, a formal cost-effective analysis of labor induction among multiparous women may be of significant interest, as a study of this kind was not possible with the current configuration of our database.

REFERENCES

Angiogenic factors for the prediction of preeclampsia in high-risk women

Tiffany A. Moore Simas, MD, MPH; Sybil L. Crawford, PhD; Matthew J. Solitro, MD; Sara C. Frost, MD; Bruce A. Meyer, MD, MBA; Sharon E. Maynard, MD

OBJECTIVE: The objective of the study was to evaluate angiogenic factors for the prediction of preeclampsia in high-risk women.

STUDY DESIGN: We collected serial serum specimens from 94 women at high preeclampsia risk between 22 and 36 weeks' gestation. Soluble fms-like tyrosine kinase-1 (sFlt1) and placental growth factor (PlGF) were measured by enzyme-linked immunosorbent assay.

RESULTS: Mean serum sFlt1 and the sFlt1/PlGF ratio were higher in subjects who developed early-onset (less than 34 weeks) preeclampsia, as compared with subjects without preeclampsia, from 22 weeks gestation onward. In subjects who developed late-onset (34 weeks or later) preeclampsia, sFlt1 was significantly increased after 31 weeks' gestation. The sFlt1/PlGF ratio at 22-26 weeks was highly predictive of early-onset preeclampsia. The within-woman rate of change of the sFlt1/PlGF ratio was predictive of overall preeclampsia risk.

CONCLUSIONS: In high-risk women, serum sFlt1 and the sFlt1/PlGF ratio are altered prior to preeclampsia onset and may be predictive of preeclampsia. Larger studies are needed to confirm these findings.

Key words: angiogenic factors, placental growth factor, preeclampsia, soluble fms-like tyrosine kinase-1

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P reeclampsia, characterized by new-onset of hypertension and proteinuria after 20 weeks' gestation, is a major cause of maternal and perinatal morbidity and mortality worldwide.1 Although most cases of preeclampsia occur in healthy, nulliparous women, several well-recognized factors are associated with substantially increased risk. These include pregnancy-associated conditions, such as multiple gestation or preeclampsia in a prior pregnancy, and maternal conditions such as adolescent age, diabetes mellitus, chronic hypertension, chronic kidney disease, and obesity.2-4 Currently there is no widely accepted screening test for the prediction of preeclampsia in individual women. The development of an accurate biomarker for preeclampsia in high-risk women has the potential to substantially improve care by allowing closer prenatal monitoring, recognition of preeclampsia earlier in the disease course, expeditious administration of steroids for fetal lung maturity, and appropriate antihypertensive therapy. Such a biomarker would also allow for investigation of targeted strategies for the prevention of preeclampsia in women who are at especially high risk.

Altered placental expression of soluble fms-like tyrosine kinase-1 (sFlt1) and placental growth factor (PlGF) appear to contribute to the pathogenesis of preeclampsia.5,6 Cross-sectional studies suggest that changes in maternal serum levels of these biomarkers antedate the onset of clinical symptoms by 5-8 weeks in healthy, nulliparous women and may be useful for screening or early diagnosis.7-10 There is little data on sFlt1 or PlGF in pregnant women with major preeclampsia risk factors such as chronic hypertension, diabetes mellitus, obesity, or chronic kidney disease.

Here we report a prospective, longitudinal study of maternal serum sFlt1 and PlGF in a cohort of pregnant women with at least 1 major preeclampsia risk factor. The primary objective was to determine the utility of maternal serum sFlt1 and PlGF for the prediction of preeclampsia in this high-risk population.

Materials and Methods

Study population

All women presenting for prenatal care at UMass Memorial Health Care be-
began May 2004 and January 2006 were considered for enrollment. Inclusion criteria were pregnancy less than 24 weeks’ gestation at enrollment and at least 1 of the following risk factors for preeclampsia: pregestational diabetes mellitus, chronic hypertension, chronic kidney disease, maternal age 18 years or younger, obesity, systemic lupus erythematosus, antiphospholipid antibody syndrome, or prior history of preeclampsia.

Diagnosis of pregestational diabetes mellitus required oral hypoglycemic or insulin therapy prior to conception. Diagnosis of chronic hypertension required use of antihypertensive agents or the presence of blood pressure 140/90 mm Hg or greater on at least 2 occasions at least 4 hours apart, prior to 20 weeks’ gestation. Women with proteinuria (300 mg or greater by 24 hour urine collection) or serum creatinine greater than 1.5 mg/dL prior to 20 weeks’ gestation were considered to have chronic kidney disease. Obesity was defined as body mass index 30 kg/m2 or greater based on self-reported prepregnancy weight and height. Diagnosis of systemic lupus erythematosus or antiphospholipid antibody syndrome was based on clinical diagnosis documented in the medical record.

The presence of preeclampsia in a prior pregnancy was confirmed through patient interview or chart review, using diagnostic criteria outlined in the next section. The Institutional Review Board of the University of Massachusetts Medical School approved the study, and all subjects provided informed consent.

**Serum sampling and immunoassay**

Women were enrolled before the 24th week of gestation. Serum was collected approximately every 4 weeks between the 22nd and 36th gestational week. All specimens were collected prior to pre eclampsia onset, considered to be the time of first elevated blood pressure or urinary protein measurement leading to diagnosis. Enzyme-linked immunosorbent assays (ELISA) for human sFlt1 and free PI GF were performed in duplicate using commercial kits (R&D Systems, Minneapolis, MN) as previously described by an investigator blinded to pregnancy outcome. Interassay and intraassay coefficients of variation were 17.0% and 4.9%, respectively, for sFlt1 and 9.5% and 5.6% for PI GF.

**Diagnosis of preeclampsia**

After completion of pregnancy, the prenatal record and labor and delivery records of each subject were reviewed to determine whether preeclampsia or gestational hypertension developed and the gestational age at which it was diagnosed. Preeclampsia was defined according to published guidelines. In women without baseline hypertension or proteinuria, preeclampsia was defined as new onset of hypertension and proteinuria after 20 weeks’ gestation. Hypertension was defined as either a systolic blood pressure 140 mm Hg or greater or a diastolic blood pressure 90 mm Hg or greater on 2 occasions at least 4 hours apart.

Proteinuria was defined as excretion of 300 mg or more protein in a 24 hour urine collection or urine dipstick 1+ or greater on 2 occasions at least 4 hours apart, with no evidence of urinary tract infection. In women with chronic hypertension, the diagnosis of preeclampsia required new-onset proteinuria after 20 weeks’ gestation. In women with chronic kidney disease (proteinuria prior to 20 weeks’ gestation), diagnosis of preeclampsia required new-onset hypertension and worsening proteinuria (doubling of 24 hour urine protein or urine protein to creatinine ratio) after 20 weeks’ gestation. In women with baseline hypertension and baseline proteinuria, diagnosis of preeclampsia required both worsening hypertension (increase in systolic or diastolic blood pressure of 30 mm Hg or greater or 15 mm Hg or greater, respectively, above baseline values) and worsening proteinuria (defined above). Gestational hypertension was defined as new onset hypertension without proteinuria after 20 weeks’ gestation.

The onset of preeclampsia was defined as the time of the first elevated blood pressure or urinary protein measurement leading to the diagnosis. A small-for-gestational-age infant was defined as an infant whose birthweight was below the 10th percentile according to US tables of birthweight for gestational age that accounted for singleton pregnancy status.

**Statistical analysis**

Continuous variables were summarized by mean and SD, and pair-wise comparisons between groups were made using Student’s t test. Categorical variables were summarized using frequency measures, and comparisons between groups were made using Fisher’s exact test. We compared geometric means of sFlt1, PI GF, and their ratio in each of 3 gestational age windows (22-26, 27-30, and 31-36 weeks) for women who did and did not develop preeclampsia, further dichotomizing women with preeclampsia into those diagnosed prior to 34 weeks (early-onset preeclampsia) and those diagnosed at or after 34 weeks gestation (late-onset preeclampsia). This 34-week cut-off between early-onset and late-onset preeclampsia was chosen based on clinical recommendations for steroid administration in situations of anticipated preterm delivery prior to this gestational age.

Within-woman correlation and right-skew of data were handled by estimating linear mixed models for natural log-transformed biomarkers and then exponentiating the means estimated on the natural-log scale (ie, geometric means). To compare within-woman trajectories for women among the 3 groups (no preeclampsia, early-onset preeclampsia, and late-onset preeclampsia), we performed linear mixed modeling of log-ratio (ln) sFlt1, ln PI GF, and ln (sFlt1/PI GF) by continuous gestational age in fractional weeks.

Receiver operator characteristic (ROC) curves were constructed from logistic regression models to evaluate the predictive potential of each biomarker and their ratio, using samples drawn during the aforementioned 3 gestational windows. ROC analyses included a woman’s first biomarker measurement in each window, to avoid within-woman correlation; this is in con-
the raw data for batch-to-batch variability in the sFlt1 and PlGF measurements; this correction did not have a significant impact on the results (data not shown). Batch-to-batch variability was assessed by including an interassay control that consisted of pooled serum from several control subjects, on each ELISA plate. To correct for variability, a multiplicative correction factor was generated for each plate on the basis of the actual standard measurement on that plate relative to the mean (phantom) for all plates.

### Results

Of 172 patients meeting inclusion criteria, 143 (83%) agreed to participate and were enrolled in the study. Of these, 16 failed to contribute at least 1 serum specimen and 3 were excluded due to lack of pregnancy outcome data. For this analysis, we also excluded 26 subjects with multiple gestations, given large differences in sFlt1 and PlGF for singleton vs multiple gestations, and 4 subjects who developed gestational hypertension without proteinuria. Analyses therefore included 94 women who contributed a

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**TABLE 1**

Characteristics and pregnancy outcomes of study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 94)*</th>
<th>No preeclampsia (n = 82)*</th>
<th>Early onset less than 34 wks (n = 5)*</th>
<th>Late onset ≥ 34 wks (n = 7)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>29.7 ± 7.5</td>
<td>29.5 ± 7.5</td>
<td>33.4 ± 6.8</td>
<td>29.4 ± 8.0</td>
</tr>
<tr>
<td>Gravity (# pregnancies)</td>
<td>2.7 ± 1.7</td>
<td>2.8 ± 1.8</td>
<td>2.2 ± 0.8</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.4 ± 8.8</td>
<td>31.2 ± 8.6</td>
<td>40.0 ± 10.2†</td>
<td>28.4 ± 7.8</td>
</tr>
<tr>
<td>Race or ethnic group (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>56 (59.6)</td>
<td>48 (58.5)</td>
<td>3 (60.0)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Black</td>
<td>11 (11.7)</td>
<td>9 (11.0)</td>
<td>1 (20.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Hispanic white</td>
<td>25 (26.6)</td>
<td>23 (28.1)</td>
<td>1 (20.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (2.1)</td>
<td>2 (2.4)</td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>70 (74.5)</td>
<td>60 (73.2)</td>
<td>4 (80.0)</td>
<td>6 (85.71)</td>
</tr>
<tr>
<td>Smoked prior to pregnancy</td>
<td>16 (17.0)</td>
<td>15 (18.3)</td>
<td>1 (20.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>8 (8.5)</td>
<td>7 (8.5)</td>
<td>0 (0.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Chronic hypertension (%)</td>
<td>26 (27.7)</td>
<td>20 (24.4)</td>
<td>5 (100.0)‡</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Pregestational diabetes (%)</td>
<td>33 (35.1)</td>
<td>28 (34.2)</td>
<td>2 (40.0)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Renal disease (%)</td>
<td>7 (7.5)</td>
<td>5 (6.1)</td>
<td>1 (20.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Maternal age younger than 18 y (%)</td>
<td>15 (16.0)</td>
<td>14 (17.1)</td>
<td>0 (0.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Obesity (body mass index 30 kg/m² or greater) (%)</td>
<td>46 (48.9)</td>
<td>40 (48.8)</td>
<td>4 (80.0)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>History of prior preeclampsia (%)</td>
<td>17 (18.1)</td>
<td>13 (15.9)</td>
<td>2 (40.0)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus (%)</td>
<td>8 (8.5)</td>
<td>6 (7.3)</td>
<td>0 (0.0)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Antiphospholipid syndrome (%)</td>
<td>2 (2.1)</td>
<td>2 (2.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>37.9 ± 2.8</td>
<td>38.2 ± 2.6</td>
<td>34.1 ± 4.9</td>
<td>37.3 ± 1.3</td>
</tr>
<tr>
<td>Mean birthweight (g)‡</td>
<td>3223 ± 835</td>
<td>3278 ± 786</td>
<td>2430 ± 1515</td>
<td>3165 ± 592</td>
</tr>
<tr>
<td>Small-for-gestational-age (%)‡</td>
<td>15 (16.3)</td>
<td>12 (15.0)</td>
<td>2 (40.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Mean placental weight (g)‡</td>
<td>453.6 ± 169.6</td>
<td>443.7 ± 179.8</td>
<td>425.0 ± 228.2</td>
<td>512.5 ± 99.5</td>
</tr>
</tbody>
</table>

*Plus-minus values are means ± SD.

*P < .05 for comparison with no preeclampsia group.

*Infant birthweight and assessment of size for gestational age available for 80 subjects without preeclampsia and all patients in both preeclampsia groups. Placental weight available for 15 subjects without preeclampsia, 3 patients with early-onset preeclampsia, and 4 with late-onset preeclampsia.
total of 385 serum samples (mean 4.1 ± 1.5 samples per subject). Of these, 82 did not develop preeclampsia, 5 developed early-onset preeclampsia (diagnosis less than 34 weeks), and 7 developed late-onset preeclampsia (diagnosis 34 weeks or later). All serum specimens were obtained prior to preeclampsia diagnosis. Baseline characteristics and pregnancy outcomes are shown in Table 1. Subjects with early-onset preeclampsia had a higher body mass index ($P = .03$) and a higher incidence of chronic hypertension ($P = .014$) as compared with those who did not develop preeclampsia.

Figure 1, A shows mean sFlt1 levels by gestational age window in subjects who developed early-onset preeclampsia and late-onset preeclampsia, as compared with those who did not develop preeclampsia. Mean sFlt1 levels were significantly higher in subjects who developed preeclampsia prior to 34 weeks, compared with subjects without preeclampsia, during each gestational window. Mean sFlt1 levels were significantly higher in subjects who developed preeclampsia after 34 weeks, compared with subjects without preeclampsia, but only in the 31-36 week gestational window. Mean PlGF levels tended to be lower for subjects who developed preeclampsia as compared with those without preeclampsia at all gestational windows. These differences reached statistical significance at 22-26 weeks for subjects with early-onset preeclampsia ($P = .009$), 27-30 weeks for subjects with late-onset preeclampsia ($P = .026$), and 31-36 weeks for both early- and late-onset preeclampsia groups ($P = .032$ and $P = .004$, respectively).

The sFlt1 to PlGF ratio is an index of antiangiogenic activity that reflects changes in the balance between sFlt1 and PlGF, which has been shown to be more strongly associated with preeclampsia than either measure alone in healthy, nulliparous women. Differences in mean sFlt1 to PlGF ratio at each gestational window for controls as compared with subjects who developed early- or late-onset preeclampsia were generally similar to those observed with sFlt1 (Figure 1, B).

In order to compare gestational changes in these biomarkers, we performed linear mixed modeling of ln sFlt1, ln PlGF, and ln sFlt1:PlGF ratio by gestational age at the time of sampling for the three groups (Table 2). Ln sFlt1 was significantly higher initially (22 week intercept) for those who developed pre-
Mixed modeling of ln sFlt1, ln PlGF, and ln (sFlt1/PlGF) by gestational age at the time of serum sampling in subjects who developed preeclampsia as compared with those who did not develop preeclampsia

<table>
<thead>
<tr>
<th></th>
<th>Intercept (SE)*</th>
<th>Slope (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ln sFlt1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No preeclampsia (n = 82)</td>
<td>6.01 (0.08)</td>
<td>0.083 (0.007)</td>
</tr>
<tr>
<td>Preeclampsia 34 wks or later (n = 7)</td>
<td>5.58 (0.28)</td>
<td><strong>0.181 (0.025)</strong></td>
</tr>
<tr>
<td>Preeclampsia less than 34 wks (n = 5)</td>
<td>6.85 (0.34)</td>
<td>0.132 (0.035)</td>
</tr>
<tr>
<td><strong>Ln PlGF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No preeclampsia (n = 82)</td>
<td>6.11 (0.10)</td>
<td>0.0135 (0.0100)</td>
</tr>
<tr>
<td>Preeclampsia 34 wks or later (n = 7)</td>
<td>6.02 (0.35)</td>
<td><strong>0.0630 (0.0337)</strong></td>
</tr>
<tr>
<td>Preeclampsia less than 34 wks (n = 5)</td>
<td>5.25 (0.43)</td>
<td>0.0141 (0.0469)</td>
</tr>
<tr>
<td><strong>Ln (sFlt1 to PlGF ratio)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No preeclampsia (n = 82)</td>
<td>-0.117 (0.131)</td>
<td>0.070 (0.013)</td>
</tr>
<tr>
<td>Preeclampsia 34 wks or later (n = 7)</td>
<td>-0.461 (0.454)</td>
<td><strong>0.244 (0.045)</strong></td>
</tr>
<tr>
<td>Preeclampsia less than 34 wks (n = 5)</td>
<td><strong>1.727 (0.561)</strong></td>
<td>0.095 (0.062)</td>
</tr>
</tbody>
</table>

* Intercept anchored at 22 weeks’ gestational age.  
† P < .05 as compared with subjects without preeclampsia.

Linear mixed-modeling analyses suggested that the within-woman rate of rise of ln sFlt1 and ln sFlt1 to PlGF ratio with advancing gestation (within-woman slope) was greater in subjects who developed preeclampsia as compared with those who did not develop preeclampsia (Table 2). Therefore, we hypothesized that the within-woman rate of change, as indicated by woman-specific time slopes from the linear models, of sFlt1 or the sFlt1 to PlGF ratio may be predictive of overall preeclampsia risk. Figure 3 shows ROC curves constructed using within-woman slopes of sFlt1 and the sFlt1 to PlGF ratio for the prediction of preeclampsia occurring at any time during pregnancy. The rate of rise of sFlt1 and the rate of rise of the sFlt1 to PlGF ratio were both predictive of preeclampsia with AUCs of 92% and 94%, respectively. Analyses using ln sFlt1 and ln sFlt1 to PlGF ratio gave similar results (data not shown).

Given recent findings suggesting that teen pregnancy is not a significant risk factor and given the prevalence of obesity, we conducted sensitivity analyses to determine whether results were similar when excluding women with no risk factors other than maternal age of 18 years or younger or obesity. Of the 94 women included in all prior analyses, 18 subjects had maternal age of 18 years or younger or obesity as their only preeclampsia risk factor. Repetition of all major analyses excluding these subjects resulted in similar findings. The P values were slightly larger, reflecting the smaller number of subjects, but the results were not qualitatively different.

**Comment**

This is the first report of maternal serum angiogenic factors, sFlt1 and PlGF, for the prediction of preeclampsia in a high-risk pregnant population, and one of the first longitudinal reports of gestational changes in these biomarkers from any pregnancy cohort. We have shown that maternal serum sFlt1 is significantly increased, and PlGF is significantly decreased, prior to disease onset in high-risk women who go on to develop preeclampsia, as compared with high-risk women who do not develop preeclampsia. Our data suggest that sFlt1, the sFlt1:PlGF ratio, and the rate of rise of these biomarkers with advancing gestation may be sensitive and specific predictors of preeclampsia in this population. Because our sample size was small, further studies will be required to validate these results and estimate the sensitivity and specificity of these biomarkers for preeclampsia screening. However, our findings suggest several patterns that may guide future studies of these biomarkers for the prediction of preeclampsia.

First, this study extends previously published findings, which have been thus far limited to healthy, nulliparous populations, confirming that these biomarkers are likely to be useful in high-risk women as well. We have also confirmed for this high-risk population that the sFlt1 to PlGF ratio is more predictive of the development of preeclampsia than either marker alone, as has been shown in healthy nulliparous women.

Second, because preeclampsia occurs over a wide range of gestational ages, no single sampling point in pregnancy will be sufficient to rule out preeclampsia. In-
stead, our data suggest that a 2-tiered screening approach may be optimal, with an initial screen in the late second trimester to detect early-onset (less than 34 weeks) preeclampsia and a second sampling in the early to middle third trimester for the detection of preeclampsia occurring closer to term.

Third, we have made the novel observation that the within-woman rate of rise of sFlt1 and the sFlt1 to PlGF ratio over time is predictive of preeclampsia. This is consistent with recent findings by Rana et al.\textsuperscript{18} and Valten et al.\textsuperscript{19} suggesting that first to second trimester changes in angiogenic biomarkers are predictive of preterm preeclampsia. To apply this clinically, at least 2 biomarker measurements at different points in gestation will be required. The first biomarker measurement, in the late second trimester, could be used to screen for early-onset preeclampsia, and establish a patient-specific baseline. A second measurement in the early to midthird trimester could allow for calculation of the rate of rise of these markers over time in an individual patient, which may prove to be predictive of overall preeclampsia risk. Future work in larger cohorts of women will be required to further explore and validate these approaches.

This study has several limitations. The absence of a gold standard for the diagnosis of preeclampsia has hampered preeclampsia research for decades. In high-risk populations, especially in women with hypertension or proteinuria at baseline, this problem is magnified. In this study, we defined preeclampsia according to published guidelines,\textsuperscript{11} which are based largely on expert opinion and clinical consensus. However, any definition of preeclampsia in this population will be controversial. The development of a deeper understanding of the pathogenesis of preeclampsia may eventually allow preeclampsia diagnosis based not on clinical signs and symptoms but instead on pathophysiologic changes specific to the disease. Until then, clinical diagnostic criteria must remain the gold standard by which any novel

Eighty-three women who contributed at least 1 serum specimen in the 22-26 week window were included in this analysis. Each woman's first biomarker measurement, within the specified gestational age window, was used to avoid within-woman correlation. Asterisk indicates ROC curves and CI denotes confidence intervals.

screening or diagnostic test must be judged.

It was our goal to study these biomarkers in a heterogeneous population of women who shared a common feature: elevated preeclampsia risk based on identifiable clinical risk factors. However, the generalizability of our findings to the intended study population may be limited by the fact that the subjects who developed early-onset preeclampsia all had chronic hypertension and were predominantly obese. Applicability of our findings, especially with regard to early-onset preeclampsia, to women without chronic hypertension is difficult. Nevertheless, women with chronic hypertension represent an especially vulnerable population, with regard to both preeclampsia risk and severity and challenges in diagnosis. Hence, an accurate screening test may be especially useful in this subgroup.

The sample size in this study is small, with only 12 patients developing preeclampsia and only 5 developing preeclampsia prior to 34 weeks’ gestation. Larger studies will be required to definitively establish the accuracy of sFlt1 and PlGF for preeclampsia prediction in this population. If validated, this should lead to future work that may have a significant impact on the management of these high-risk pregnancies. Although no intervention has yet proven effective for the prevention of preeclampsia, early detection, monitoring, and supportive care are beneficial to the patient and the fetus. Reliable prediction of preeclampsia would allow for closer prenatal monitoring, earlier diagnosis, and expeditious intervention with steroids for fetal lung maturity, magnesium for seizure prophylaxis, antihypertensive medications, or bed rest. Identification of preeclampsia prior to the onset of hypertension and proteinuria will allow for targeted studies of existing (eg, antiplatelet agents), controversial (calcium, antioxidants), or novel treatment and preventive strategies. Potential benefits of any screening test are highest in populations at greatest baseline risk of disease; hence, women in these high-risk groups would be ideal candidates for screening.

In summary, we have demonstrated that maternal serum angiogenic factors are altered prior to preeclampsia onset in women with at least 1 major preeclampsia risk factor. Elevations in the sFlt1 to PI GF ratio in the late second trimester may be predictive of early-onset (less than 34 weeks) preeclampsia, whereas a
rapid rise in the sFlt1 to PI GF ratio with advancing gestation may be predictive of preeclampsia occurring any time during gestation. A 2-tiered screening approach may prove to be a useful tool for the prediction of preeclampsia among high-risk women.21

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REFERENCES
The global network: a prospective study of stillbirths in developing countries

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OBJECTIVE: Our goal was to determine stillbirth rates in a multisite population-based study in community settings in the developing world.

STUDY DESIGN: Outcomes of all community deliveries in 5 resource-poor countries (Democratic Republic of Congo, Guatemala, India, Zambia, and Pakistan) and in 1 mid-level country (Argentina) were evaluated prospectively over an 18-month period. Births of >1000 g with no signs of life were defined as stillbirth.

RESULTS: Outcomes of 60,324 deliveries were included. Stillbirth rates ranged from 34 per 1000 in Pakistan to 9 per 1000 births in Argentina. Increased stillbirth rates were associated significantly with lower skilled providers, out-of-hospital births, and low cesarean section rates. Maceration was present in 17.2% of stillbirths.

CONCLUSION: The stillbirth rates among births of ≥1000 g in these developing countries were substantially higher than reported stillbirth rates in developed countries (3-5/1000 births). Because most developed countries define stillbirth as ≥20 weeks of gestation or ≥500 g and because almost one-half of all stillbirths are <1000 g, the developing/developed country difference is actually larger than apparent from this study. Maceration was uncommon, which indicates that most of the deaths probably occurred during labor. The low rates of physician attendance, hospital delivery, and cesarean section deliveries suggest that stillbirth rates could be reduced by access to higher quality institutional deliveries.

Key words: developing country, intrapartum stillbirth, stillbirth

S

stillbirths generally account for one-half of all perinatal deaths, with an estimated 4 million occurring worldwide each year. More than 97% of these stillbirths take place in developing countries.1 For many reasons, stillbirths have been understudied, underreported, and rarely have been considered in attempts to improve adverse pregnancy outcomes in developing countries.1,2

Recent estimates suggest that stillbirth rates of >30 per 1000 births are common among the least developed countries, especially in Sub-Saharan Africa and Southeast Asia. By comparison, rates of 3-5 per 1000 deliveries have been documented in the United States and other developed countries, and rates of 10-15 per 1000 are reported in mid-level countries, such as those in South and Central America.3,4 Although the World Health Organization has attempted to standardize the definition of stillbirth by recommending 1000 g as the lower limit for international comparisons (corresponding to approximately 28 weeks of gestation), the lower limit of the gestational age or birthweight that is reported varies widely. In developed countries, stillbirth has been defined generally as fetal loss beyond 20 weeks of gestation; however, some developed countries (such as Sweden) still use 28 weeks of gestation as the...
lower cutoff. In less developed countries, a gestational age of 28 weeks or a birthweight of 1000 g is often the lower cutoff that is used.\(^5\)

The timing of stillbirth in relation to delivery also varies from developed to developing countries. Stillbirths that occur more than 12-24 hours before delivery have skin that is “macerated.”\(^2,6\) Although those stillbirths that occur in the intrapartum period or immediately before or during delivery are generally normal in appearance and are often called fresh stillbirths. In developed countries, intrapartum stillbirths comprise less than 10% of all stillbirths; in some of the least developed countries, up to one-half of all stillbirths are thought to occur intrapartum.\(^2,6\) When intrapartum stillbirths occur, they likely represent inadequate access to or poor quality of essential obstetric care.\(^7,8\)

Because data on stillbirths are not collected routinely in many countries and most of the stillbirth research has been hospital-based, much is still unknown about the prevalence, timing, and circumstances that are associated with stillbirths in developing countries, where over one-half of all deliveries occur at home. Understanding the burden of stillbirth has important programmatic and resource implications, which are of particular concern in very low-resource settings. Our goal in this study was to determine population-based stillbirth rates and to characterize healthcare at delivery in prospective, well-defined community-based birth cohorts in developing countries. On the basis of a review of previous studies of stillbirth,\(^4\) we hypothesized that home birth and delivery with unskilled attendant (traditional birth attendant or family) would be associated with higher rates of stillbirth.

**METHODS**

The study was conducted as part of the Global Network for Women’s and Children’s Health Research (Global Network), a National Institutes of Health-funded, multisite research network that represents partnerships of US and international investigators. Prospective data registries were created to establish baseline delivery rates as part of a larger study of neonatal resuscitation in developing countries that was conducted in 6 countries: Argentina, Democratic Republic of Congo, Guatemala, India (1 site in Orissa and 1 site in Belgaum), Pakistan, and Zambia. The study was reviewed and approved by the institutional ethics review committees of all participating foreign sites, the partner institutions in the United States, and the data center at Research Triangle Institute. Consent was obtained at the community level; women provided informed verbal consent.

The outcomes of all deliveries in the communities, defined as a distinct geographic region whose birth attendants did not overlap with other communities, were collected. All birth attendants (n = 3676) were trained prospectively to collect data and assess basic clinical variables and outcomes, which included differentiation of stillbirths and neonatal deaths at birth, type of stillbirth, and assessment of gestational age. Birth attendants were trained to identify maceration using pictures to standardize reporting of this condition. Data collection was overseen by trained community coordinators (nurses or physicians) who oversaw data collection of all birth attendants in the community.

Each Global Network site included 10-28 communities, with approximately 300-500 deliveries per community annually. The sites that were studied were distinct geographic entities and included rural areas in Orissa, India; Thatta, Pakistan; Kafue, Zambia; and Equateur, Democratic Republic of Congo, all with very limited access to health care services, to Belgaum, India which had more access to healthcare, to the most developed geographic area, in Argentina.

Women were registered by 24-28 weeks of pregnancy. After delivery, the community coordinator collected the data that were recorded by the birth attendant. Data included basic information on maternal demographics and neonatal and maternal outcomes at delivery. A stillbirth was defined as any delivery of \(\geq 1000\) g, corresponding to approximately 28 weeks of gestation, in which no signs of life (breathing, crying, heartbeat, movement) were evident. The type of delivery attendant included physician, nurse or nurse-equivalent, traditional birth attendant (TBA), family, or unattended. Location of delivery included hospital, health center, home (including the TBA’s home), or other (in transit). Prenatal care was defined as at least 1 visit with a health provider. Finally, the birthweight was taken within 48 hours of delivery with scales that were provided for the study.

All data were entered centrally at each study site; data edits, which included inter- and intraform consistency checks, were performed at entry, with additional edits performed by the data center. The data were analyzed with SAS software (version 9.0; SAS Institute Inc, Cary, NC). Relative risks were calculated with the Mantel-Haenszel procedure for the cross-tabulation identified variables that were associated with stillbirth. Reference categories were defined as those categories that were associated with the lowest stillbirth rates.

**RESULTS**

From March 2005 to December 2006, 60,324 deliveries were recorded in 103 communities in the participating Global Network sites; consent was obtained from 60,154 women (99.7%) whose pregnancy outcomes were included in this study (Table 1). Most women (89.0%) received at least 1 prenatal care visit. In Argentina, 68.9% of the deliveries were conducted by a physician; in 3 countries (Guatemala, Democratic Republic of Congo, and Zambia), <1% of deliveries were conducted by a physician. Most deliveries (66.3%) were conducted in a home setting (family or birth attendant’s home). The site of delivery ranged from 100% of deliveries in a hospital or health clinic in Argentina to 99.9% of the deliveries in a home setting in Guatemala. Cesarean delivery rates ranged from 19.1% in Argentina to 0 in the communities in Guatemala and Orissa, India. Birthweights were available for 76% of stillbirths and 91% of the live births.

A total of 1472 stillbirths were recorded (Table 2). The mean stillbirth rate was 24 per 1000 deliveries, which
ranged from 9 per 1000 in Argentina to 34 per 1000 deliveries in Pakistan. Signs of maceration were reported in 17.2% of stillbirths (range between sites was 3.6%–45.8%). The mean birthweight for the stillbirths was 2221 g ± 744 g. In comparison, the mean birthweight for live births was 2918 g ± 520 g (P ≤ .001); 63.6% of the stillbirths were ≥2000 g.

Women who were >35 years of age at delivery, who had no formal education, who were primiparous or multiparous (≥4th pregnancy) had a higher relative risk of stillbirth (Table 3). In addition, women who had no prenatal care, who had a lower level of care provider at delivery, and who delivered out of hospital were more likely to have a stillbirth than women without these characteristics. Of the perinatal characteristics, infants who were male, preterm, and <2500 g all had a higher risk of stillbirth. Less than 1% of all stillbirths had documented congenital abnormalities at the time of delivery.

**COMMENT**

The major strength of this study was that we prospectively collected population-based delivery outcomes for distinct, geographically defined communities in 6 countries that represented different levels of care. Data collectors received standardized formal training and ongoing oversight by community coordinators, who verified all pregnancy outcome data. We are not aware of any multicountry study of stillbirth with this level of data standardization or study oversight. In addition, most previous studies of stillbirths in developing countries have neither been prospective nor population-based.
Table 3: Characteristics by stillbirth

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n)</th>
<th>Stillbirths per 1000 (n)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>27,814</td>
<td>22</td>
<td>0.9 (0.8, 1.0)</td>
</tr>
<tr>
<td>25-35*</td>
<td>27,739</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;35</td>
<td>3,625</td>
<td>36</td>
<td>1.5 (1.2, 1.8)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>26,849</td>
<td>30</td>
<td>1.6 (1.4, 1.8)</td>
</tr>
<tr>
<td>Any formal education*</td>
<td>32,639</td>
<td>19</td>
<td>1.0</td>
</tr>
<tr>
<td>Living children (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14,999</td>
<td>29</td>
<td>1.4 (1.2, 1.5)</td>
</tr>
<tr>
<td>1-4*</td>
<td>37,874</td>
<td>21</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;4</td>
<td>4,011</td>
<td>29</td>
<td>1.4 (1.1, 1.6)</td>
</tr>
<tr>
<td>Prenatal Care</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≥1 Visit*</td>
<td>53,248</td>
<td>22</td>
<td>1.0</td>
</tr>
<tr>
<td>No prenatal care</td>
<td>6,590</td>
<td>44</td>
<td>2.0 (1.8, 2.3)</td>
</tr>
<tr>
<td>Birth attendant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician*</td>
<td>9,486</td>
<td>19</td>
<td>1.0</td>
</tr>
<tr>
<td>Nurse/midwife</td>
<td>16,036</td>
<td>25</td>
<td>1.3 (1.1, 1.6)</td>
</tr>
<tr>
<td>TBA/family/unattended</td>
<td>34,563</td>
<td>26</td>
<td>1.3 (1.1, 1.6)</td>
</tr>
<tr>
<td>Delivery location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home/other</td>
<td>39,839</td>
<td>26</td>
<td>1.2 (1.1, 1.4)</td>
</tr>
<tr>
<td>Clinic/hospital*</td>
<td>20,282</td>
<td>22</td>
<td>1.0</td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31,497</td>
<td>28</td>
<td>1.2 (1.0, 1.3)</td>
</tr>
<tr>
<td>Female*</td>
<td>28,554</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37</td>
<td>7,002</td>
<td>45</td>
<td>2.9 (2.5, 3.4)</td>
</tr>
<tr>
<td>≥37*</td>
<td>32,305</td>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2500</td>
<td>8,089</td>
<td>84</td>
<td>4.6 (4.4, 4.9)</td>
</tr>
<tr>
<td>≥2500*</td>
<td>47,217</td>
<td>7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Reference category.

The mean stillbirth rate of 24.0 per 1000 deliveries is >5-fold higher than stillbirth rates in most developed countries. Stillbirths of <1000 g were not included in this study but are included in the US rates and in the US account for 50% of the stillbirths. In addition, women who did not register and experienced a stillbirth before 28 weeks of gestation may never have reported their loss. Thus, the disparity between the developed/developing country stillbirth rates is even larger than indicated by the comparison described earlier. Within our study, stillbirth rates ranged from 9 per 1000 in the Argentinian communities to 34 per 1000 in the Pakistani communities.

Similar to studies in developed countries, maternal age of >35 years and lower socioeconomic status were associated with higher stillbirth rates. In addition, higher stillbirth rates were associated with less prenatal care, unattended deliveries or deliveries by TBAs, out-of-hospital births, and lower rates of cesarean section delivery. Cesarean section delivery rates of at least 5% are considered necessary to reduce stillbirth and prevent maternal death. Although cesarean section delivery may be a proxy for many healthcare quality factors, in this study, the site with the highest cesarean section delivery rate, Argentina, also had the lowest stillbirth rate. As another example, the Asian sites that had no access to cesarean section delivery (Orissa, India; and Thatta, Pakistan) had significantly higher stillbirth rates than did Belgaum, India, which had a 3% cesarean section delivery rate.

Because previous studies have also reported an association between lower level providers and adverse pregnancy outcomes, ensuring increased access to skilled delivery attendants has been used in an attempt to improve adverse pregnancy outcomes. However, because skilled providers are unavailable in many of the least developed geographic areas, also studies have examined a strategy of training traditional birth attendants. For example, a cluster-randomized trial in Pakistan found that training traditional birth attendants in basic delivery skills significantly reduced the stillbirth rates (50 per 1000 in the intervention clusters vs 71 per 1000 in the control clusters).

Most studies of stillbirth in developing countries have not included the birthweight, which is an important proxy for viability, especially where reliable gestational age dating is unavailable. Birthweight of stillbirths has been difficult to collect, often because of cultural barriers. A few hospital-based studies have reported birthweight for stillbirths in less developed countries, however, population-based stillbirth birthweights are not available. We found that the mean birthweight for stillbirths was lower than that of the live births, but more than one-half of the stillbirths were ≥2000 g and thus were likely to represent near-term...
or term deliveries. Furthermore, in this study, most of the stillbirths were fresh and are likely to have occurred during labor.

The acquisition of more knowledge about stillbirths is important because of its significant contribution to adverse pregnancy outcomes. In this study, the mean stillbirth rate of >24 per 1000 represents a >5-fold increase compared with developed country rates. Importantly, in the less developed communities, where nearly all deliveries occurred in home settings without trained health providers, rates were as high as 34 per 1000, compared with the rates in Argentina of 9 per 1000, where nearly all deliveries were fresh and occurred in hospital settings. Although our data suggest that higher quality of healthcare at delivery, especially access to high level healthcare providers and cesarean delivery, is associated with lower stillbirth rates, more research on the specific causes of these stillbirths would assist in planning appropriate interventions. The fact that most of the stillbirths were fresh and that many were term or near-term suggests that stillbirth rates could be reduced substantially by higher quality intrapartum care.

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REFERENCES
Signature pathways identified from gene expression profiles in the human uterine cervix before and after spontaneous term parturition

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OBJECTIVE: This study aimed to discover “signature pathways” that characterize biologic processes, based on genes differentially expressed in the uterine cervix before and after spontaneous labor.

STUDY DESIGN: The cervical transcriptome was characterized previously from biopsy specimens taken before and after term labor. Pathway analysis was used to study the differentially expressed genes, based on 2 gene-to-pathway annotation databases (Kanehisa Laboratories, Kyoto University, Kyoto, Japan) and Metacore software (GeneGo, Inc, St. Joseph, MI). Overrepresented and highly impacted pathways and connectivity nodes were identified.

RESULTS: Fifty-two pathways in the Metacore database were enriched significantly in differentially expressed genes. Three of the top 5 pathways were known to be involved in cervical remodeling. Two novel pathways were plasmin signaling and plasminogen activator urokinase signaling. The same analysis with the Kyoto Encyclopedia of Genes and Genomes database identified 4 significant pathways that the impact analysis confirmed. Multiple nodes that provide connectivity within the plasmin and plasminogen activator urokinase signaling pathways were identified.

CONCLUSION: Three strategies for pathway analysis were consistent in their identification of novel, unexpected, and expected pathways, which suggests that this approach is both valid and effective for the elucidation of biologic mechanisms that are involved in cervical dilation and remodeling.

Key words: cervical dilation, cervical remodeling, cervix, gene signature network, labor, microarray, parturition, pathway analysis, plasmin, systems biology


L
abor, delivery, and the postpartum period are accompanied by dramatic changes in the uterine cervix. Adverse pregnancy outcome in term (or the rest of dilation) and preterm (cervical insufficiency and preterm labor) gestation may occur as a result of cervical disease. Thus, insights into the processes that are involved in cervical dilation and remodeling are critical to the understanding of abnormal labor. The current knowledge of the biological functional of the cervix has been derived from the study of human cervical biopsy specimens with hypothesis-driven research. However, the mechanisms that underlie these processes are not completely understood.

Most research on the biology of the uterine cervix during pregnancy has been conducted with a reductionist approach. Reductionism is the study of a phenomenon by identifying the individual components of a system. The assumption is that a complex system can be understood by investigating the units of the whole. In the case of a biological process, these parts may be represented, for example, by the study of individual genes, proteins, carbohydrates, and lipids. Although there has been a great deal of progress made to date with a reductionist approach in physics, biology, and...
The cervical transcriptome before and after term labor with an unripe cervix (term delivery with an unripe cervix) was studied.

A cross-sectional study was performed in women not in labor at term and in women who underwent spontaneous vaginal delivery (term labor). The cervical transcriptome before and after labor was profiled in cervical biop-
Pathway analysis

To identify pathways relevant to cervical biology during parturition, we considered 2 repositories of pathways and 2 algorithms for analysis. The 2 pathway databases used in this study to map genes to predefined pathways are (1) Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa Laboratories, Kyoto University, Kyoto, Japan), which provides a database of metabolic, regulatory, and disease pathways; and (2) MetaCore software (GeneGo, Inc, St Joseph, MI), which is a proprietary, manually curated database containing the probability of having the human protein-protein, protein-DNA and protein compound interactions, metabolic and signaling pathways, and the effects of bioactive molecules. KEGG contains approximately 250 canonical signaling and metabolic pathways; MetaCore software contains approximately 450 such pathways.

The 2 approaches used to analyze the pathways were: (1) statistical analysis for overrepresentation and (2) a novel impact analysis. The first method assesses the probability of having the observed number of differentially expressed genes on a given pathway just by chance. A Fisher exact test was performed to evaluate these probabilities, using R (www.r-project.org). The impact analysis, performed with Pathway Express, takes into consideration the number of differentially expressed genes on each pathway, the position of the genes within the pathway, and the signaling interactions between various genes as described by the pathway. Signaling interaction refers to the situation in which the change in the activity of a given gene affects the expression of another gene in a consistent way. Signaling pathways from KEGG define a number of signaling interactions that include, for example, activation, repression, inhibition, and phosphorylation. The exact definitions for all these types of signaling interactions can be found at http://www.genome.jp/kegg. An impact value and probability value are assigned to each pathway. The probability values that were obtained from both analyses were adjusted with the use of the false discovery rate method; a probability value of <.05 was considered statistically significant.

Network connectivity analysis

An additional analysis was carried out to evaluate the potential importance of individual nodes in protein interaction networks for providing connectivity among differentially expressed genes.

To identify such “topologically significant” proteins, the set of differentially expressed genes was used to construct the shortest path network that connected corresponding nodes in the global database of protein interactions (MetaCore software). Next, the number of all paths that traversed each node in this shortest path network that was specific for genes differentially expressed in labor was computed. For each node, this number was compared with the total number of all paths going through the same node in the global network. With these numbers and the relative size of the differential gene set, probability values were calculated for each node in the shortest path network. A conservative Bonferroni correction was used to correct for multiple testing, and a probability value of <.01 was deemed significant. The probability value for a node indicates the likelihood that the node provides connectivity among the original set of differentially expressed genes. The nodes that are deemed significant by this method are displayed on pathways maps, where their functional roles can be evaluated further. A detailed description of the connectivity analysis is available as supplementary material on the web site of the Journal.
Real time quantitative real-time reverse transcriptase polymerase chain reaction assays

Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assays of selected genes were performed on a set of cervical biopsy samples different from those used in the microarray analysis. Patients who were included were those who underwent elective cesarean section with an unripe cervix (not in labor at term) and patients after spontaneous vaginal delivery (term labor). A detailed description of the methods and analysis is available as supplementary material on the web site of the Journal.

Results

Pathway analysis

Over-representation method on signaling and metabolic pathways. Fifty-two of the 450 pathways in the MetaCore database were significant based on the overrepresentation analysis \((P < .05)\). The top 20 of these are listed in Table 1; the entire list can be found as supplementary material on the web site of the Journal. The 5 most significant pathways were (1) chemokines and adhesion, (2) extracellular matrix remodeling, (3) plasmin signaling, (4) plasminogen activator, urokinase (PLAU) signaling (Figure 1), and (5) vascular endothelial growth factor (VEGF)–family signaling. The figures of the remaining pathways can be found as supplementary material on the web site of the Journal.

The overrepresentation analysis performed on the KEGG pathways identified, 4 significant pathways were: cytokine–cytokine receptor interaction, complement and coagulation cascades, calcium signaling pathway, and arginine and proline metabolism (Table 2).

Impact analysis on KEGG signaling pathways. Impact analysis restricted to KEGG signaling pathways that were performed with Pathway Express identified 7 significant pathways, among which there were 2 pathways that were not significant according to the overrepresentation method: leukocyte transendothelial migration and epithelial cell signaling (Table 3).

Plasmin/PLAU signaling pathways. The plasmin and PLAU signaling pathways were among the most highly significant. Further investigation with the MetaCore database identified multiple transcription factors that activate the expression of plasminogen activator, tissue-type (PLAT): JunB, JunD, FOSL2, FosB, CREM, and c-FOS (Figure 2).

The PLAU signaling pathway (Figure 1) displays plasminogen activator urokinase binding to its receptor on the cell surface, subsequent binding to JAK1, and phosphorylation of STAT1 that leads to activation. The pathway is truncated at this point in the MetaCore pathway database. Thus, the relationship between activation of STAT1 and the differentially regulated genes in the uterine cervix before and after labor was explored by network analysis. Network analysis indicated that activation of STAT1 is linked to regulation in expression of several genes that are listed on the right side of Figure 3. JAK1 provides an essential network conduit between plasminogen activator urokinase receptor and several differentially expressed targets of STAT1.

Connectivity Analysis of MetaCore Pathways. Multiple nodes within the plasmin and PLAU signaling pathways were found to be topologically significant on the basis of their function of providing connectivity (Figure 1).

qRT-PCR

qRT-PCR assays were performed to measure messenger RNA levels of selected genes that are involved in the plasmin and PLAU signaling pathways. PLAU receptor (PLAUR), plasminogen activator inhibitor type 1 (SERPINE2), serine protease inhibitor (LEKT1), and fibroblast growth factor 2 (FGF2) were selected for further study. When we examined the top 5 pathways, FGF2 and LEKT1 were found to be specific to the plasmin and/or PLAU signaling pathways.

Consistent with the microarray analysis, qRT-PCR revealed FGF2, PLAUR, and SERPINE2 to be significantly up-regulated in term labor patients, when compared with term no labor. LEKT1 was significantly downregulated after term labor (Figure 4).

Comment

Principle findings of this study are: 1) cervical dilation and remodeling after term labor is associated with specific gene signature networks; 2) fifty-two MetaCore database metabolic and signal-
ing pathways involve the activity of the genes of the cervical transcriptome of spontaneous term labor; 3) the 5 most significant of these pathways were chemokines and adhesion, extracellular matrix remodeling, plasmin signaling, PLAU signaling, and VEGF-family signaling; 4) a network analysis identified multiple transcription factors that activate the expression of PLAT; 5) the same network analysis also indicated that JAK1 provides an essential network conduit between PLAUR and several differentially expressed targets of STAT1; and 6) qRT-PCR confirmed the involvement of the plasminogen/plasmin system in the process of cervical dilation and remodeling after labor.

The use of pathway analysis to derive gene “signature networks” allows for the following: 1) the ability to transition from biology at the molecular level to a more global systems approach to disease/biological processes; 2) the identification of key regulators or transcription factors that may not have been identified by microarray analysis; and 3) further interpretation of gene expression data by providing information on the protein-protein interaction, metabolic, signaling, and transcription-regulatory networks.

It has been proposed that cervical changes during pregnancy occur in 4 phases: softening, ripening, dilation, and changes that occur after parturition. We present a unique report of the signaling and metabolic pathways that are involved in phase 4 of this process: cervical dilation and remodeling after term labor. In the current study, 52 pathways that are documented by the Metacore database were noted to be significant. Some of them represent canonical pathways that can be found also in KEGG, although others are proprietary. The use of novel pathway analysis methods (Impact Factor [Pathway Express] and Connectivity [Metacore]) confirmed the involvement of several of these signaling pathways and genes that lead to a consistent result of what represents the processes that are involved in cervical dilation and remodeling after term spontaneous labor.

Several pathways that were found to be significant (chemokine and adhesion, extracellular matrix remodeling, cytokine-cytokine interaction, VEGF-family signaling) contain genes that have been previously suspected as involved in cervical dilation and remodeling.
lar matrix in human amnion, choriodecidua, and placenta during and after labor. In addition, plasminogen activator inhibitor activity is increased in the serum of pregnant women near term, when compared with nonpregnant women and decreased after delivery. 

Degradation of the cervical extracellular matrix may occur as a result of the activation of the plasminogen system. The finding of upregulation of multiple transcription factors that are involved in the activation of PLAT provides further support for the involvement of this pathway in cervical dilation and remodeling after term labor.

It is noteworthy that the macrophage migration inhibiting factor (MIF) in innate immune response pathway was significant. MIF encodes a lymphokine that is involved in cell-mediated immunity, immunoregulation, and inflammation. It plays a role in the regulation of the macrophage function in host defense through the suppression of antiinflammatory effects of glucocorticoids. Elevated amniotic fluid concentrations of MIF are associated with intraamniotic inflammation, histologic chorioamnionitis, and shorter amniocentesis-to-delivery interval in patients in preterm labor.

Interestingly, this study has identified Shc and GRB2 as significant nodes. Both are important mediators in MAPK-related signal transduction. Both mediate response to various growth factors and inflammatory response. The involvement of MAPK cascade during parturition has been demonstrated recently. The role of these pathways in the mechanisms that are involved in cervical dilation and remodeling in term labor require further investigation.

The understanding of the gene signature pathways before and after labor in the uterine cervix is central to the molecular elucidation of the mechanisms responsible for cervical insufficiency, preterm labor, and arrest of dilation. Thus far, these common complications of pregnancy have eluded pathophysiologic definition.

A major strength of this study is that this report represents the first pathway analysis of the uterine cervix before and after human term labor and delivery. In addition, several significant pathways were noted to be consistently important when evaluated by several methods. The results of these analyses are consistent with previous results. However, we also highlight pathways that had not been implicated previously. The report of the plasminogen activation cascade and the pathways of innate immunity playing substantial roles in cervical dilation and remodeling is novel. Changes in the expression of several genes involved in some of the reported pathways were confirmed by qRT-PCR.

A limitation of this investigation is that we were unable to follow temporally the changes that are seen in an individual’s cervix as labor progresses because of the obvious constraints of human research. Of note, the 2 patient groups differ in their obstetric history. Although it is not certain that this could account for any changes that are seen in gene expression, we wish to point out this difference.

Other types of pathway analyses have been previously used to examine the changes in myometrium in animal models. Salomonis et al examined the mouse myometrium in the non-pregnant, mid gestation, late gestation, and postpartum states and defined gene expression changes under these conditions. Hierarchical-ordered partitioning and collapsing hybrid and GenMAPP 2.0 pathway (Gladstone Institutes, University of California at San Francisco, CA) analysis identified term quiescence, term activation, and postpartum involution expression patterns. There are no previous reports of the mechanisms that are involved in cervical dilation and remodeling after spontaneous labor and delivery with the use of pathway analysis.

The processes of cervical dilation and remodeling are the result of the activation of several pathways that have been implicated in the common terminal pathway of parturition. The pathways that are involved in this complex process include networks that are involved in chemokine and adhesion activation, cytokine-cytokine activity, extracellular matrix remodeling, the plasminogen system, recognition by the innate immune system, and the complement and coagulation cascade.

This investigation provides a unique global view of the changes that are seen in the uterine cervix after term labor and delivery. Remaining unanswered questions include the timing of the activation of each cascade, the role of each pathway in patients with cervical disease that result in abnormal term (arrest of dilation) and preterm birth (preterm labor, cervical insufficiency), and effective treatment strategies for cervical disease in pregnancy. The understanding of the pathways that lead to the changes in the cervix during labor and delivery may be critical to unraveling the solutions for the treatment of cervical disease in pregnancy. Future investigation of effective treatments for cervical disease in pregnancy should be targeted to the processes that have been identified as playing a critical role in the metabolic and signaling pathways that have been identified. In addition, future studies should focus on the remaining 3 components of system-level understanding: system dynamics, control, and design.

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Are women who have had a preterm twin delivery at greater risk of preterm birth in a subsequent singleton pregnancy?

Francesca L. Facco, MD; Kate Nash, BA; William A. Grobman, MD, MBA

OBJECTIVE: The purpose of this study was to determine whether preterm birth of twins is associated with an increased risk of preterm birth in a subsequent singleton pregnancy.

STUDY DESIGN: All patients who delivered a twin gestation and a subsequent singleton pregnancy at Northwestern Memorial Hospital during a 10-year period were identified. We used a cohort study design, comparing the outcomes of the singleton pregnancies in women with preterm twin deliveries to those pregnancies with term twin deliveries.

RESULTS: One hundred sixty-seven women delivered twins followed by a singleton pregnancy. Women whose twin delivery was preterm (n = 99) were more likely than those who had delivered a term twin pregnancy (n = 68) to deliver a subsequent preterm singleton pregnancy (13.1% vs 2.9%; odds ratio, 5.0; 95% CI, 1.1, 22.9).

CONCLUSION: Preterm birth of twins is associated with an increased risk of preterm delivery in a subsequent singleton pregnancy.

Key words: premature, preterm birth, twin gestation

In the United States, 12% of births are preterm.1 Preterm birth is the leading cause of neonatal death and birth-related morbidity.2 Of the multiple risk factors for preterm birth that have been delineated, the 1 factor that has been shown to have the highest population attributable risk is “previous preterm delivery.”3 More specifically, multiple investigators have demonstrated that an idiopathic preterm delivery of a singleton fetus is associated with a high risk of idiopathic preterm delivery in a subsequent singleton pregnancy.3,4

However, the risk of subsequent preterm delivery is uncertain when the previous preterm delivery is of a twin gestation. This question is of particular relevance because the frequency of twin gestations has been increasing steadily and because the indication for prophylactic progesterone supplementation is a history of preterm birth.1,5 Studies to determine whether a preterm twin delivery is associated with an increased risk of a preterm singleton birth have reported conflicting results. Menard et al6 found that preterm birth of a twin gestation was associated with a nearly 3-fold increased risk of a preterm delivery in a subsequent singleton pregnancy. Rydhstroem7 and Bloom et al8 did not observe an association between a premature twin birth and the subsequent risk of a premature delivery in a singleton pregnancy. We sought to investigate further the risk of preterm birth in women with this history.

Materials and Methods
All women who delivered a twin pregnancy followed by a singleton pregnancy at ≥20 weeks of gestation at Northwestern Memorial Hospital between June 1, 1995, to May 31, 2005, were identified by a search of a database of all hospital discharges for the appropriate V-codes for outcome of delivery. Delivery records were then obtained and reviewed. Preterm delivery was defined as delivery at <37 completed weeks of gestation. Women were excluded from further analysis if either their twin or singleton pregnancy was delivered iatrogenically preterm (eg, after an induction for pre-eclampsia) or had a fetus with a major anomaly or an intrauterine death. Also excluded were women who, before their pregnancies during the study period, had other premature deliveries. This exclusion was performed so that any associations would not be confounded by previous preterm births.

The data were analyzed in 2 groups: those who had a term twin delivery and those who had a preterm twin delivery. Demographic data and pregnancy outcomes were abstracted from the medical record. Univariable comparisons were made with the Student t test and chi-square analysis for continuous and categoric variables, respectively. All statistical tests were 2-sided, and a probability value of <.05 was used to define the statistical significance of associations. All analyses were performed with Minitab software (version 13; Minitab, Inc, State College, PA). This study was approved by the institutional review board of Northwestern University.

Results
Two hundred thirty-six women with the required history of a previous twin birth...
followed by a singleton birth were identified. Of these, 21 women (9%) were excluded because either the twin or singleton pregnancy was delivered iatrogenically preterm; 34 women (14%) were excluded because of a delivery at <20 weeks of gestation, fetal anomalies, or an intrauterine fetal death; and 5 women (2%) were excluded because of a history of a preterm birth. There were 176 women who met inclusion criteria and had a spontaneous singleton delivery. Of these, the medical records of 9 women (5%) did not allow reliable ascertainment of gestational age at delivery in at least 1 pregnancy; thus, these women were also excluded from further analyses.

Of the 167 women who delivered twins before a singleton infant, the initial twin delivery was preterm in 99 cases (59%). These preterm deliveries were related to premature preterm rupture of the membranes in 49 women (49.5%) and spontaneous labor in the remaining 50 women (50.5%). Demographic characteristics of the cohort, which were stratified by whether their twin delivery was preterm or term, are presented in the Table. As expected, the mean gestational age at delivery of the twin pregnancy was significantly different between the 2 groups. Other demographic characteristics, which included maternal ages of the 2 groups at their subsequent singleton delivery (33.1 ± 4.4 years vs 33.2 ± 4.7 years, respectively; 7 = .7) were not different.

Of the 99 women who had delivered preterm twins, 13 women (13.1%) delivered prematurely in a subsequent singleton pregnancy. Of the 68 women who had delivered term twins, 2 women (2.9%) delivered subsequent premature singleton infants. Delivery of a preterm twin pregnancy was associated significantly with the delivery of a subsequent preterm singleton pregnancy (odds ratio, 5.0; 95% CI, 1.1, 22.9; 7 = .039).

**COMMENT**

Preterm birth of a singleton infant is associated consistently with a significantly increased risk of a preterm delivery in a subsequent singleton gestation. The preterm delivery of twins has been associated inconsistently with an increased risk for preterm birth of a subsequent singleton infant. We found that the idiopathic preterm birth of twins is associated with an increased risk of idiopathic preterm birth in a subsequent singleton infant.

Our results are consistent with data from a retrospective review of twin deliveries followed by a singleton gestation reported by Menard et al, who found that preterm birth of a twin gestation was associated with a significantly increased risk of preterm delivery in a subsequent singleton pregnancy (relative risk, 2.87; 95% CI, 1.02-8.09). Previous obstetric history preceding the index twin delivery was not described. We excluded women with a preterm birth before the index twin pregnancy.

Our results differ from those of Rydhstroem, who found that a preterm twin birth did not significantly affect the gestational duration of a subsequent singleton pregnancy. No explicit comparison of the frequency of preterm birth in singleton gestations that occurred after a previous preterm twin birth with those pregnancies that occurred after a previous term twin birth was made. Rydhstroem’s study also did not exclude women whose preterm births were iatrogenic. Because some indications for iatrogenic preterm delivery may not be recurrent, the inclusion of this patient population in the study may obscure the true recurrence risk of idiopathic preterm delivery. In addition, this was a population-based study from Sweden; therefore, the conclusions may not be generalizable to the current US population, in which the preterm birth rate is significantly higher than that in Sweden.

Bloom et al reported results similar to those of Rydhstroem’s from a population in Dallas, Texas, and concluded that a previous twin delivery at <35 weeks of gestation did not significantly increase the risk of preterm birth in a subsequent singleton pregnancy. However, their sample size was 82 twin pregnancies and did not have sufficient power to detect the difference in the preterm delivery rates that we detected in our study population.

Given the significant number of multiple gestation deliveries at Northwestern Memorial Hospital, we were able to identify a large population of women who met our study criteria and who could allow the discernment of a difference in outcomes. We are also confident that we were able to eliminate the con-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preterm twin delivery (n = 99)</th>
<th>Term twin delivery (n = 68)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at twin delivery (wks)*</td>
<td>32.3 ± 4.6</td>
<td>38.3 ± 0.7</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Maternal age at twin delivery (y)*</td>
<td>30.9 ± 4.9</td>
<td>30.0 ± 4.5</td>
<td>.2</td>
</tr>
<tr>
<td>White (n)</td>
<td>69 (69.7%)</td>
<td>48 (70.6%)</td>
<td>.9</td>
</tr>
<tr>
<td>Significant medical history (n)†</td>
<td>9 (9.1%)</td>
<td>9 (13.2%)</td>
<td>.4</td>
</tr>
<tr>
<td>Cesarean delivery (n)</td>
<td>33 (33.3%)</td>
<td>22 (32.4%)</td>
<td>.9</td>
</tr>
<tr>
<td>Previous term birth (n)</td>
<td>28 (28.3%)</td>
<td>21 (30.9%)</td>
<td>.7</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD.
† History of hypertension, pregestational diabetes mellitus, or autoimmune disease.
foundating issue of iatrogenic preterm birth by reviewing each subject’s original medical record and excluding from the analysis any subjects with an iatrogenic preterm delivery of either their twin or singleton gestation.

We also excluded women with a preterm delivery before their twin gestation in an effort to prevent possible confounding of our results by other nontwin preterm deliveries. It is possible that the causes that led to preterm birth in this excluded population are different than those in our cohort because the biologic condition of women with a history of a previous preterm singleton vs twin birth may differ. For example, preterm singleton deliveries may be related to inflammation; preterm twin births may be attributed partially to excessive myometrial stretch.\textsuperscript{9-13} If these patients were not excluded, it is possible that the association between preterm twin delivery and subsequent singleton delivery could be even higher.

A possible limitation of this study is the potential for referral bias. Our hospital is a tertiary care and referral center. Therefore, it is possible that our study cohort represents a particularly high-risk population with results that are not applicable to a more general population. However, the rate of preterm delivery in twin gestations that were recorded in our population is similar to that reported in the general population, which suggests that external validity should be intact. Moreover, the rate of preterm delivery in singleton pregnancies is 10.4%, which is a frequency that is very similar to the overall 9% (15/167) frequency seen in our study.\textsuperscript{1}

In conclusion, the data suggest that preterm birth of a twin pregnancy is associated with a higher risk of preterm delivery in a subsequent singleton pregnancy. These data can help physicians and patients better quantify the risk of preterm delivery and aid in the counseling of patients with a history of preterm birth of twins. Meis et al\textsuperscript{14} studied women with a previous preterm delivery of a singleton pregnancy and demonstrated that weekly injections of 17 alpha-hydroxyprogesterone caproate resulted in a significant reduction in the rate of recurrent preterm delivery in a subsequent singleton gestation. It remains uncertain whether women with a previous preterm delivery of twins, currently pregnant with a singleton fetus, would benefit from treatment with progesterone.

REFERENCES

Reduced third-trimester levels of soluble human leukocyte antigen G protein in severe preeclampsia

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OBJECTIVE: Recently, lower maternal plasma human leukocyte antigen (HLA)-G protein levels in preeclampsia (PE) in the first and second trimester was reported. Thus, we sought to evaluate the levels of HLA-G protein in patients with severe PE during the third trimester.

STUDY DESIGN: In this prospective case control study, amniotic fluid and maternal and cord blood samples were aspirated from 50 pregnant women during the third trimester. The study group included 26 pregnant women diagnosed with severe PE and 24 women without PE serving as controls. A soluble HLA-G–specific enzyme-linked immunosorbent assay was used to measure protein levels. Statistical analysis included the Student t test and simple regression analysis.

RESULTS: Maternal serum HLA-G levels in PE pregnancies were found to be significantly lower as compared with normal pregnancies (10.97 ± 6.55 vs 36.05 ± 34.53 μg/mL; P = .003).

CONCLUSION: A reduced level of maternal HLA-G protein was associated with severe PE during the third trimester. This finding may suggest an essential role for HLA-G in normal and preeclamptic pregnancies.

Key words: maternal HLA-G protein, severe preeclampsia


Preeclampsia (PE) is a multisystem disorder unique to human pregnancies and a leading cause for maternal and fetal mortality and morbidity that complicates approximately 2-7% of pregnancies.1,2 Although PE is one of the most studied topics in obstetrics, it still remains one of the most enigmatic because of its ambiguous etiology.1

Several studies have established that PE is a maternal inflammatory response commonly manifested clinically at the third trimester.3,6 However, its pathogenesis may be related to early abnormal trophoblast invasion into the maternal vascular tissue.7-9 Normal trophoblast invasion is concomitant with the expression of human leukocyte antigen (HLA)-G protein on trophoblasts. This nonclassical HLA class I antigen is expressed at high levels on the surface of extravillous trophoblasts, which are in direct contact with maternal tissue and lymphocytes.10,11 The morphology of the HLA-G antigen is nearly monomorphic; thus, it is presumably recognized as “self” and as such is not expected to evoke a maternal immune response. The function of HLA-G is not entirely clear, but it may act as an inhibitory ligand by interacting with the Ig-like transcript (ILT)-2 and ILT4 receptors. These receptors are the human inhibitory immune leukocytes immunoglobulin (Ig)-like receptors (ILIR) B1 (also called LIR1, ILT2, or CD85j) and ILIRB2 (ILT2/ILT4/CD85d).12 In addition, a soluble form of HLA-G (sHLA-G) protein derived from an alternatively spliced form of mRNA13 may participate in the vascular remodeling of maternal spiral arteries during pregnancy through an interaction with CD160.14

In PE in which poor placentation occurs, the placental expression of HLA-G protein was found to be attenuated.15,16 Furthermore, Yie et al17 reported lower maternal serum levels of sHLA-G as early as the first trimester in women who subsequently developed PE. Recently it was reported that HLA-G protein can also be found in amniotic fluid and cord blood.18-20 Thus, we sought to evaluate the levels of third-trimester sHLA-G antigen derived from maternal and fetal serums and amniotic fluid in pregnancies diagnosed as severe PE.

Materials and Methods

Study design
A prospective, institutional review board–approved case-control study was performed. The study consisted of 50 singleton third-trimester pregnancies admitted to Rabin Medical Center and Soroka Medical center from January 2003 to August 2005. The case group consisted of 26 pregnant women diagnosed with severe PE, and 24...
Women without PE served as controls. All women underwent cesarean section. The control group was matched for gestational age ± 13 days, gravity, and parity. Pregnancies were dated by either a certain and accurate last menstrual period consistent with fetal biometry at the time of the anatomical scan in the second trimester, early first-trimester crown-rump length, or a known day of fertilization. Pregnancies with questionable dating were excluded from the study.

Exclusion criteria were preexisting proteinuria and hypertension, multiple gestations, deliveries less than 34 weeks of gestation, active labor, and a known genetic or congenital malformation. In the control group, indications for cesarean section delivery less than 37 weeks of gestation were fetal distress and imminent active labor (ie, regular contractions with no cervical dilation or rupture of membrane without contractions or dilation) in an already scheduled cesarean section because of repeated cesarean section or malpresentation or at the woman’s request.

The definition used for establishing a severe PE diagnosis was based on the criteria of the American College of Obstetricians and Gynecologists of a normal blood pressure until 20 weeks of gestation and whether 1 or more of the following criteria were present: (1) blood pressure of 160 mm Hg systolic or higher or 110 mm Hg diastolic or higher on 2 occasions at least 6 hours apart while the patient is on bed rest; (2) proteinuria of 5 g or higher in a 24-hour urine specimen or 3+ or greater on 2 random urine samples collected at least 4 hours apart; (3) oliguria or less than 500 mL in 24 hours; (4) cerebral or visual disturbances; (5) pulmonary edema or cyanosis; (6) epigastric or right-upper-quadrant pain; (7) impaired liver function; (8) thrombocytopenia; and (9) fetal growth restriction.21

Written informed consent was obtained from all patients.

**Blood and amniotic fluid samples**

Before delivery, samples of maternal serum were collected from all patients. Maternal cord blood and amniotic fluid were collected from the women during the cesarean section. All samples were centrifuged at 3000 g for 5 minutes and stored at -20°C.

**Detection of soluble HLA-G protein**

Using an HLA-G–specific enzyme-linked immunosorbent assay (ELISA), we measured the concentration of sHLA-G in maternal serum, cord serum, and amniotic fluid from both groups. Concentrations of sHLA-G protein were measured using a sandwich ELISA with 2 HLA-G–specific antibodies, one that recognizes both the membrane and soluble forms of HLA-G and a second that recognizes only the soluble form. This assay is specific for the soluble form of the full-length HLA-G protein because it uses an antibody that detects the unique carboxyl terminus found only on sHLA-G.13,22 Each well of high binding polystyrene 96-well micro plates (Costar, Corning, NY) was coated with 50 μL of capturing antibody minimum essential medium-G/9 5 μg/mL in 0.1 M carbonate buffer (pH 9.6) at 4°C overnight. The plates were washed 3 times with phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBS-T). The wells were blocked for 3 hours at room temperature with 100 μL of 10% skim milk in PBS, followed by washing 5 times with PBS-T after each incubation step. Standards and standard protein solutions were applied at 50 μL volumes in triplicate and incubated at 4°C overnight. Standard protein was sHLA-G isolated from supernatants of 0.221-Gs as described15 or recombinant protein produce in Escherichia coli refolded with recombinant β2m and peptide as described.23

Essentially similar standard profiles were obtained using either source of protein. Standard was diluted from 300 to 1 ng/mL serially in human serum AB (Gemini Bio-Products, Woodland, CA) depleted of HLA-G proteins by column purification with monoclonal antibody 87G. Biotinylated detecting antibody 16G1 0.5 µg/mL in PBS with 2% bovine serum albumin (BSA) was applied and incubated for 3 hours at room tempera-
One hundred microliters of NeutrAvidin-horseradish peroxidase (Molecular Probes, Eugene, OR) at 0.5 μg/mL in PBS with 2% BSA was applied and incubated for an hour at room temperature followed by the addition of 100 μL of Amplex UltraRed (Molecular Probes) at 50 μM in PBS with 0.67 mM hydrogen peroxide and incubation at room temperature for 30 minutes protected from light. The fluorescence from each well was measured with Fluoroskan Ascent (Thermo Electron Co, Vantaa, Finland) using filters at 544 nm for excitation and 590 nm for emission. The minimal detection level was 10 mL/ng as determined using control samples.

All measurements were carried out in triplicate with intraplate variances as summarized in the Figure. Interplate variance was tested using control samples.

Calculations were performed with JMP version 5 program (SAS Institute, Inc, Cary, NC).

### Results

Maternal serum was obtained from 24 of 26 women in the study group (SG) and from 22 of 24 women in the control group (CG). Amniotic fluid was obtained from all pregnant women participating in the study. Cord blood was obtained from 24 of 26 women in the SG and all women in the CG. When the SG and CG were compared, there were no statistical differences in gestational age (GA) (36.1 ± 2.8 [± SD] weeks’ gestation and 36.1 ± 3.1 [± SD] weeks’ gestation, respectively; P = .72); gravidity (3.3 ± 2.9 [± SD] vs 3.8 ± 2.4 [± SD], respectively; P = .49); and parity (2.4 ± 2.9 [± SD] vs 2.9 ± 2.4 [± SD], respectively; P = .49).

From measurements of maternal serum, we found sHLA-G levels in PE pregnancies were significantly lower than those of normal pregnancies (10.97 ± 6.55 vs 36.05 ± 34.53 μg/mL; P = .003; Figure). However, mean fetal serum and amniotic fluid sHLA-G levels in PE pregnancies were not significantly different in normal pregnancies. These results are summarized in the Table.

In intragroup analysis, in a comparison of amniotic fluid with fetal serum, mean sHLA-G levels within each group were significantly higher in both PE and normal pregnancies. Furthermore, mean sHLA-G levels in maternal serum from normal pregnancies was significantly higher than in fetal serum or amniotic fluid from the same group. Conversely, in PE pregnancies mean sHLA-G levels in maternal serum were not significantly different when compared with fetal serum; however, they were significantly higher than those found in amniotic fluid. These results are shown in the Table.

In summary, maternal serum sHLA-G levels in PE pregnancies were found to be constantly lower in the third trimester (1 of 26 above 20 ng/mL) as compared with the control group in which the distribution was clearly skewed (13 of 22 showed levels above 20 ng/mL; Figure). Using a simple regression analysis, there was no significant correlation between the gestational age and levels of sHLA-G in the SG and the CG (P = .3).

### Comment

The major finding of our prospective study indicates that sHLA-G levels in maternal serum from women in their third trimester of pregnancy are lower in women with PE than among normal controls. Recently, Yie et al reported lower sHLA-G protein levels in serum samples from 12 women with PE during the first and second trimesters. Although they observed a trend toward lower third-trimester plasma HLA-G in the study group, the small sample size did not allow a statistically significant conclusion to be drawn. By expanding the study population, we were able to demonstrate significantly lower levels during the third trimester in women suffering from severe PE.

This observation is paralleled by several studies reporting lower placental HLA-G expression and a report of lower levels in postpartum serum in women with PE. Based on a worldwide litera-

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**Table shHLA-G levels in severe PE and in normal pregnancies including intragroup analysis**

<table>
<thead>
<tr>
<th></th>
<th>Severe PE</th>
<th>Normal pregnancies</th>
<th>P value</th>
</tr>
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<tr>
<td>Maternal serum</td>
<td>10.97 ± 6.55</td>
<td>36.05 ± 34.53</td>
<td>.003*</td>
</tr>
<tr>
<td>Cord blood</td>
<td>5.39 ± 6.42</td>
<td>7.19 ± 10.42</td>
<td>.47</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>−4.11 ± 6.59</td>
<td>−1.35 ± 9.86</td>
<td>.25</td>
</tr>
<tr>
<td>Maternal serum vs amniotic fluid</td>
<td>10.97 ± 6.55 vs −4.1 ± 6.6*</td>
<td>36.05 ± 34.53 vs −1.35 ± 9.86*</td>
<td>.25</td>
</tr>
<tr>
<td>Maternal serum vs cord blood</td>
<td>10.97 ± 6.55 vs 7.19 ± 10.42</td>
<td>36.05 ± 34.53 vs 7.19 ± 10.42*</td>
<td>.25</td>
</tr>
<tr>
<td>Cord blood vs amniotic fluid</td>
<td>5.39 ± 6.42 vs −4.1 ± 6.6*</td>
<td>7.19 ± 10.42 vs −1.4 ± 9.8*</td>
<td>.25</td>
</tr>
</tbody>
</table>

* P < .05

Statistical analysis used: Student t test.
In addition to the distinguishing levels of HLA-G in maternal serum, we found that the levels of sHLA-G in normal third-trimester pregnancies were highest in the maternal compartment, followed to a lesser extent by the fetal compartment, and were at minimal and frequently undetectable levels in amniotic fluid. It is noteworthy that in PE no difference was found between maternal and cord serum, whereas in amniotic fluid the HLA-G levels were again significantly lower. Combined with recent reports of sHLA-G protein expression in fetal and amniotic tissues, these results might contribute to an understanding of the origin and distribution of sHLA-G. Whereas the main source of maternal serum sHLA-G is probably the extravillous cytotrophoblasts, the expression of sHLA-G in the mother, fetus, and amniotic tissue and its distribution remains to be elucidated.

It is well established that parturition is an inflammatory process manifested by a cascade of inflammatory substances such as prostaglandins, interleukins, and cytokines. Sacks et al showed a decreasing level in maternal serum sHLA-G protein during the three trimesters in both normal and PE pregnancies. Furthermore, we previously reported a higher amniotic fluid HLA-G levels at second-trimester as compared with the third trimester in normal pregnancies. The data presented by us combined with that of Yie suggest that maternal serum HLA-G protein levels decrease with gestational age. The reduced immunosuppressant HLA-G levels in a normal third trimester and PE combined with a decrease in the number and function of cytotrophoblasts may support the idea that both parturition and PE constitute maternal systemic immune-mediated inflammatory processes.

Because PE pathogenesis is related to early abnormal trophoblastic invasion, several studies have attempted to identify early-pregnancy proteins that might be predictive of PE. Recently, Levine et al reported that placental growth factor urine levels were lower in the second trimester in women who later developed PE and that soluble fms-like tyrosine kinase 1 levels increased approximately 5 weeks before the onset of disease. Similarly, maternal serum HLA-G is significantly low in PE in early pregnancy and as such may be useful in predicting the onset of PE. Further additional longitudinal and large population studies and analysis are needed to establish a diagnostic role for sHLA-G in PE.

The role of HLA-G in maternal-fetal immune tolerance has been extensively studied in a number of different contexts. In PE decreased HLA-G expression levels have been consistently observed, prompting speculation that lower HLA-G levels might be related to early partial failure of maternal immune tolerance, which in turn is manifested later as an inflammatory response. Although it is important to point out that no study has provided evidence distinguishing lower HLA-G levels as a cause or effect of PE, this study adds to a growing body of evidence that HLA-G expression at the level of both membrane-bound and soluble protein of women with PE is lower during all 3 trimesters of pregnancy. Therefore, based on the likely immunologic role of HLA-G in pregnancy and the results presented here and in related studies, we propose that HLA-G has an etiologic role in PE. Furthermore, measurements specifically detecting sHLA-G in maternal serum at various stages of pregnancy might contribute a potential diagnostic and clinical role in the treatment of PE.

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phoblasts and found in amniotic fluid are due to unusual glycosylation. J Immunol 1998;160: 5922-8.
Stillbirths in an urban community in Pakistan

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OBJECTIVE: The purpose of this study was to determine stillbirth risk factors and gestational age at delivery in a prospective developing country birth cohort.

STUDY DESIGN: At 20-26 weeks of gestation, 1369 Pakistani women were prospectively enrolled in the study; the gestational age was determined by ultrasound evaluation, and risk factors and pregnancy outcomes were assessed.

RESULTS: The stillbirth rate was 33.6 of 1000 births, despite the fact that 96% of the women received prenatal care, 83% of the women were attended by skilled providers in the hospital, and a 20% of the women underwent cesarean delivery. Fifty-one percent of stillbirths occurred at 37 weeks of gestation and 19% occurred from 34-36 weeks of gestation. Only 4% of the births had congenital anomalies. Hemoglobin of <8 g/dL, vaginal bleeding, and preeclampsia were associated with increased stillbirth risk.

CONCLUSION: In this developing country with reasonable technical resources defined by hospital delivery and a high cesarean delivery rate, stillbirth rates were much higher than rates in the United States. That most of the stillbirths were term and did not have congenital anomalies and that the death appeared to be recent suggests that many Pakistani stillbirths may be preventable with higher quality obstetric care.

Key words: developing country, obstetric care, stillbirth

Stillbirth is one of the most common adverse outcomes of pregnancy. Each year, 3.3 million stillbirths are reported; 97% stillbirths occur in developing countries.1 Because registries are available in only 4% of the developing world and underreporting is a common problem,2 it is likely that an additional 1-2 million stillbirths occur that are not reported.

Stillbirth rates vary by geographic region and socioeconomic status. Rates of 5 per 1000 are seen in the United States and most developed countries; stillbirth rates in the range of 30-40 per 1000 births are common in the least developed countries.3 South Asia has the world’s largest numeric stillbirth burden; the rates range from 25-40 per 1000 births. Within Pakistan, reported stillbirth rates vary from 36 per 1000 to 70 per 1000 in some rural areas.4,5,6 In contrast, the World Health Organization reports a Pakistani stillbirth rate of 22 per 1000 births.5 One reason for the discrepancy among reports is that the lower limit of the gestational age or birthweight varies widely. Many developed countries use 20 weeks as the lower gestational age cutoff for stillbirth, but some developing countries (such as Sweden) still use 28 weeks as the lower cutoff. In developing countries, the most commonly used cutoffs are 28 weeks or 1000 g.5,7,10,11

Stillbirths that occur in the peripartum period are generally normal in appearance and are often called fresh stillbirths. When the skin is not intact, or “macerated,” it implies that death occurred at >24 hours before delivery.2 In developed countries, intrapartum stillbirths comprise <10% of all stillbirths; in many developing countries, higher proportions of the stillbirths are thought to occur during birth.2 The occurrence of an intrapartum stillbirth in a developed country is considered the result of inadequate care.12 Intrapartum stillbirths in developing countries may represent inadequate access to essential obstetric care and inadequate care.13

Other factors that are associated with the high stillbirth rates in developing countries are infection, which includes congenitally acquired infections such as syphilis, Gram-negative sepsis, malaria, birth injury, hypertensive disease (especially when associated with poor management of preeclampsia/eclampsia), poor nutritional status, previous stillbirth, congenital anomalies, and sickle cell disease.5,8 Because most of the research has been hospital-based, rather than population-based, much is still unknown about the prevalence and cause of stillbirth in developing countries. Thus, our goal in this study was to determine the stillbirth rate, the risk factors for stillbirth, and gestational age at delivery in a well-defined, prospective urban developing country stillbirth cohort.

METHODS

The study was reviewed and approved by the Aga Khan University Ethical and Re-
The LHWs tracked all enrolled women until the pregnancy was completed, and the pregnancy outcome was recorded by the study staff. The study team developed a liaison with all the public and private delivery facilities and the local home birth attendants to capture all birth outcomes in the study cohort. Once a delivery was reported to the study clinic (either by the LHW, the hospital staff, or the woman/family), the research medical officer and a study nurse visited the woman at her home or at the health facility within 48 hours of delivery to collect the maternal delivery and postnatal data and to confirm the birth outcome.

A stillbirth was defined as an infant who was born after enrollment for whom no sign of life (breathing, crying, heartbeat) was evident. Whether the infant was macerated or not was determined. For all reported stillbirths, a physician who was trained in general research methods and specifically in data collection that was related to this study visited the home and collected information from the family about the circumstances related to the event and the treatment provided. For the deliveries that occurred in a hospital, this information was supplemented and verified by the hospital records. Finally, the records of all stillbirths were reviewed by a neonatologist (S.S.) and the primary author (I.J.).

The primary obstetric cause of stillbirth was assigned jointly, using the Patterson et al\textsuperscript{14} adaptation of the Aberdeen Classification\textsuperscript{15} for developing countries. The primary cause of death was defined as the obstetric antecedent factor or event that initiated the process or sequence of events that led to the death of the fetus. The classification system is nonhierarchic and allows for the identification of the following primary causes, with the use of set criteria and definitions: intrapartum asphyxia, spontaneous preterm labor, antepartum hemorrhage, intrauterine infections, intrauterine growth retardation (including postmaturity), hypertension, fetal abnormality, maternal disease, trauma, and unexplained intrauterine death.\textsuperscript{14} A single cause is assigned to each stillbirth.

Data management and analysis
All data were entered centrally; data edits that included inter- and intraform consistency checks were performed at entry; additional edits were performed by an independent data center. The data were analyzed with SAS software (version 9.0; SAS Institute Inc, Cary, NC). Descriptive analyses were performed; chi-square and Fisher’s exact tests were completed, and relative risks (RRs) and 95% CIs were calculated for the prospectively identified variables that were associated with stillbirth.

RESULTS
From September 2003 to August 2005, 2205 pregnant women were registered by the LHWs in the study area (Figure 1).
Eighty-five percent (n = 1879 women) met the prescreening criteria (resident of the area and <26 weeks of gestation) and were referred to the clinic for further screening. Of the 1606 women who attended the clinic, 1369 pregnant women (85%) were confirmed to be between 20 and 26 weeks of gestation, were willing to participate, and were enrolled in the study. Birth outcomes and follow-up data were ascertained for 1280 of the women (94%) enrolled in the study. Among the reasons for nonascertainment of outcome were delivery outside the area and loss to follow-up evaluation (5%) and refusal of subsequent visits (1%).

There were 43 stillbirths; the overall stillbirth rate was 33.6 per 1000 births. Table 1 lists the demographic and maternal risk factors in the study population. Most of the women who enrolled were Urdu speaking (81%); Punjabi, Pushto, and Sindhi ethnicities represented most of the remaining population. One-third of the population had a household monthly income of <600 rupees ($10 US), and another one-third had a household income of between 600 and 1000 rupees per month. Approximately one-half of the women lived with an extended family; the other one-half of the women lived within a nuclear family arrangement. Eighty-five percent of the women who enrolled were between 20 and 35 years old, with only 10% >35 years. Nearly one-third of the population had no formal education, and only 18% had >10 years of formal education. Nearly three-fourths of the women had a birth interval of >24 months; 23% of the women had experienced at least 1 pregnancy loss; 20% of the women were primigravid, and 25% were gravid >5. These characteristics were not significant risk factors for stillbirth, although there was a trend toward a previous pregnancy loss and high parity being risk factors for stillbirth.

Table 2 describes the characteristics of labor, delivery, and birth in the overall population and among the stillbirths (n = 43). Nineteen percent of liveborn deliveries were preterm (<37 weeks of gestation) vs 49% for stillbirths (RR, 3.9; 95% CI, 2.2-6.9). Fifty-one percent of all stillbirths were term; 19% of the stillbirths were late preterm (34-36 weeks of gestation) and thus potentially salvageable. Of those stillbirths with available data, 96% had no evidence of gross congenital anomalies, and 73% were fresh, which suggests a peripartum death. Sixty percent of the stillbirths were male, compared with 51% of the live births (P = .3250).

Of the prenatal risk factors that were examined at 20-26 weeks of gestation, approximately 92% of enrolled women were anemic with hemoglobin concentrations ≤11 gm/dL; of these, 89% had hemoglobin levels between 8 and 11 gm/dL. Although values between 8 and 11 were not associated with an increased risk for stillbirth, hemoglobin levels of <8 gm/dL were a significant risk factor for stillbirth (RR, 3.8; 95% CI, 1.6-9.2). Labor and delivery characteristics that were associated with stillbirth included foul-smelling amniotic fluid (RR, 4.6; 95% CI, 2.1-9.8), cloudy or meconium-stained fluid (RR, 12.1 [95% CI, 5.6-25.8] and 4.2 [95% CI, 1.8-9.7], respectively), and excessive bleeding during delivery (RR, 5.5; 95% CI, 2.7-11.2). However, neither prolonged labor (11% of stillbirths and 13% of live births) nor maternal fever (6% of stillbirths and 9% of live births) were associated significantly with an increased risk of stillbirth.

Access to medical care was similar for women with stillbirths and live births (Table 3). Ninety-five percent of all women received at least 1 prenatal care visit; 72% of the live births received >4 visits, compared with 69% of women with a stillbirth (P = not significant). Seventy nine percent of live births, compared with 84% of stillbirths (P = not significant), were conducted at a hospital, clinic, or health center; the remaining 20% were conducted in a home setting. Sixty percent of live birth deliveries were attended by a physician; 22% were attended by a nurse or equivalent provider; 11% were attended by a traditional birth attendant, and 5% were attended by a family member. Seventy-one percent of all deliveries and 14% of stillbirths were performed by cesarean delivery.

The stillbirths in this study were associated commonly with antepartum hemorrhage (23%), intrapartum asphyxia (23%), spontaneous preterm labor (18%), and maternal disease (8%; Table 4). Seven intrauterine fetal deaths (18%) were classified as unexplained because these occurred at term, were without a congenital anomaly, and were mostly macerated and because no cause could be determined. Almost 90% of antepartum hemorrhages were associated with a clinical abortion. Elevated blood pressure (140/90 mm Hg) on admission was recorded for 56% of the women with a history of antepartum hemorrhage. These cases are classified as abortion with hypertension and not as hypertensive disorders in accordance with the classification system we used. Although 9 of the 43 fetal deaths were
noted to have foul-smelling amniotic fluid, each of these cases had factors other than infection that were thought more likely to be causal. Among women who delivered with maternal disease who had a stillbirth, 2 women had diabetes mellitus, and 1 woman had maternal jaundice, the cause and type of which was unknown.

**COMMENT**

This study had a number of strengths and weaknesses. The strengths include the recruitment of women from a specific geographic area, the prospective nature of the study, the high follow-up rate, ultrasound determination of gestational age, the determination of the outcomes (regardless of whether the delivery occurred at home or in the hospital), and the attempt to identify specific causes of death. Among the weaknesses, the relatively small number of stillbirths and the absence of autopsies stand out.

The most striking finding of this study was the high rate of stillbirth (33.6 per 1000 deliveries) in a community in

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### TABLE 1
**Demographic and maternal risk factors***

<table>
<thead>
<tr>
<th>Variable</th>
<th>Live births (n)</th>
<th>Stillbirths (n)</th>
<th>Relative risk (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total enrolled</td>
<td>1237</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td></td>
<td></td>
<td></td>
<td>.2415</td>
</tr>
<tr>
<td>&lt;20</td>
<td>69 (6%)</td>
<td>1 (2%)</td>
<td>0.5 (0.1, 3.3)</td>
<td></td>
</tr>
<tr>
<td>20-34</td>
<td>1045 (85%)</td>
<td>34 (81%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>117 (10%)</td>
<td>7 (17%)</td>
<td>1.8 (0.8, 4.0)</td>
<td></td>
</tr>
<tr>
<td>Household monthly income per capita (rupees; 60 = $1 US)</td>
<td></td>
<td></td>
<td></td>
<td>.4998</td>
</tr>
<tr>
<td>Low (&lt;600 rupees)</td>
<td>215 (32%)</td>
<td>4 (20%)</td>
<td>0.5 (0.2, 1.8)</td>
<td></td>
</tr>
<tr>
<td>Middle (600-1000 rupees)</td>
<td>250 (38%)</td>
<td>9 (45%)</td>
<td>1.0 (0.4, 2.7)</td>
<td></td>
</tr>
<tr>
<td>Upper (&gt;1000 rupees)</td>
<td>197 (30%)</td>
<td>7 (35%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Maternal education (y)</td>
<td></td>
<td></td>
<td></td>
<td>.7298</td>
</tr>
<tr>
<td>None</td>
<td>412 (34%)</td>
<td>15 (36%)</td>
<td>1.0 (0.4, 2.3)</td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>214 (17%)</td>
<td>9 (21%)</td>
<td>1.1 (0.4, 2.9)</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>390 (32%)</td>
<td>10 (24%)</td>
<td>0.7 (0.3, 1.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>215 (18%)</td>
<td>8 (19%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>.7914</td>
</tr>
<tr>
<td>Urdu</td>
<td>998 (81%)</td>
<td>36 (86%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Other (Punjabi, Pashto, Sindhi)</td>
<td>233 (19%)</td>
<td>6 (14%)</td>
<td>0.7 (0.3, 1.7)</td>
<td></td>
</tr>
<tr>
<td>Family structure</td>
<td></td>
<td></td>
<td></td>
<td>.0855</td>
</tr>
<tr>
<td>Extended</td>
<td>635 (52%)</td>
<td>16 (38%)</td>
<td>0.6 (0.3, 1.1)</td>
<td></td>
</tr>
<tr>
<td>Nuclear-only</td>
<td>596 (48%)</td>
<td>26 (62%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Birth interval (mo)</td>
<td></td>
<td></td>
<td></td>
<td>.0797</td>
</tr>
<tr>
<td>&lt;12</td>
<td>6 (1%)</td>
<td>0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>12-24</td>
<td>205 (27%)</td>
<td>1 (5%)</td>
<td>0.1 (0.0, 1.1)</td>
<td></td>
</tr>
<tr>
<td>≥24</td>
<td>553 (72%)</td>
<td>19 (95%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
<td>.0506</td>
</tr>
<tr>
<td>Primigravid</td>
<td>221 (18%)</td>
<td>9 (21%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>388 (31%)</td>
<td>7 (17%)</td>
<td>0.5 (0.2, 1.2)</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>322 (26%)</td>
<td>9 (21%)</td>
<td>0.7 (0.3, 1.7)</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>299 (24%)</td>
<td>17 (41%)</td>
<td>1.4 (0.6, 3.0)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy loss</td>
<td></td>
<td></td>
<td></td>
<td>.0572</td>
</tr>
<tr>
<td>At least 1 previous loss</td>
<td>222 (22%)</td>
<td>12 (36%)</td>
<td>1.9 (1.0, 3.9)</td>
<td></td>
</tr>
<tr>
<td>No previous loss</td>
<td>775 (78%)</td>
<td>21 (64%)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* Totals for each category may be less because of missing values; percentages may not add up to 100% because of rounding.
### TABLE 2
#### Labor and delivery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Live births (n)*</th>
<th>Stillbirths (n)</th>
<th>Relative risk (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>597 (51%)</td>
<td>22 (60%)</td>
<td>1.4 (0.7, 2.6)</td>
<td>.3250</td>
</tr>
<tr>
<td>Female</td>
<td>566 (49%)</td>
<td>15 (41%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age at birth</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Preterm</td>
<td>232 (19%)</td>
<td>21 (49%)</td>
<td>3.9 (2.2, 6.9)</td>
<td></td>
</tr>
<tr>
<td>Term</td>
<td>1003 (81%)</td>
<td>22 (51%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple gestation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>1175 (99%)</td>
<td>30 (97%)</td>
<td>0.3 (0.0, 1.8)</td>
<td>.2482</td>
</tr>
<tr>
<td>Twin</td>
<td>10 (1%)</td>
<td>1 (3%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Gross congenital abnormality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>120 (11%)</td>
<td>1 (4%)</td>
<td>0.3 (0.0, 2.3)</td>
<td>.3506</td>
</tr>
<tr>
<td>No</td>
<td>1000 (89%)</td>
<td>27 (96%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Signs of maceration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>Not available</td>
<td>22 (63%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Macerated</td>
<td></td>
<td>8 (23%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Not known</td>
<td></td>
<td>5 (14%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td>.0281</td>
</tr>
<tr>
<td>&lt;8</td>
<td>39 (3%)</td>
<td>5 (12%)</td>
<td>3.8 (1.6, 9.2)</td>
<td></td>
</tr>
<tr>
<td>8-11</td>
<td>1096 (89%)</td>
<td>34 (81%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>&gt;11</td>
<td>94 (8%)</td>
<td>3 (7%)</td>
<td>1.0 (0.3, 3.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Length of labor</strong></td>
<td></td>
<td></td>
<td></td>
<td>1.0000</td>
</tr>
<tr>
<td>Prolonged</td>
<td>157 (13%)</td>
<td>4 (11%)</td>
<td>0.8 (0.3, 2.4)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1024 (87%)</td>
<td>31 (89%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Smell of amniotic fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Foul</td>
<td>94 (9%)</td>
<td>9 (27%)</td>
<td>4.6 (2.1, 9.8)</td>
<td></td>
</tr>
<tr>
<td>Not foul</td>
<td>971 (89%)</td>
<td>19 (58%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Not known</td>
<td>30 (3%)</td>
<td>5 (15%)</td>
<td>7.4 (2.9, 18.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Color of amniotic fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Clear</td>
<td>899 (82%)</td>
<td>13 (39%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Meconium-stained</td>
<td>141 (13%)</td>
<td>9 (27%)</td>
<td>4.2 (1.8, 9.7)</td>
<td></td>
</tr>
<tr>
<td>Other unclear</td>
<td>53 (5%)</td>
<td>11 (33%)</td>
<td>12.1 (5.6, 25.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Fever in labor</strong></td>
<td></td>
<td></td>
<td></td>
<td>.7758</td>
</tr>
<tr>
<td>Presence of fever</td>
<td>105 (9%)</td>
<td>2 (6%)</td>
<td>0.6 (0.2, 2.6)</td>
<td></td>
</tr>
<tr>
<td>No fever</td>
<td>1042 (91%)</td>
<td>32 (94%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Not known</td>
<td>2 (&lt;1%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vaginal bleeding</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Excessive bleeding</td>
<td>71 (6%)</td>
<td>10 (29%)</td>
<td>5.5 (2.7, 11.2)</td>
<td></td>
</tr>
<tr>
<td>Normal bleeding</td>
<td>1053 (93%)</td>
<td>24 (69%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Not known</td>
<td>8 (1%)</td>
<td>1 (3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Totals for each category may be less because of missing values; percentages may not add up to 100% because of rounding.
* Defined as prolonged if it was >12 hours for primiparous women or >8 hours for multiparous women.
* Defined as excessive, based on clinical opinion of birth attendant.

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12 hours for primiparous women or 8 hours for multiparous women.
which most of the women were delivered at a hospital facility by a doctor or midwife, with an overall 20% cesarean delivery rate. Moreover, more than one-half of the stillbirths in this population were term, and another 19% of stillbirths were late preterm (34-36 weeks of gestation). There were few congenital anomalies among the stillbirths (4%), and most stillbirths were without maceration, which indicates that many of the stillbirths occurred in the peripartum period and thus were potentially salvageable during the time of labor and delivery. Lown et al note that appropriate cesarean section should prevent many of the stillbirths are preterm. Our study reinforces findings from other recently published studies that report a failure of Pakistani health facilities to offer essential and comprehensive obstetric care; deficiencies in staff competence have also been reported. We therefore speculate that upgrading health system performance will reduce the high stillbirth rates and other adverse pregnancy outcomes, even in populations with adequate access to maternity care.

In the United States and other developed countries, most of the stillbirths occur antenatally and frequently are macerated. In this study, most stillbirths were fresh, which indicates that most fetal deaths occurred close to delivery. A recent population-based study in rural areas of Pakistan, where the stillbirth rate was 47 per 1000 births, reported that 75% were fresh, which are results that are similar to this study. Based on these and other data, it appears that there are major differences in the timing of stillbirths between developed and developing countries, with a far greater percentage of stillbirths in developing countries occurring in the peripartum period.

In the United States, 50% of the stillbirths occur at <28 weeks of gestation (approximately 1000 g), and nearly 80% of the stillbirths are preterm. The gestational age distribution of stillbirths in most developing countries is unknown, in part because gestational age is not assessed routinely. However, in this study, all the gestational ages were assessed by mid-trimester ultrasound examination.
That 51% were term and another 19% were late preterm suggests that the gestational age pattern in Pakistan is different from that found in developed countries with a far greater likelihood that a stillbirth, when it occurs, will occur at or close to term. Nevertheless, even with a high proportion of stillbirths at or near term, in this study, preterm birth was significantly associated with stillbirth.

In developed countries, even with an autopsy and histologic evaluation of the placenta, the cause of many stillbirths remains unknown. In this study, these evaluations were unavailable, and the cause of death was determined based on questions asked of the delivery attendant, family members, and the mother and, if available, a review of the medical record. Therefore, our results likely will approximate only causes that were assigned with the use of more sophisticated methods. Nevertheless, in these stillbirths, it appears that severe antenatal anemia is a relatively potent risk factor; the constellation of asphyxia, hypertension, antepartum hemorrhage, and abortion also contributed to the risk for stillbirth. The frequent observation in the stillbirths of foul-smelling amniotic fluid suggests that intraamniotic infection plays an important role also.

In Hyderbad, Pakistan, stillbirths are a common adverse pregnancy outcome that occur in ≥3% of births. The risk factors appear similar to those that are seen in other geographic areas. Because so few stillbirths had congenital anomalies and most were term or near term and fresh, this study suggests that most of those stillbirths should be preventable with better obstetric care. Because the quality of care in this setting is relatively high for a developing country (with the provision of prenatal care, skilled birth attendance, and a high rate of cesarean delivery seen in both the overall and stillbirth population), it is likely that the quality of care would need to be improved for the stillbirth rate to be substantially reduced. For example, the interventions that are used in many developed countries to identify women at risk for stillbirth (which include fundal height measurements and third-trimester ultrasound examination to search for fetal growth retardation, routine screening for diabetes mellitus, and fetal kick counts and nonstress testing to identify fetuses at risk of dying) rarely are used in Pakistan and were not in routine use in Hyderbad during the course of this study.

In developed countries, a perinatal death review in which each death is examined for cause and preventability is often the first step that is undertaken to develop appropriate interventions to reduce adverse outcomes. Because it appears that the medical resources are available to achieve a lower stillbirth rate in Hyderbad than we observed, we recommend the initiation of an ongoing perinatal death review to better define the causes of stillbirth, to determine which stillbirths may be preventable, and to direct interventions and resources to improve perinatal outcomes. If undertaken, it is likely that such a review would find a general lack of appropriate monitoring for women who are most at risk for stillbirth (eg, those women with growth retardation, hypertension, diabetes mellitus, and hemorrhage) and fail to perform appropriately timed cesarean deliveries for those women at high risk of fetal death.


Preterm birth (PTB) is the leading cause of infant mortality and the largest single cause of cerebral palsy. Of the PTBs in the United States, about 80% are spontaneous. The risk of recurrence ranges between 15% and 80%, depending on the number of prior PTBs and how early in gestation the PTB occurred. For example, women with 1 prior PTB are 2-4 times more likely to deliver another preterm infant, and women with 2 prior PTBs have a 4- to 6-fold increased risk of delivering preterm compared to multiparous women with no prior PTB.

Objective: The purpose of this study was to determine effectiveness of 17 alpha-hydroxyprogesterone caproate (17 P) prophylaxis by gestational age (GA) at 17 P initiation.

Study Design: Singleton gestations with ≥ 1 preterm birth (PTB) treated with 17 P prophylaxis for recurrent preterm birth before 27 weeks were identified from a database. Data were stratified by GA at 17 P initiation (16-20.9 [n = 599] weeks and 21-26.9 [n = 307] weeks) and number of PTB (1, 2, > 2). Outcome variables were PTB at < 37, < 35, and < 32 weeks.

Results: No significant differences were found in gestational age at delivery or rates of recurrent PTB < 37, < 35, and < 32 weeks between those women initiating 17 P at 16-20.9 weeks or 21-26.9 weeks, or when stratified by number of prior preterm deliveries.

Conclusion: Initiation of 17 P prophylaxis at 21-26.9 weeks is as effective as initiation at 16-20.9 weeks of gestation.

Key words: preterm delivery, 17 alpha-hydroxyprogesterone caproate prophylaxis

Materials and Methods

We performed a retrospective analysis of prospectively collected clinical data from women enrolled from February 2004 through March 2006 in an outpatient 17 P administration program provided by Matria Healthcare. The data included medical and pregnancy history, current pregnancy risk factors and diagnoses, biometric clinical data, medication administration records, and pregnancy outcomes. All information was collected using standard operating procedures, forms, and proprietary software systems. In addition to the care provided by the patient’s physician, outpatient clinical management was provided by skilled perinatal nurses utilizing standardized plans of treatment, educational materials, and nursing interventions. Our Institutional Review Board (IRB) determined that analysis of these data does not require additional IRB approval. All patients received weekly home nursing assessments and injections, as well as telephonic perinatal nursing and pharmacist support available 24 hours a day, 7 days a week for any pregnancy-related concerns. The patients’ health care provider prescribed all outpatient services. At initiation of the service, patients received information concerning informed consent to allow their deidentified data to be used for research and reporting purposes and were asked to sign a consent form if they wished to participate.

Eligible for inclusion were patients with singleton gestation, history of at least 1 prior PTB, and who were between 16.0-26.9 weeks’ gestation with no symptoms or diagnosis of preterm labor.
at initiation of 17 P prophylaxis. We excluded women with cervical cerclage and those withdrawing from the program after receiving only the initial test injection. The 17 P was compounded by a qualified compounding pharmacy following USP <977> standards utilizing an ISO Class 5 Clean Room to the specifications and formulation as the 17 P used in the NICHD-sponsored trial, including the vehicle (castor oil). Adequate quality control procedures and documentation to assure sterility and potency of each vial was followed.

Data were divided into 2 groups according to initiation of 17 P at 16-20.9 weeks, or 21-26.9 weeks' gestation and stratified according to the number of prior PTB (1, 2, or > 2). Various \( \chi^2 \) and nonparametric methods were used as appropriate to compare maternal demographics, neonatal mortality, and pregnancy outcomes among the 3 groups overall and within strata. A 2-sided \( P \) value of < .05 was considered significant. The primary study outcome was the incidence of recurrent PTB according to the number of initiation of 17 P prophylaxis. Secondary analyses were performed for effect of the time of initiation of treatment according to the number of prior PTBs.

**Results**

There were 906 women who met inclusion criteria. Five hundred and ninety-nine women (66%) initiated 17 P at 16-20.9 weeks' gestation, and 307 (34%) initiated 17 P at 21-26.9 weeks' gestation. Maternal characteristics are presented in Table 1. Women who initiated 17 P at 21-26.9 weeks were more likely to have more than 1 previous PTB. As expected, women who initiated 17 P at 16-20.9 weeks received more weekly injections when compared with women who initiated 17 P at 21-26.9 weeks.

Neonatal mortality was infrequent in both groups. There were 3 stillbirths (0.5%) and 3 neonatal deaths in the early initiation group and 2 stillbirths and 1 neonatal death in the late initiation group. Pregnancy outcomes are presented in Table 2 according to early or late initiation of 17 P, for all enrolled, and according to the number or previous PTB (1 vs 2 vs > 2). In the primary analysis, no significant differences were found in GA at delivery or rates of recurrent PTB < 37, < 35, and < 32 weeks between those women initiating 17 P at 16-20.9 weeks or 21-26.9 weeks overall or when stratified by number of prior preterm deliveries.

Within each GA at 17 P group we also examined the impact of number of prior PTB. For women with initiation of 17 P at 16-20.9 weeks, differences were noted among those women with 1 versus 2 and > 2 prior PTB for SPTB at < 37 weeks (27% vs 44% and 59%, respectively) and < 35 weeks (9% vs 23% and 20%, respectively) (all \( P < .05 \) compared to 1 prior PTB group). No differences were noted between women with 1, 2, or > 2 prior PTB for delivery at < 32 weeks (4% vs 9% vs 7%, respectively). For women with initiation of 17 P at 21-26.9 weeks, differences were noted between those with 1 versus > 2 prior PTB for delivery < 35 weeks (11% vs 30%, respectively, \( P < .05 \)). Women with > 2 prior PTB initiating 17 P at 21-26.9 weeks had a higher rate of SPTB at < 32 weeks than women with 1 and 2 prior PTB (2% and 2% vs 18%, respectively, both \( P < .05 \)).

**Comment**

**Principal findings of the study**

In this retrospective study, we found that women with a history of PTB, initiation of 17 P prophylaxis between 21 and 26.9 weeks' gestation was as effective as initiation between 16 and 20.9 weeks. Our findings support those in a recently published study in a similar patient population, suggesting that benefit could still be derived with later initiation of 17 P. We further show that later initiation still imparts benefit for women with 2 and > 2 prior PTBs who are at especially high risk for recurrent PTB.

**Clinical implication of the study**

The observation of a prophylactic effect of 17 P initiated between 21 and 26.9 weeks that is apparently equivalent to earlier initiation suggests that use may be expanded to women with prior spontaneous PTB who present for their first prenatal care
during this time period. Our data are observational and thus may have been influenced by unknown factors. Nevertheless, if confirmed by additional study, our findings may allow effective prophylaxis for a significant number of at-risk women who previously might not have been thought to be eligible for 17 P.

It is generally accepted that the presence of progesterone is required for the maintenance of pregnancy. The hypothesis that progesterone was, in fact, the hormone for maintaining pregnancy dates back to 1950. Enthusiasm for 17 P as prophylaxis for recurrent PTB was based on a substantial reduction in recurrence risk in a study of 43 women, but use declined until 2 recent randomized controlled trials demonstrated a significant reduction in PTB with either weekly injections of 17 P or daily vaginal progesterone suppositories among women with a history of prior SPTB. In the NICHD trial, 6 weekly IM injections of 17 P, the synthetic caproate ester analog of the naturally occurring progesterone metabolite, or placebo were initiated at 16-20 weeks' gestation in a total of 463 women (17 P in 310 women and placebo in 153). Women receiving 17 P compared with those receiving placebo experienced a reduced incidence of deliveries at <37 weeks (36.7% vs 54.9%), <35 weeks (20.6% vs 30.7%), and <32 weeks (11.4% vs 19.6%) of gestation, respectively. Our retrospective observational study achieved similar success rates: 34% delivered at <37 weeks, 16% delivered at <35 weeks, and 5% delivered at <32 weeks. There were 89 women in the NICHD trial who had 2 or more prior PTB. We report data on 274 women who had 2 or more prior PTB. In the NICHD trial, tocolytic therapy was administered to 17.3% of women treated with 17 P and to 15.9% of women who received placebo (15.9%). Tocolysis was given to just 11% of the women treated with 17 P in our study population.

### Strength and weakness of the study

The major strength of the present study is the large database from which we drew the study population, which allowed us to compare the outcome of women initiating 17 P treatment at 16-20.9 and 21-26.9 weeks' gestation and to stratify by number of prior PTB (1, 2, < 2). The limitation of our study is its retrospective nature. We did not have specific data such as baseline cervical length, the GA or circumstances of the prior PTB, maternal race, and complete neonatal outcomes.

### Future areas of investigation

Future large multicentered, randomized trials will be required to further investigate whether later initiation of 17 P, between 21 and 27 weeks, is effective in the prevention of PTB. We also need long-term data to assure the safety of this treatment in utero.

### References


### Table 2

<table>
<thead>
<tr>
<th>Pregnancy outcomes</th>
<th>All patients</th>
<th>Early 17 P start at 16-20.9 weeks (n = 330)</th>
<th>Late 17 P start at 21-26.9 weeks (n = 46)</th>
<th>P value</th>
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<tr>
<td>Delivery ≤ 37 wk (%)</td>
<td>41.9</td>
<td>42.0</td>
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<tr>
<td>SPTB ≤ 37 wk (%)</td>
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<td>&lt; 35 wk (%)</td>
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<td>&lt; 32 wk (%)</td>
<td>5.8</td>
<td>4.2</td>
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### Table 2

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<th>Late start (n = 92)</th>
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<td>&lt; 35 wk (%)</td>
<td>12.3</td>
<td>15.1</td>
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<td>&lt; 32 wk (%)</td>
<td>4.8</td>
<td>2.6</td>
<td>.296</td>
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### Table 2

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<th>Late start (n = 82)</th>
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<tr>
<td>SPTB ≤ 37 wk (%)</td>
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<td>39.0</td>
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<tr>
<td>&lt; 35 wk (%)</td>
<td>26.5</td>
<td>14.6</td>
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<td>&lt; 32 wk (%)</td>
<td>9.7</td>
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### Table 2

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<th>Late start (n = 53)</th>
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<td>65.2</td>
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<td>.066</td>
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<tr>
<td>SPTB ≤ 37 wk (%)</td>
<td>58.7</td>
<td>39.4</td>
<td>.113</td>
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<tr>
<td>&lt; 35 wk (%)</td>
<td>21.7</td>
<td>30.3</td>
<td>.438</td>
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<tr>
<td>&lt; 32 wk (%)</td>
<td>6.5</td>
<td>18.2</td>
<td>.154</td>
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Data presented as percentage as indicated.
Clinical characteristics of women prescribed 17 alpha-hydroxyprogesterone caproate in the community setting

Charles Rittenberg, MD; Scott Sullivan, MD; Niki Istwan, RN; Debbie Rhea, MPH; Gary Stanziano, MD; Roger Newman, MD

OBJECTIVE: The objective of the study was to describe clinical characteristics and pregnancy outcomes of women in a community setting prescribed 17 alpha-hydroxyprogesterone caproate (17P) prophylaxis for prevention of preterm delivery (PTD).

STUDY DESIGN: A retrospective review was conducted of data collected from patients enrolled for weekly outpatient 17P administration and nursing assessment between April 2004 and January 1, 2006 (n = 1979). Pregnancy history, referral indication, labor/delivery onset (spontaneous or indicated), and gestational duration were identified.

RESULTS: Almost 80% of women prescribed 17P had a prior preterm delivery, although only 711 of the study population (35.9%) met National Institute of Child Health and Human Development (NICHD) study criteria for 17P including initiation of treatment at 16 to 20.9 weeks. Spontaneous PTD occurred in 37.3%; 22.1% delivered less than 35 weeks; and 9.0% less than 32 weeks. More than one quarter of patients (26.9%) discontinued 17P at less than 34 weeks and prior to delivery.

CONCLUSION: In community practice, only one third of patients receiving 17P met strict NICHD study criteria. Early initiation and adherence to completion of therapy are clinical issues related to 17P prophylaxis.

Key words: 17 alpha-hydroxyprogesterone caproate, preterm birth prevention, progesterone

patients’ history did not include prior preterm delivery, further consultation between the Matria medical director and the patient’s provider occurred prior to accepting the patient for 17P administration services. Written authorization is received from patients at enrollment, allowing the use of their deidentified data for research and reporting purposes.

Matria’s 17P outpatient administration service includes a one-on-one education session with an experienced obstetrical nurse related to high risk pregnancy and signs or symptoms of preterm labor. This education includes written patient education materials related to pregnancy, preterm labor, and 17P. Weekly skilled nursing visits are arranged for further patient assessment and 17P administration. Nurses and pharmacists are available 24 hours a day, 7 days a week for patient questions and concerns. The 17P medication was compounded by a qualified pharmacy with substantial quality control procedures and documentation to assure sterility and potency of each vial. The qualified pharmacy compounded 17P following US Pharmacopeia Chapter 797 standards using an International Organization for Standardization class 5 clean room to the specifications and formulation as the 17P used in the Meis/NICHD trial, including the vehicle (castor oil). Unit dose (250 mg), preservative-free vials were delivered to the patient’s home for administration by the perinatal nurse. The 17P was administered using the Z-track injection method to reduce medication leakage and increase patient comfort with the injection.

Pregnancy history, reason for 17P treatment, labor/delivery onset (spontaneous or indicated), gestational duration, and program compliance were identified for this analysis. Data were divided into groups based on gestation type (singleton or multiple gestations) and history of prior preterm delivery.

**RESULTS**

Overall, 2159 women were identified as having been prescribed 17P. We included for analysis 1979 women (91.7% of overall women identified) having documented pregnancy outcomes. Within the study group, 79.5% of women prescribed 17P (1573 of 1979) had a history of prior preterm delivery (1517 singleton and 56 multiple gestations). However, just 63.6% of women (1258 of 1979) met patient inclusion criteria for 17P as outlined in the NICHD study by Meis et al\(^1\) (singleton gestation with a history of preterm delivery without cerclage at 17P initiation). Of the patients meeting NICHD inclusion criteria (n = 1258), only 711 (56.5%) initiated 17P at 16 to 20.9 weeks as in the Meis study design. Consequently, in the present analysis, only 35.9% of women (711 of 1979) who received 17P in the community practice setting met the exact patient inclusion criteria and followed the same treatment protocol outlined in the NICHD prospective randomized trial including initiation of treatment at 16 to 20.9 weeks’ gestation.

Referrals for 17P services were received from 1196 physicians. The majority of patients (88.3%) were receiving care at community hospitals. Of women receiving care at community hospitals 79.9% had a history of prior preterm delivery, compared with 79.4% of women receiving care at academic centers. In the community hospital setting 38.9% of women prescribed 17P met the same criteria as in the NICHD study including gestational age at initiation of treatment, compared with 35.5% of women treated at academic centers. Of the 1979 patients receiving 17P in the study period, 414 (21%) had Medicaid coverage and 1565 (79%) had private insurance or were self-pay.

Data were further stratified by singleton and multiple gestations. Maternal characteristics and 17P treatment summary are presented for each group stratified by previous preterm delivery status in Table 1. The majority (91.7%, 1814 of 1979) of women enrolled for 17P services had a singleton gestation. Within the singleton group, 83.6% of women (1517 of 1814) had a history of prior preterm delivery. Of women with a prior preterm delivery, 259 of 1517 of singletons (17.1%) had a cerclage in place at initiation of 17P. Whereas 1258 (69.3%)
of women with singleton gestations met the NICHD patient inclusion criteria for 17P (no cerclage, prior preterm delivery), initiation of 17P occurred within the recommended time interval of 16 to 20.9 weeks in only 711 (56.5%) women, whereas for 547 (43.5%), 17P initiation occurred at 21 weeks or later. Initiation of 17P for indications other than prior preterm delivery occurred in 16.4% of women (297 of 1814). These other indications included: cerclage (23.2%, 69 of 297), current preterm labor (44.8%, 133 of 297), or history of spontaneous abortion or preterm labor in a prior pregnancy (32.0%, 95 of 297).

One hundred sixty-five women enrolled for outpatient 17P administration services were identified as having multiple gestations of which 79.4% (131 of 165) were twins, 17.6% (29 of 165) were triplets, 1.8% (3 of 165) were quadruplets, and 1.2% (2 of 165) were quintuplets. Of these women with multiple gestations, 33.9% (56 of 165) had experienced a prior preterm delivery.

Compliance with treatment was assessed by identification of those patients who refused to continue 17P injections for reasons other than delivery despite physicians’ orders to continue treatment until at least 34 weeks of gestation. Three percent (59 of 1979) of women discontinued 17P for reasons other than delivery after a single injection, and an additional 24.0% (474 of 1979) of women electively discontinued weekly 17P injections prior to 34 weeks of gestation or the occurrence of preterm delivery. The mean gestational age at discontinuation of 17P was 28.9 ± 4.7 weeks for those women refusing to continue weekly treatment.

Overall, 44.3% (877 of 1979) of women that received 17P prophylaxis experienced preterm labor with or without preterm delivery and 37.3% (738 of 1979) of women experienced spontaneous preterm delivery at less than 37 weeks gestations; 22.1% delivering at less than 35 weeks; and 9.0% at less than 32 weeks. Pregnancy outcomes are compared for singleton and multiple gestations with and without prior preterm delivery in Table 2A and 2B. Rates of preterm delivery were similar between women with or without a history of prior preterm delivery for both singleton and multiple gestations.

**Comment**

In this review of data collected from patients prescribed 17P prophylaxis in a community setting, more than 20% of women received 17P for reasons other than prior preterm delivery. This is consistent with the findings from a recent MFM practice survey by Ness et al.3 showing that among physicians prescribing progesterone, 38% recommended its usage for indications other than previous spontaneous preterm birth. Although the ACOG committee opinion recommends restriction of progesterone treatment to only those patients with a history of spontaneous preterm birth,2 it would appear that clinicians have adopted wider usage patterns than those in the sentinel prospective, randomized trial by Meis et al.1 Perhaps this is related to excitement on the part of the clinician in this age of nihilism that there may actually be a way to reduce the incidence of prematurity for high-risk patients.

The use of 17P for these expanded indications is not necessarily wrong or inappropriate; rather, it just cannot be supported by the currently available evidence. Would 17P benefit a woman with a prior preterm birth who now has a cerclage or twins? The truth is that we do not know because of the limitations of the current literature.

Expanding the clinical indications for any therapy into untested or unproven areas has the potential to waste resources or may even result in adverse outcomes. Although a new drug application has been submitted to the Food and Drug Administration for 17P, the drug has not yet been approved. A recent presentation of further NICHD-MFMU Network data at the 2007 meeting of the Society for Maternal–Fetal Medicine of a randomized clinical trial of 17P in twin gestations revealed a lack of efficacy in this population.4 Additional clinical trials sponsored by the NICHD are currently underway involving 17P for women with

**TABLE 2A**

<table>
<thead>
<tr>
<th></th>
<th>Singletons with PPTD (n = 1517)</th>
<th>Singletons without PPTD (n = 297)</th>
</tr>
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<tbody>
<tr>
<td>GA delivery (wks)</td>
<td>36.4 ± 3.5</td>
<td>36.6 ± 3.5</td>
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<tr>
<td>Less than 37 wks</td>
<td>681 (44.9%)</td>
<td>120 (40.4%)</td>
</tr>
<tr>
<td>SPTD less than 37 wks</td>
<td>549 (36.2%)</td>
<td>93 (31.3%)</td>
</tr>
<tr>
<td>SPTD less than 35 wks</td>
<td>225 (14.8%)</td>
<td>39 (13.1%)</td>
</tr>
<tr>
<td>SPTD less than 32 wks</td>
<td>91 (6.0%)</td>
<td>19 (6.4%)</td>
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Data presented as mean ± SD or n (percent) as indicated.

**TABLE 2B**

<table>
<thead>
<tr>
<th></th>
<th>Multiple gestation with PPTD (n = 56)</th>
<th>Multiple gestation without PPTD (n = 109)</th>
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<tr>
<td>GA delivery (wks)</td>
<td>32.5 ± 3.8</td>
<td>33.3 ± 3.5</td>
</tr>
<tr>
<td>Less than 37 wks</td>
<td>51 (91.1%)</td>
<td>102 (93.6%)</td>
</tr>
<tr>
<td>SPTD less than 37 wks</td>
<td>36 (64.3%)</td>
<td>60 (55.0%)</td>
</tr>
<tr>
<td>SPTD less than 35 wks</td>
<td>29 (51.8%)</td>
<td>44 (40.4%)</td>
</tr>
<tr>
<td>SPTD less than 32 wks</td>
<td>13 (23.2%)</td>
<td>19 (17.4%)</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or n (percent) as indicated.
a shortened cervix (less than 25 mm) in the midtrimester, and others are urgently needed to evaluate the use of 17P for indications other than history of spontaneous preterm delivery.

Timely initiation of 17P therapy and adherence to completion of the treatment regimen in the community setting also appear to be greater problems than have been reported in the available controlled clinical trials. In the community setting, only 52% of women initiated 17P treatment between the recommended 16 to 20.9 weeks’ gestation. There is evidence to support this later initiation. Gonzalez-Quintero et al,5 including 3 of the authors of the current paper, found no statistically significant difference in outcomes between women who initiated 17P between 16 and 20.9 weeks and those who initiated treatment between 21 and 26.9 weeks. In the present study, more than one quarter discontinued weekly injections prior to 34 weeks for reasons other than delivery. Although early discontinuation was by patient request, it is unknown whether treatment was discontinued because of medication side effects, discomfort from weekly injections, or a result of physician discouragement or reassurance that she was “out of the woods” despite having not yet achieved 34 weeks’ gestation. This finding is troubling, given that early discontinuation of 17P has been shown to increase the risk for recurrent preterm delivery among women with singleton gestations having a prior preterm delivery.6

Lack of patient willingness to complete the course of treatment may ultimately impact the ability of the treatment to reduce the overall incidence of preterm delivery. Early initiation of treatment and adherence to completion of therapy are issues that need to be addressed with regard to prophylactic 17P therapy. These issues, along with appropriate indications for treatment, are concerns that arise in clinical practice beyond the findings of prospective, randomized clinical trials.

Although the current study is limited by the nature of a descriptive analysis, we believe it is representative of prophylactic 17P usage patterns in a community setting. It is important to note that use of 17P outside the usual indications is not a result of marketing by the service provider. Matria does not market or encourage the use of 17P for any indications other than that of a prior preterm birth. When another indication is given, Matria contacts the provider to inform him or her that this is not an approved indication and to confirm their desire to proceed with 17P treatment. If the provider still wishes to prescribe 17P, its administration is not currently withheld. It remains to be seen what role pharmacies, vendors, and payers will have in approving use of 17P in light of the evolving literature. Of note, the rate of 17P for indications beyond prior preterm delivery was 20% in this study, compared with 38% in a survey of MFM providers performed by Ness et al3 under the sponsorship of the March of Dimes. These percentages should not be directly compared because the present study analyzed the proportion of patients receiving 17P, whereas the study by Ness et al3 analyzed the proportion of providers who have prescribed 17P for indications outside the Meis protocol.

REFERENCES
A short interpregnancy interval is a risk factor for preterm birth and its recurrence

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OBJECTIVE: We tested the hypothesis that short interpregnancy intervals (IPIs) increase the risk for preterm birth (PTB), recurrence of PTB, and delivery at early extremes of gestational age.

STUDY DESIGN: Using the Missouri Department of Health’s birth certificate database, we performed a population-based cohort study of 156,330 women who had 2 births from 1989-1997. The association between IPI and subsequent pregnancy outcome was assessed.

RESULTS: The shortest IPIs (≤6 months) increased the risk of extreme PTB (adjusted odds ratio, 1.41; 95% CI, 1.13-1.76). IPIs of ≤6 months and 6-12 months increased the overall risk of PTB (adjusted odds ratios, 1.48 [95% CI, 1.37-1.61] and 1.14 [95% CI, 1.06-1.23], respectively) and PTB recurrence (adjusted odds ratios, 1.44 [95% CI, 1.19-1.75] and 1.24 [95% CI, 1.02-1.50], respectively).

CONCLUSION: The risk of PTB and its recurrence increases with short IPIs, even after adjustment for coexisting risk factors. This highlights the importance of counseling women with either an initial term or preterm birth to wait at least 12 months between delivery and subsequent conception.

Key words: birth spacing, interpregnancy interval, preterm birth, recurrence


As the rate of preterm birth continues to rise,1,2 the search for more predictive and reliable risk factors that may lead ultimately to effective intervention strategies for its prevention intensifies. The association between a short period of time between birth and subsequent conception (interpregnancy interval [IPI]) with adverse perinatal outcomes (such as preterm birth, low birthweight, intrauterine growth restriction, and fetal death) has been demonstrated in numerous studies over the past 3 decades.3 The findings of most of these studies, though, have been limited by methodologic restrictions such as small sample size, lack of control for important confounding factors, and analysis of the interbirth rather than IPI.3

Despite consistent suggestive evidence of the association between a short IPI and preterm birth, little is known about the association of a short IPI with preterm birth when it is accompanied by other important preterm birth risk factors. One of the strongest predictors of preterm birth is a history of preterm delivery.4-6 The concomitant effect of a short IPI and a previous preterm delivery on a mother’s risk of delivering a preterm neonate is unknown. We performed this study to test the hypothesis that a short interval of time between delivery and subsequent conception not only increases the overall risk of preterm birth but also the risk of preterm birth in an especially high-risk group of women, those with a previous preterm delivery. Furthermore, we sought to evaluate the effect of a short IPI on preterm births that occur at the early extremes of gestational age (<28 weeks of gestation) when neonatal morbidity and mortality are the highest.

MATERIAL AND METHODS

Study design
A study protocol was approved by the Missouri Department of Health and Senior Services to analyze the state’s maternally linked birth-death certificate database. The dataset provided by the Missouri Department of Health and Senior Services, Section of Public Health Practice and Administrative Support, included all births and fetal deaths that occurred in the state of Missouri from 1978-1997. All protected health information was deidentified. The study was deemed exempt from institutional review board review from Washington.
University School of Medicine in St. Louis.

We excluded cases of intrauterine fetal death from our analysis because our primary interest was to determine the influence of IPI on live preterm birth. We also excluded births with a major congenital malformation and births that occurred at <20 weeks of gestation. Births that resulted from a multifetal gestation were excluded because of their known tendency to deliver preterm. Our analysis was restricted to births that occurred between 1989 and 1997 because of an unacceptable amount of missing data for births that occurred before 1989. There was minimal data missing (<0.5%) for each of the variables that were analyzed in the remaining cohort. Because this was a retrospective analysis, the individuals who collected the database information that was later used for outcome measures (gestational age) and to calculate the IPI (delivery dates and gestational age) were blind to the study premise. The investigator (E.A.D.) who performed the statistical analysis was not blind to the study premise, but this is unlikely to have affected the results because the exposure and outcome of interest were objective measures that were defined before the study.7

To calculate IPI, our analysis was limited to only those births to mothers who delivered >1 infant during the study period. In the case of mothers who delivered >2 infants during the study period, only the outcome of the second birth in relation to the first birth that occurred during the study period was examined. Multiple IPIs to the same mother were not included in this study to avoid the effect of clustering. Multiple births to the same mother were linked by a unique identifier called a sibship number. Methods for constructing and evaluating a database with live birth and fetal death records that are organized into sibships based on maternal association have been described.8

We conducted a retrospective population-based cohort study on the subcohort of second pregnancies for women who were linked by sibships during the study period. The association between preterm birth and 4 IPI categories (<6 months, 6-12 months, 12-18 months, and >18 months) were evaluated. We performed our primary analysis on preterm births that occurred at <35 weeks of gestation to avoid inclusion of borderline gestational ages, thus minimizing misclassification bias, and to identify the population of neonates at risk for poorer outcomes. The >18-month IPI group was the reference category that was used for comparison in these analyses.

To better understand the association of short IPIs with various subcategories of preterm birth, we subdivided preterm births into the ordinal categoric variables of moderate preterm birth (32-346/7 weeks of gestation), very preterm birth (28-316/7 weeks of gestation), and extreme preterm birth (<28 weeks of gestation). We evaluated the association of IPI with all preterm births that occurred at <35 weeks of gestation and then separately with preterm births that occurred in each of the 3 gestational age strata that were noted.

Previous preterm birth is known to increase the risk of subsequent preterm birth. To evaluate the effect of IPI on recurrent preterm birth, we examined the association between preterm birth (<35 weeks of gestation) and IPI in women with a preceding delivery at <35 weeks of gestation. To further evaluate the effect of birth spacing on pregnancy outcomes in multiparous mothers, we analyzed the association between preterm birth (<35 weeks of gestation) and IPI in women with a preceding delivery at >35 weeks of gestation.

Definitions

IPI was defined as the period of time between the preceding delivery and subsequent conception. Delivery dates, gestational age at delivery, and last menstrual period dates were available in the database that was used for this study. Using birth certificate data, the IPI was first calculated with the number of weeks between the first delivery and the last menstrual period when recorded for the second pregnancy. This method of calculating IPI yielded an unacceptable number of impossible intervals (ie, negative intervals), likely reflecting the inaccuracy of last menstrual period reporting. We then calculated the IPI by subtracting the number of weeks of gestational age of the second pregnancy from the number of weeks between the 2 deliveries. This second method of calculating the IPI was used for all of the analyses in this study. Gestational age was defined as the best estimate with combined information from the last menstrual period and clinical data.

Primary preterm birth was defined strictly in this study as a preterm birth that occurred at >35 weeks of gestation that was preceded by a birth at <35 weeks of gestation during the study period. Recurrent preterm birth was defined as >1 preterm birth (<35 weeks of gestation) to the same mother during the study period. No prenatal care indicated a lack of prenatal visits. Race was self-reported by the mother.

Statistical analysis

Data were analyzed with SPSS software (version 14.0; SPSS Inc, Chicago, IL). The unit of analysis was the individual birth, and rates of preterm birth in various preterm gestational age categories per 100 live births were calculated for the entire cohort and separately for births according to the 4 IPI subcategories. The rates of birth at <35 weeks of gestation were also calculated for pregnancies with and without a preceding preterm birth at <35 weeks of gestation for each of the IPI categories.

Demographic characteristics were compared with the use of analysis of variance for continuous variables and χ² or Fisher exact tests for categoric variables. Bivariate techniques were used to assess the association between preterm birth and the IPI categories. The risks of preterm birth in each of the IPI categories were expressed as unadjusted odds ratios (ORs) with 95% CIs.

Logistic regression models were constructed to assess further the association of preterm birth with the IPI categories. First, bivariate techniques were used to identify factors that were associated significantly with preterm birth. These potential confounding factors were then included in the logistic regression model to
examine the association of IPI with all cases of preterm birth (<35 weeks of gestation) and then with each preterm birth subcategory (32-35, 28-32, and <28 weeks of gestation). Final regression models were established by adding or removing covariates and testing for differences between hierarchical models with the likelihood ratio test or Wald test. The final explanatory models for the study outcomes were those that included the most significant and parsimonious variables. Adjusted odd ratios and 95% CIs were reported for each of the preterm birth and IPI categories.

Finally, to confirm the linear trend between interval length and preterm birth risk that was identified in the logistic regression analysis, with IPI modeled as a 4-stratum categorical variable, we also analyzed IPI as a continuous variable in the multivariable model.

**RESULTS**

A total of 711,015 births were recorded in Missouri between 1989 and 1997. After the exclusions, we identified 156,330 mothers with 2 consecutive live births that occurred during the study period. This original cohort therefore comprised a total of 312,660 live births. The perinatal outcomes that were analyzed in this study were evaluated from the latter of the 2 births to each mother (n = 156,330).

The baseline demographic characteristics of the study population are listed in Table 1. Women with shorter IPIs were younger and had fewer years of education. Maternal black race was significantly more common in women with short IPIs, as was a preceding preterm birth at <35 weeks of gestation. Women with shorter intervals between pregnancies were also more likely to be unmarried, receive state-funded program support (Medicaid, food stamps, WIC), smoke cigarettes, and have no prenatal care.

Table 2 demonstrates the risk of preterm birth by IPI length. The overall rate of preterm birth at <35 weeks of gestation for this cohort of singleton, liveborn, nonanomalous infants was 3.7%. The rate of preterm birth at <35 weeks of gestation was highest in women with the shortest IPIs (<6 months), 6.9%. This increased risk of preterm birth persisted after being controlled for other important sociodemographic factors that are associated with preterm birth (previous preterm birth, no prenatal care, black race, Medicaid, maternal age <18 years). IPIs of <6 months were associated with a 48% increase in the risk of preterm birth at <35 weeks of gestation; intervals of 6-12 months increased the risk 14%, but intervals of >12 months had no significant association with preterm birth risk. To further analyze the association of short IPIs with early preterm births, when neonatal outcomes are worse, we divided the preterm births into 3 categories: moderately preterm, very preterm, and extremely preterm. The rate of preterm birth was 2.3% at 32-35 weeks of gestation, 1.0% at 28-32 weeks of gestation, and 0.5% at <28 weeks of gestation. The risk of preterm birth in each of these subcategories increased when the IPI was <6 months (adjusted OR, 1.41-1.57). Short IPI did not appear to have a differential effect on risk among severity subtypes of preterm birth (eg, moderate vs extreme), because the magnitude and precision of ORs for preterm birth were similar across preterm birth severity types within a short IPI category (Table 2).

We subdivided the cohort into women whose first birth was >35 or <35 weeks of gestation to estimate the risk of a short IPI on preterm birth in women who were already at high risk, those with a previous preterm birth. Of 156,330 births in our cohort, 6181 births (4.0%) occurred to

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**TABLE 1**

Demographic characteristics by IPI

<table>
<thead>
<tr>
<th>Demographic</th>
<th>IPI (mo)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;6</td>
<td>6-12</td>
</tr>
<tr>
<td>N</td>
<td>15,200</td>
<td>27,405</td>
</tr>
<tr>
<td>Maternal age (y)*</td>
<td>24.1 ± 5.4</td>
<td>25.6 ± 5.5</td>
</tr>
<tr>
<td>Maternal education (y)*</td>
<td>12 ± 2.2</td>
<td>12.7 ± 2.3</td>
</tr>
<tr>
<td>Gestational age at birth (wk)*</td>
<td>38.6 ± 2.8</td>
<td>38.9 ± 2.4</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>3264 ± 613</td>
<td>3378 ± 576</td>
</tr>
<tr>
<td>Maternal black race (%)</td>
<td>27.9</td>
<td>19.6</td>
</tr>
<tr>
<td>Married (%)</td>
<td>58.6</td>
<td>70.1</td>
</tr>
<tr>
<td>Medicaid (%)</td>
<td>58.4</td>
<td>46</td>
</tr>
<tr>
<td>Food stamps (%)</td>
<td>45.2</td>
<td>33.4</td>
</tr>
<tr>
<td>WIC (%)</td>
<td>56.3</td>
<td>43.9</td>
</tr>
<tr>
<td>Cigarette smoking: mother (%)</td>
<td>29.4</td>
<td>23.8</td>
</tr>
<tr>
<td>No prenatal care (%)</td>
<td>4.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Previous preterm birth (%)</td>
<td>6.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
women with a preceding preterm birth. The rate of subsequent preterm birth at <35 weeks of gestation for women with a preceding preterm birth was 16.5% (OR, 5.96; 95% CI, 5.54-6.41). The highest risk of recurrent preterm birth was to women with the shortest IPIs, <6 months. Women with a previous preterm birth and an IPI of <6 months had a 44% increase in risk of recurrent preterm birth after adjustment for coexistent risk factors for preterm birth. IPIs of 6-12 months also increased the risk of recurrent preterm birth (Table 3). IPIs of 12-18 months were associated with an increased risk of recurrent preterm birth in the unadjusted analysis, but this finding was not statistically significant after accounting for confounding risk factors for preterm birth in this group of women. Although having a preceding birth at >35 weeks of gestation decreased the absolute risk of preterm birth at <35 weeks of gestation (OR, 0.85; 0.72-0.97).

**TABLE 2**

Risk of preterm birth at <35 weeks of gestation by IPI

<table>
<thead>
<tr>
<th>Variable</th>
<th>IPI (mo)</th>
<th>&lt;6</th>
<th>6-12</th>
<th>12-18</th>
<th>&gt;18*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>15,200 (9.7%)</td>
<td>27,405 (17.5%)</td>
<td>27,523 (17.6%)</td>
<td>86,202 (55.1%)</td>
</tr>
<tr>
<td>Preterm birth &lt;35 wk of gestation (n)**</td>
<td>1042 (6.9%)</td>
<td>1190 (4.3%)</td>
<td>908 (3.3%)</td>
<td>2691 (3.1%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.28 (2.12-2.46)</td>
<td>1.41 (1.31-1.51)</td>
<td>1.06 (0.98-1.14)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.48 (1.37-1.61)</td>
<td>1.14 (1.06-1.23)</td>
<td>1.0 (0.93-1.09)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Moderate preterm 32-35 wk of gestation (n)**</td>
<td>613 (4%)</td>
<td>713 (2.6%)</td>
<td>574 (2.1%)</td>
<td>1717 (2%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.11 (1.92-2.31)</td>
<td>1.32 (1.21-1.44)</td>
<td>1.05 (0.93-1.15)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.46 (1.32-1.62)</td>
<td>1.11 (1.01-1.22)</td>
<td>1.01 (0.92-1.12)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Very preterm 28-32 wk of gestation (n)**</td>
<td>287 (1.9%)</td>
<td>327 (1.2%)</td>
<td>229 (0.8%)</td>
<td>645 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.62 (2.28-3.02)</td>
<td>1.61 (1.41-1.85)</td>
<td>1.11 (0.96-1.30)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.57 (1.35-1.83)</td>
<td>1.22 (1.06-1.41)</td>
<td>1.04 (0.89-1.22)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Extreme preterm 20-28 wk of gestation (n)**</td>
<td>141 (0.9%)</td>
<td>150 (0.5%)</td>
<td>105 (0.4%)</td>
<td>329 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.55 (2.09-3.10)</td>
<td>1.45 (1.20-1.76)</td>
<td>1.00 (0.80-1.25)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.41 (1.13-1.76)</td>
<td>1.12 (0.91-1.38)</td>
<td>0.88 (0.7-1.11)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* Reference.
** N = 5831; 3.7%.
† N = 3617; 2.3%.
§ N = 1488; 1.0%.
† Covariates included in the logistic regression model: previous preterm birth (at <35 weeks of gestation), no prenatal care, black race, Medicaid, maternal age <18 years.
* N = 726; 0.5%.

**TABLE 3**

Risk of preterm birth in women with a preceding birth at <35 or >35 weeks of gestation by IPI

<table>
<thead>
<tr>
<th>Variable</th>
<th>IPI (mo)</th>
<th>&lt;6</th>
<th>6-12</th>
<th>12-18</th>
<th>&gt;18*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preceding PTB (n = 6181)</td>
<td>1004 (16.2%)</td>
<td>1223 (19.8%)</td>
<td>1022 (16.5%)</td>
<td>2932 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>Recurrent PTB (n = 1091; 16.5%)*</td>
<td>218 (21.7%)</td>
<td>223 (18.2%)</td>
<td>169 (16.5%)</td>
<td>409 (13.9%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.71 (1.42-2.05)</td>
<td>1.38 (1.15-1.64)</td>
<td>1.22 (1.00-1.49)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.44 (1.19-1.75)</td>
<td>1.24 (1.02-1.50)</td>
<td>1.17 (0.96-1.44)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Preceding birth &gt;35 weeks (n = 150,149)</td>
<td>14,196 (9.5%)</td>
<td>26,182 (17.4%)</td>
<td>26,501 (17.6%)</td>
<td>83,270 (55.5%)</td>
<td></td>
</tr>
<tr>
<td>Primary PTB (n = 4812; 3.2%)*</td>
<td>824 (5.8%)</td>
<td>967 (3.7%)</td>
<td>739 (2.8%)</td>
<td>2282 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>2.19 (2.01-2.37)</td>
<td>1.36 (1.26-1.47)</td>
<td>1.02 (0.94-1.11)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Adjusted OR (95% CI)**</td>
<td>1.50 (1.37-1.64)</td>
<td>1.12 (1.04-1.22)</td>
<td>0.97 (0.89-1.06)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* Overall risk of recurrent preterm birth with a preceding preterm birth at <35 weeks of gestation: OR, 5.96; 95% CI 5.54-6.41.
† Covariates included in the logistic regression model: no prenatal care, black race, Medicaid, maternal age <18 years.
§ Overall risk of primary preterm birth with a preceding birth at >35 weeks of gestation: OR, 0.85; 95% CI 0.84-0.86.
Comment

Birth spacing is an important consideration for parents when planning a family. Obstetric care providers have strong and consistent data to support counsel ing mothers to wait an appropriate amount of time between delivery and the next conception to avoid increasing the risk of adverse pregnancy outcomes.\(^3\)

The optimal amount of time to wait between gestations does vary between studies, but most large analyses conclude that IPIs of either 0-6 or 12 months are associated with an increased risk of preterm birth.\(^9\) - \(^16\)

A recent metaanalysis of retrospective studies that included outcomes of >10 million births found that the risk of preterm birth increased 1.9% for each month <18 months between pregnancies.\(^3\) In this study, the risk of preterm birth was increased 40% with intervals <6 months, but only 7% when the interval was 12-17 months. Based on this information, a woman at average risk of preterm birth in the United States (eg, 10%) would increase her absolute risk by only 4% to 14% if conception occurred <6 months from her last delivery. A more complicated question is: How long should a woman who is already at high risk of preterm birth, such as a woman who has just delivered a preterm infant, delay her next pregnancy?

To our knowledge, the association between a short IPI and recurrence of preterm birth has not been reported. Our findings demonstrate that, when compared with an IPI of >18 months, an interval of <6 months increases the risk of recurrent preterm birth at <35 weeks of gestation by 44%, even after adjustment for concomitant risk factors. This contributes to a rate of preterm birth of >20% for this high-risk group of women. Likewise, we find that IPIs of 6-12 months also increase the risk of recurrent preterm birth, but to a lesser degree. We also found that women at relatively low risk of preterm birth (those with a preceding birth at >35 weeks of gestation) increase their risk of preterm birth by short birth spacing. These women had a low rate of birth <35 weeks of gestation (2.7%) with an IPI of >18 months, but the rate was increased >2-fold to 5.8% with a shorter interval of <6 months.

Some of the limitations of our study are related to the data source. When vital statistics records are used for research purposes, the validity of the data is often scrutinized.\(^17\) The possibility of underreporting and/or data inaccuracy, which may or may not be random, should be considered because much of the birth certificate information is obtained by maternal recollection. Also, levels of medical knowledge are widely variable among individuals who obtain birth certificate data. Because the exposure (IPI) and outcome (preterm birth) in this study are variables that are based on fairly objective measures, we believe that this limitation is minimal in our analysis. Validity of the recorded gestational age is another potential weakness of studies from birth certificate data.\(^18\) The use of the best clinical estimate of gestational age, rather than last menstrual period data alone, enhanced the precision of this variable in our analysis.\(^19\)

The recent metaanalysis of studies that evaluated the association of birth spacing with adverse perinatal outcomes outlined criteria that the authors believed to be important to the methodologic quality of studies such as this. Those criteria include (1) use of the IPI (time from delivery to subsequent conception) rather than interbirth interval, (2) examination of 4-6 categories of IPIs, (3) measurement of outcomes and birth spacing by direct measures (medical records), (4) blinding of birth spacing status and ascertainment of outcomes, (5) minimal loss to follow-up or nonvalid exclusions, and (6) control for pertinent confounding factors.\(^7\) We believe that the findings of our study are both valid and generalizable on the basis of our adherence with the aforementioned criteria and the population-based nature of the data source. The risk increase that is associated with short IPIs in our study are similar to those demonstrated in similar studies on large population-based birth databases, which increased the validity of our findings.\(^11\) - \(^13\), \(^15\) - \(^16\), \(^20\) - \(^21\) This increased risk of preterm birth associated with short IPIs is not as robust as it is with other risk factors, such as maternal black race (OR, 4.11; 95% CI, 3.78-4.47) or lack of prenatal care (OR, 3.0; 95% CI, 2.69-3.37), but similar in effect to important socioeconomic risk factors for preterm birth, such as low level of maternal education (OR, 1.33; 95% CI, 1.23-1.44) or

95% CI, 0.84-0.86) relative to patients with a previous preterm birth, a short IPI of either <6 months or 6-12 months increased the risk of primary preterm birth (adjusted ORs, 1.50 and 1.12, respectively).

We identified a linear (inverse) relationship between preterm birth risk and IPI in women with a previous preterm birth at <35 weeks of gestation (P value for trend = .012) and in women with a previous birth >35 weeks of gestation (P value for trend = .021). Stated differently, as the IPI decreased, preterm birth risk increased. To confirm this apparent trend that was found between the multi-level categorical IPI variable and preterm birth risk, we developed a second multi-variable model, with IPI expressed as a continuous variable. This analysis confirmed the inverse linear relationship between progressively shorter IPIs and increasing preterm birth risk, with probability values for trend of <.001 for both groups (women with and without a previous birth at <35 weeks of gestation). The Figure shows the relative effect of short IPIs on subsequent pregnancies to women whose preceding births were >35 weeks of gestation.

![Figure](image-url)
We concur with previous studies that short IPIs are associated with an increased risk of preterm birth. We find that, even after adjustment for other important factors that are associated with preterm birth, a short IPI increases the risk of preterm birth. Furthermore, short IPIs increase preterm birth risk in both high-risk women (those with a previous preterm delivery) and low-risk women (with a preceding birth at >35 weeks of gestation). This information regarding the association of increasing risk of preterm birth with short IPIs is important when preconception counseling is provided. Our results suggest that women may benefit from a period of at least 12 months between delivery and subsequent conception, regardless of the gestational age of the preceding birth.

REFERENCES
Any investigations have focused on looking for strategies to prevent mental retardation and developmental disabilities in different pathologic conditions. In our previous work, we demonstrated that prenatal or postnatal treatment with 2 synthetic peptides that are derived from neuroprotective proteins can enhance learning in healthy adults and aged mice. These small 8 and 9 amino acid peptides, SALLRSIPA (SAL) from activity-dependent neurotrophic factor (ADNF) and NAPVSIPQ (NAP) from activity-dependent neuroprotective protein (ADNP) mimic the activity of their parent proteins. These parent proteins (ADNF and ADNP) are released by glial cells and are regulated by vasoactive intestinal peptide, which is a central nervous system neurotransmitter and neuromodulator with neurotrophic properties.

Numerous studies have demonstrated the neurotrophic activity of the peptides NAP and SAL. Prenatal or postnatal administration of NAP + SAL prevented alcohol-induced learning deficit in a model for fetal alcohol syndrome (FAS). In a model for FAS, the peptides have been shown to act through the prevention of changes in the expression of N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA) receptor subunits. The GABA and the NMDA receptors for glutamate are pentameric and tetrameric ion channels, respectively, the function of which is determined by their subunit
Composition.\textsuperscript{11} These receptors are key components of a brain circuit that leads to learning.\textsuperscript{12} Specifically, the GABA receptor subunits GABA\textsubscript{A}B\textsubscript{3} and GABA\textsubscript{A}\alpha5, and the NMDA receptor subunits NR2A and NR2B, play an important role in the learning process.

Our objective was to see whether learning enhancement of aged mice with NAP + SAL also acts through modification of the NMDA and GABA receptor subunits.

Materials and Methods

Fourteen-and-a-half month old C57B16/J male mice (The Jackson Laboratory, Bar Harbor, ME) were kept in a 12-hour light/12-hour dark regimen, with food and water available at all times. Mice received humane animal care in compliance with the National Institutes of Health Guidelines for Care and Use of experimental animals. The protocol was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee. Following previously published methods,\textsuperscript{1} mice were withheld food and water for 3 hours before treatment every day to avoid interference with drug absorption. For 10 consecutive days mice were treated by gavage with either D-NAP (20 \textmu g) + D-SAL (20 \textmu g; SynPep, Dublin, CA; n = 6 from 4 litters) or placebo (n = 5 from 3 litters). The all-D amino acid configurations of the peptides were used that allow for oral administration. D-NAP was diluted in 50 \mu L of dimethyl sulfoxide and diluted in 2.5 mL of filtered Dulbecco’s phosphate-buffered saline solution; D-SAL was dissolved in the same solution. On day 11, brains from NAP + SAL and control groups were collected with micro-electrophysiologic mechanism of learning enhancement of aged mice with NAP + SAL did not result in a modification in the expression of the peptides during development in a contrast with findings of the administration of the peptides during development in a model for FAS, in which alterations in these subunits were found in prevention of learning deficit.\textsuperscript{9,10}

Results

Here we show that, in the brains from aged male mice, a 10-day treatment with the peptides D-NAP + D-SAL did not result in a modification in the expression of the NMDA receptor subunits NR2A and NR2B (P > .05; Figure 1). Similarly, the GABA\textsubscript{A} receptor subunits GABA\textsubscript{A}\alpha5 and GABA\textsubscript{A}B\textsubscript{3} expression was not modified by treatment with the peptides (P > .05; Figure 2; control, 6 pups from 4 litters) and D-NAP + D-SAL (n = 5 from 3 litters). As previously reported, the animals treated with the peptides learned better than the controls in the Morris water maze, a model of spatial learning.\textsuperscript{1}

Comment

Postnatal oral treatment with the peptides NAP + SAL resulted in learning enhancement in aged mice that is not mediated through the NMDA and GABA receptor subunits NR2A, NR2B, GABA\textsubscript{A}\alpha5 and GABA\textsubscript{A}B\textsubscript{3}. This is in contrast with findings of the administration of the peptides during development in a model for FAS, in which alterations in these subunits were found in prevention of learning deficit.\textsuperscript{9,10} Long-term potentiation (LTP) is the electrophysiologic mechanism of learn-
ing.\textsuperscript{13} NMDA activation has been shown to be the first step for the cell membrane depolarization and thus activation of LTP.\textsuperscript{14} NMDA firing is regulated by the inhibitory tone of the neurotransmitter GABA towards the GAB\textsubscript{A} receptor.\textsuperscript{12} The NR2B subunit has been shown to promote LTP and to be particularly abundant in states of high firing, as during development, whereas the NR2A subunit is known to reduce LTP and to be predominant with aging.\textsuperscript{14} In a model for FAS, we have shown that prenatal treatment with the peptides NAP + SAL resulted in an increase in NR2B and a decrease in NR2A after alcohol exposure, with respect to the alcohol group, which may explain the prevention of learning deficit in FAS. The subunits GAB\textsubscript{A}\textsubscript{2}\textsubscript{5} and GAB\textsubscript{A}B\textsubscript{3} also have been shown to be involved in learning.\textsuperscript{15,16} In a model for FAS, NAP + SAL administration after alcohol-exposure resulted in an overexpression of these subunits, compared with the controls, that indicated that the peptides are able to modify the expression of these GAB\textsubscript{A} subunits.\textsuperscript{9,10}

In this set of experiments, we have shown that postnatal treatment with NAP + SAL in aged mice resulted in learning enhancement that is not mediated through the modification of these subunits expression. It may be that the peptides act through these receptors only in the presence of a perturbing agent or that the receptors are not modifiable beyond the development period. A previous study showed that LTP induction was altered not only by NAP alone but also by the interaction of alcohol and NAP.\textsuperscript{17} Another possibility is that different subunits in these receptors may have triggered the enhancement, because other subunits in the NMDA and GABA receptors have been targeted as important for LTP and learning.\textsuperscript{14,18}

Alternatively, it may be that, in the postnatal period when the synapses are already formed and the system is not perturbed by toxic agents (as alcohol), the peptides act through a different pathway. It has been shown that their action may reside in the potential to stabilize the cytoskeleton molecule tubulin after zinc intoxication.\textsuperscript{19} Furthermore, ADNF increases the activity of the transcription factor CREB and of protein kinase A, thus contributing to the regulation of transcription.\textsuperscript{20} All these studies were conducted in systems that were perturbed either by a toxic agent or a disease process, and the peptides have shown to prevent some of their sequelae. In addition, the peptides may work through inhibition of oxidative damage. The production of oxygen free radicals that results in oxidative damage is a known mechanism of aging neurodegeneration. Previously, the peptides have been shown to prevent alcohol-induced alterations in oxidative stress,\textsuperscript{21} as assessed by glutathione levels.\textsuperscript{22} This may be a mechanism of the learning enhancement in the aged animals. We have shown that the neurotrophic properties of these peptides are also active in healthy subjects.\textsuperscript{1,12} Further studies are needed to elucidate their mechanism of action in this population.

REFERENCES


APPENDIX

For RNA extraction, samples were thawed and immediately homogenized with a sonicator (Janke & Kunkel, Wilmington, NC). The samples were pro...
cessed with SV Total RNA Isolation System (Promega, Madison, WI). A 5-L aliquot was taken for spectrophotometric determination of RNA content. The remaining sample was stored at −80°C. With the use of 5 µg of total RNA, the reverse transcriptase reaction was performed (Applied Biosystems, Foster City, CA) in a final volume of 150 µL. For real-time PCR, the NR2A, NR2B, GABA_A_3, GABA_A_5 primer pair was designed and synthesized by TIB Molbiol (Adelphia, NJ). The receptor subunits primer sequences were: GABA_A_3: 5′-CGA GTT GCC CTT GGG ATT AC-3′ (sense) and 5′-GAT ATT CCC GTG AGC ACT GTG GTC ACT CC-5′ (antisense); GABA_A_5: 5′-GGC AGA CAG TAG GCA CTG AG-3′ (sense) and 5′-GTC AGC ACT GTG GTC ACT CC-5′ (antisense); NR2B: 5′-CCG CAG CAC TAT TAGA GAA CA-3′ (sense) and 5′-ATC CAT GTG TAG CCG TAG CC-5′ (antisense); NR2A: 5′-GGA AGT TGG ACG CTT TCA TC-3′ (sense) and 5′-TCT TCC ATC TCA CCG TCA CC-3′ (antisense). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard with the primer pair synthesized by Integrated DNA Technologies (Coralville, IA). GAPDH primer sequence was 5′-TGC ACC ACC AAC TGC TTA-3′ (sense) and 5′-GGA TGC AGG GAT GAT GTT C-3′ (antisense). With the use of the Fast Start DNA Master SYBR green 1 dye-base detection (Roche Diagnostics Corp, Indianapolis, IN), target genes and GAPDH expression were measured by real-time PCR with the LightCycler with relative quantification software (Roche Diagnostics Corp) and with melting point analysis to assess the specificity of the amplified genes. Optimization for NR2B, NR2A, GABA_A_3, GABA_A_5, and GAPDH-specific primers was performed with separate runs by varying magnesium chloride and primer concentrations, the amount of template, and annealing temperature; optimized conditions were used as previously published.9,10

The presence and purity of target gene sequence expression in the real-time PCR reaction were confirmed by gel electrophoresis. To further eliminate the risk of cross contaminations LightCycler Uracil-DNA Glycosylase (Roche Diagnostics Corp) was added to the master mix in all the PCR experiments. The final volume of the reverse transcriptase PCR reaction was 20 µL.
Effects of acute alcohol intoxication in the second trimester of pregnancy on development of the murine fetal lung

Xiangyuan Wang, MD; Prasra Gomutputra, BS; Debra J. Wolgemuth, PhD; Laxmi Baxi, MD

OBJECTIVE: We hypothesized that administration of alcohol during the second trimester of gestation at the pseudoglandular phase of lung development might lead to aberrant differentiation and growth, similar to that seen in congenital cystic adenomatoid malformation in human. We further hypothesized that these effects would be apparent morphologically and by altered Hoxb5 expression.

STUDY DESIGN: C57BL/6J mice, exposed to ethanol at embryonic day (E) 11.5 to E13.5, which corresponds to the pseudoglandular stage of lung development, were examined at E18.5. The lungs were analyzed histologically by immunostaining.

RESULTS: The average body and lung weight of alcohol-exposed (AE) fetuses were lower than those of control fetuses, the reduction in lung mass being more than the body weight. Histology showed that AE lungs were less developed and exhibited higher expression of Hoxb5 in AE lungs than controls.

CONCLUSION: Alcohol exposure at E13.5 affected fetal lung development, with delayed differentiation and increased Hoxb5.

Key words: acute alcohol exposure, Hoxb5, lung development, lung malformation, murine

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It is well known that alcohol exposure relatively early in pregnancy can lead to craniofacial abnormalities and neural defects associated with fetal alcohol spectrum disorders, such as fetal alcohol syndrome. Although extensive studies have been done on acute and chronic alcohol exposure during the late first trimester, much less information is known about the consequences of the exposure at later periods. The lung is one of the organs that develops during this later gestation and could be affected. There have been reports of a higher incidence of respiratory problems in children who were exposed to alcohol in utero, and we published a case report of congenital cystic adenomatoid malformation (CCAM) following in utero exposure to alcohol. Recently, an epidemiological study by Baumann et al reported that congenital anomalies of the respiratory tract, excluding those of diaphragm, were common in children of women >30 years of age who abused alcohol in pregnancy.

CCAM, a congenital malformation of the lung affecting the lower respiratory system, is characterized by localized arrest of lung development at the pseudoglandular or canalaricular stage. The most common CCAM, CCAM1, is a malformation of bronchial type epithelium, which arises during the pseudoglandular stage of lung development during 7-16 weeks’ gestation, characterized by repeated dichotomous branching that leads to formation of bronchial airways. It has been proposed that CCAM is caused by an imbalance of cell proliferation and apoptosis; however, the molecular pathways and gene alterations leading to this condition is still unclear. One molecule that has been implicated in congenital lung malformations that are characterized by abnormal airway patterning during branching morphogenesis, such as CCAM, is Hoxb5. Immunoblot analysis revealed that Hoxb5 protein levels are higher in normal lung tissue as compared with age- and developmentally matched CCAM tissue.

We have previously hypothesized that CCAM could result from an acute high exposure to alcohol during the period of time when lungs are at the pseudoglandular stage of development. To test this hypothesis experimentally, we studied the effect of acute high exposure of alcohol during the late midgestation in a mouse model on the development and differentiation of fetal lungs. At this stage, the lungs are at pseudoglandular phase of development, similar to 7-17 weeks’ gestation in humans. We further hypothesized that the acute alcohol intoxication during this period may disrupt lung development by altering the expression of genes involved in modulating lung development, such as Hoxb5.

Materials and Methods

Alcohol exposure

For timed matings, the day of the detection of the vaginal plug was considered embryonic day (E) 0.5, and the developmental stage was determined according to Theiler. Pregnant C57BL/6J mice, a
strain known to be susceptible to ethanol were injected intraperitoneally with 2 doses of either 25% ethanol (3.75 g/kg; alcohol exposed [AE]) or with Ringer’s solution (controls [C]) at a 4-hour interval at day 11.5, 12.5, and 13.5 of gestation (E11.5, E12.5, and E13.5). The animals quickly recovered from the alcohol treatment and resumed normal movement and feeding. Fetuses were retrieved at E18.5, weighed, measured, and the numbers of viable and resorbed embryos recorded. The lung tissue was then fixed in 4% buffered paraformaldehyde and processed for histological analysis, according to our routine procedures. Immuno staining for Hoxb5

The paraffin-embedded tissues were then sectioned at 6 μm and analyzed by immunostaining using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Tissue sections were deparaffinized and hydrated through a graded series of ethanol and distilled water then placed in 0.01 M citrate acetate buffer at pH 6.0 and processed for 20 minutes at 4°C, washed with 1 × phosphate-buffered saline with 0.1% Triton X-100 and blocked for 1 hour with 1% normal goat serum. Incubation with Hoxb5 (a gift from Dr. MaryAnn Volpe, Department of Pediatrics, Tufts University School of Medicine) antibody at a 1:100 concentration was carried out overnight at 4°C. The slides were incubated with biotinylated antirabbit IgG (1:200) for 2 hours at room temperature followed by incubation in ABC reagent for 2 hours. Immunostaining was visualized using 0.2 mg/mL 3,3′-diaminobenzidine and 0.01% hydrogen peroxide in 0.1 M Tris buffer (pH 7.2). The sections were counterstained with hematoxylin. The stained slides were viewed on a Nikon 800 photomicroscope (Nikon, Tokyo, Japan) under bright-field optics.

RESULTS

The resorption rate, body weight, and lung weight of the alcohol-exposed fetuses compared with control fetuses

The pregnant C57BL/6J mice were injected intraperitoneally with either ethanol (alcohol exposed-AE) or Ringer’s solution (control) during specific gestation stages (E11.5, E12.5, and E13.5), and fetuses were retrieved at E18.5 (AE: E11.5, n = 25; E12.5, n = 31; E13.5, n = 73; C: E11.5, n = 9; E12.5 n = 7; and E13.5, n = 24). As shown in the Table, we observed that the AE mice exhibited a higher embryonic resorption rate, regardless of the gestation stage that they were exposed to alcohol, than the C mice (60% at E11.5, 13% at E12.5, and 38.5% at E13.5 in AE mice vs 0% at all stages in C mice). Further analysis was done on the E13.5 fetuses. None of the embryos exhibited craniofacial abnormalities seen in mice exposed to alcohol at earlier stages of gestation. However, both the average body weight and the average lung weight of these AE fetuses were less than that of the control fetuses at the same gestation age (0.94 g vs 1.13 g and 0.034 g vs 0.047 g, respectively; P < .001). Interestingly, the decrease in lung mass in AE fetuses was greater than the decrease in their total body mass. Whereas the lung weight accounted for 4.2% of the total body weight in the control fetuses, it was accounted for only 3.6% of the total body weight in the AE fetuses. Thus, the lungs were preferentially affected by the acute treatment of alcohol as compared with the overall reduction in body size.

Morphology of lungs as compared with control lungs

Histology examination with hematoxylin-eosin stain of the AE lungs (ethanol exposed at E13.5 and killed at E18.5) revealed that not only were these lungs smaller but also were developmentally immature as compared with the C lungs. In mice, the canalicular stage occurs during E14.2 to E16.6, whereas the saccular stage occurs during E16.6 to postnatal day 5. Our results showed that the AE lungs only reached the canalicular stage, whereas the C lungs were already at the saccular stage (Figure 1). The control lungs were characterized by the widening of the airways into saccules, which in turn generated alveolar ducts and sacs (Figure 1, A and B). The epithelial cells also differentiated into type I flat cells and type II larger cells; however, the AE lungs clearly lacked the widening of the airways and type I flat cells (Figure 1, C and D). In addition, the columnar epithelium lining the airways illustrated the canalicular stage.

Hoxb5 immunostaining of AE and control lung tissues

One of the genes implicated in lung development in the mouse model is Hoxb5, a homeobox-containing gene, in particular for patterning of airway branches. Furthermore, as mentioned before, elevated levels of human Hoxb5 have been reported in CCAM lung tissue. Therefore we examined the

<table>
<thead>
<tr>
<th>Gestation stage</th>
<th>Total # of embryos</th>
<th>Resorbed embryos</th>
<th>Resorption rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E11.5 Alcohol-exposed</td>
<td>11.5</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>11.5</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>E12.5 Alcohol-exposed</td>
<td>12.5</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>12.5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>E13.5 Alcohol-exposed</td>
<td>13.5</td>
<td>73</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>13.5</td>
<td>24</td>
<td>0</td>
</tr>
</tbody>
</table>
expression of Hoxb5 in the AE and C mouse lung specimens (Figure 2). The mesenchymal cells in AE lungs expressed higher level of Hoxb5 than the C lungs. This observation correlated well with the developmental delay of the AE lungs that we observed morphologically. That is, Hoxb5 has been shown by others to be expressed on or before E14.5, peaking at E15.5 and declining thereafter.16-19

**COMMENT**

In the present study, we showed that high acute alcohol exposure at the pseudoglandular development phase of lungs does in fact affect lung development. The disproportionate reduction in the mass of the AE lungs as compared with the C lungs suggested that lung might be particularly sensitive to the acute alcohol exposure during this period of time; that is, during the pseudoglandular phase. Furthermore, the morphologic analysis showed that at the same gestational stage, the AE lungs were developmentally delayed, compared with the C lungs, at the same gestation stage.

Hoxb5 is a homeobox-containing gene that has been shown to be important in the patterning of airway branches during mouse lung morphogenesis.10,12-15 Strong expression of Hoxb5 is found in lung mesenchyme during branching morphogenesis, and as the saccular stage begins, its expression falls to almost undetectable levels. Its role in bronchiolar patterning was further evidenced in studies in which the induction of Hoxb5 expression with retinoic acid resulted in elongation of bronchioles, whereas inhibition of Hoxb5 with antisense oligonucleotides prevented branch elongation.12 The human ortholog Hoxb5 has also been implicated during human lung development and in the etiology of congenital lung malformations such as bronchopulmonary sequestration and congenital cystic adenomatoid malformation.11 Although we are far from elucidating the molecular pathway of the malformations we have observed, there is a commonality with regard to the persistence of expression of Hoxb5 between our findings on exposure to...
alcohol and Hoxb5 expression in human CCAM mesenchyme in human tissue.9

REFERENCES

OBJECTIVE: The purpose of this study was to evaluate potential associations between vascular endothelial growth factor (VEGF) gene polymorphisms and preeclampsia.

STUDY DESIGN: One hundred ten patients with preeclampsia and 209 healthy pregnant control subjects were enrolled in the study. After peripheral blood was obtained from all women and the genomic DNA was isolated, we genotyped +936C/T polymorphisms in the 3′-untranslated region of the VEGF gene, using polymerase chain reaction and restriction fragment length polymorphism techniques.

RESULTS: The distribution of genotypes of the +936C/T polymorphism was significantly different between women with preeclampsia and the control group (P < .001). Carriage of the +936T allele was significantly more frequent in preeclamptic patients than in control subjects (odds ratio, 2.06; 95% CI, 1.38-3.08). Logistic regression analysis on VEGF genotype and clinical parameters such as age, educational status, body mass index, and neonatal gender showed carriage of the 936T allele to be significantly more frequent in preeclamptic patients than in control subjects (adjusted odds ratio, 2.23; 95% CI, 1.46–3.42).

CONCLUSION: Carriage of the +936T allele of the VEGF gene may be associated with increased susceptibility to the development of preeclampsia and may be an independent risk factor for preeclampsia.

Key words: polymorphism, preeclampsia, restriction fragment length polymorphism, vascular endothelial growth factor

Vascular endothelial growth factor gene +936 C/T polymorphism is associated with preeclampsia in Korean women

Jae-Yoon Shim, MD; Jong Kwan Jun, MD; Bok-Kyung Jung, MS; Sung Hoon Kim, MD; Hye-Sung Won, MD; Pil Ryang Lee, MD; Ahm Kim, MD

Vascular endothelial growth factor (VEGF) is a major angiogenic factor and plays an important role as a regulator of endothelial cell proliferation and vascular permeability. Previous studies have shown reduced circulating concentrations of VEGF in preeclamptic patients and increased VEGF expression that is associated with hypoxia in the placenta of preeclamptic patients. VEGF has also been shown to affect early events in pregnancy, which lead to trophoblast invasion and placenta. The VEGF gene is located in the chromosome region 6p21.3 and consists of 8 exons and 7 introns. Many polymorphisms of the VEGF gene have been identified. In a study that involved 23 healthy young men, a common 936 C/T polymorphism in the 3′-untranslated region (UTR) of the VEGF gene was shown to affect VEGF plasma levels, and carriers of a 936 T allele had significantly reduced levels.

Considering the important roles of VEGF in pregnancy, functional polymorphisms in the VEGF gene are potentially important as genetic markers of susceptibility to preeclampsia. Based on genetic predisposition, this relationship may be strengthened by showing an association between genetic polymor-
phisms of VEGF and an increased risk of developing preeclampsia.

We performed a genetic association study of +936 C/T polymorphisms of the VEGF gene in preeclamptic patients and control subjects.

**Materials and Methods**

**Study population**

In this study, 110 patients with preeclampsia and 209 healthy unrelated pregnant control subjects were enrolled at the Asan Medical Center between July 2004 and December 2005. All women were nulliparous of Korean origin and hence comprised a single ethnic group. The study protocol was approved by the institutional review board of the Asan Medical Center. Each woman reviewed and voluntarily signed a written informed consent form before study entry.

The diagnosis of preeclampsia was based on the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy\(^1\) (ie, blood pressure \(\geq 140/90\) mm Hg that persisted for at least 6 hours and proteinuria of \(\geq 1+\) (dipstick) or \(\geq 300\) mg/24 hours after 20 weeks of gestation). Exclusion criteria in each group included multiple pregnancies, chronic hypertension, chronic renal disease, pregestational or gestational diabetes mellitus, or other underlying medical disease (such as a history of endometriosis, rheumatoid arthritis, and systemic lupus erythematosus).

**Genotyping of the VEGF gene polymorphisms**

After peripheral blood was obtained from each patient and the genomic DNA was isolated with the QIAamp DNA Blood Midi kit (QIAGEN, Hilden, Germany), we genotyped +936 C/T polymorphisms at the 3’-UTR of the VEGF gene, using polymerase chain reaction (PCR) and restriction fragment length polymorphism assays. The PCR primers for the +936 C/T polymorphism were 5’-AAGGAAGAGGAGACTCTGCG-CAGAGC-3’ (forward) and 5’-TAAAATGTATGATGTGGGCTGGTGCTACAGG-3’ (reverse). PCR was performed in 25-μL reaction volumes that contained 0.1 μg genomic DNA, PCR buffer (10 mmol/L Tris pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 μmol/L dNTPs, 0.25 μmol/L of each of the forward and reverse primers, and 1 U Taq DNA polymerase (Takara, Shuzo, Kyoto, Japan). After an initial denaturation step (5 minutes at 94°C), 32 cycles of PCR that consisted of denaturation at 94°C for 40 seconds, were annealed at 62°C for 60 seconds, and extensions at 72°C for 60 seconds were performed. PCR products were digested with restriction endonuclease Nla III (New England Biolabs, Beverly, MA) at 37°C for 3 hours and were separated by electrophoresis on a 1.5% agarose gel with ethidium bromide (0.1 μg/mL). The +936 T allele was cut into 2 fragments of 122 and 86 base pair, whereas the +936 C allele remained uncut with a length of 208 base pair.

The genotyping was repeated twice in all samples; if the discrepancy persisted, gene sequencing was performed with the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotyping of the blood samples was performed by an experienced laboratory staff member (B.K.J.), who was blinded to cases and control subjects.

**Statistics**

Assuming that the frequency of the +936 T allele would be 15% in the control group and 30% in the preeclampsia group and that the ratio of numbers of each group would be 1:2, our sample size provides at least 80% power and a type I error (α) of 0.05 with post hoc power analysis. The clinical and demographic data of the study groups were compared with the use of the Student t test, chi-square test, and Fisher’s exact test, depending on whether the data were continuous or categoric. Hardy-Weinberg equilibrium for the polymorphism was tested by the chi-square test. Genotype distribution and allele frequency between groups were compared by the chi-square test and Fisher’s exact test. Logistic regression analyses were performed to assess the independent role of the VEGF genotype and other variables, which included age, body mass index (BMI), education, and neonatal sex. All statistical analyses were performed with SPSS software (version 11.0; SPSS Inc, Chicago, IL).

**Results**

Baseline demographic and clinical data are shown in Table 1. There were significant differences in prepregnancy BMI and high-grade education (more than high school) between groups.

Table 2 shows the genotype distribution and allele frequency of the VEGF +936 C/T polymorphism. There is no significant deviation from Hardy-Weinberg equilibrium in each group (P > .05). The distribution of genotypes of the +936 C/T polymorphism was significantly different between women with preeclampsia and the control group (P < .001).

**Genotypic distribution of the VEGF gene**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preeclampsia (n = 110)</th>
<th>Control subjects (n = 209)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)*</td>
<td>31.1 ± 3.6</td>
<td>30.3 ± 3.5</td>
<td>.058</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)*</td>
<td>22.1 ± 3.6</td>
<td>20.9 ± 2.7</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>High-grade education (n)</td>
<td>61 (55.5%)</td>
<td>167 (79.9%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>162 ± 19</td>
<td>116 ± 11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td>102 ± 12</td>
<td>74 ± 8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)*</td>
<td>34.9 ± 3.4</td>
<td>38.9 ± 2.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neonatal birthweight (g)*</td>
<td>1929 ± 741</td>
<td>3200 ± 351</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neonatal male sex (n)</td>
<td>50 (45.5%)</td>
<td>119 (56.9%)</td>
<td>.059</td>
</tr>
</tbody>
</table>

* Values expressed as mean ± SD.
of the control group in our study are not significant more frequent in preeclamptic patients than in healthy pregnant control subjects (odds ratio [OR], 2.06; 95% CI, 1.38-3.08). In a dominant model, carriers of the T allele were significantly more common in the preeclamptic group than in the control group (OR, 2.28; 95% CI, 1.41-3.69).

We performed logistic regression analyses on VEGF genotype and clinical parameters such as age, educational status, BMI, and neonatal gender. Carriage of the 936 T allele was significantly more frequent in preeclamptic patients than in healthy pregnant control subjects (adjusted OR, 2.23; 95% CI, 1.46-3.42). In a dominant model, carriers of the T allele were significantly more common in the preeclamptic group than in the control group (adjusted OR, 2.53; 95% CI, 1.51-4.23).

**TABLE 2**

| VEGF genotype distributions and allele frequencies in preeclamptic women and control subjects |
|---------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Genotype distributions (n) | Allele frequencies | Adjusted OR (95% CI)* |
| +936 C vs T | n | CC | CT | TT | P value | C | T | (95% CI) | (95% CI)* |
|---|---|---|---|---|---|---|---|---|---|---|
| Preeclampsia (n = 110) | 58 (52.7%) | 45 (40.9%) | 7 (6.4%) | .001 | 0.732 | 0.268 | 2.06 (1.38-3.08) | 2.23 (1.46-3.42) |
| Control subjects (n = 209) | 150 (71.8%) | 55 (26.3%) | 4 (1.9%) | 0.849 | 0.151 | 2.28 (1.41-3.69) | 2.53 (1.51-4.23) |
| Dominant model (CC vs CT+TT) | 2.06 (1.38-3.08) | 2.23 (1.46-3.42) |

* Adjusted by logistic regression analysis with maternal age, BMI, educational status, and neonatal sex.

**COMMENT**

Our results indicate an association between the +936 T allele in the 3’-UTR of the VEGF gene and an increased risk of preeclampsia (adjusted OR, 2.23). In our study, the +936 T allele is an independent risk factor for preeclampsia, as shown by multivariate analysis. The genotype distribution and allele frequency of the control group in our study are comparable with those of previous reports on the Korean population. Recent studies have demonstrated a possible association among +936 C/T polymorphism and breast cancer, rheumatoid arthritis, and acute respiratory distress syndrome, in which angiogenesis may play a crucial role in the development of diseases.

Actually, 1 large study and 2 small studies have investigated the association between VEGF polymorphisms and preeclampsia. One large study, however, did not investigate the 936 C/T polymorphism in the 3’-UTR of the VEGF gene, but did investigate 5 other polymorphisms. Two small studies produced conflicting results regarding the association between the +405 C/G polymorphism and preeclampsia. Whereas 1 study suggested that the G allele reduced the risk of severe preeclampsia, the other study reported no significant association between the +405 C/G polymorphism and preeclampsia but showed an association between the +936 C/T polymorphism and the severity of preeclampsia. The results of our study differ from those of previous studies. Possible explanations for these differences include the ethnic groups that were studied, different compositions of case and control groups, a small sample size with inadequate power, and whether confounding variables were considered. The results of our study may be more reliable because all participants were of Korean ethnicity, the relatively large sample size included nulliparous control subjects, and we assessed the relationship between the VEGF polymorphisms and preeclampsia by logistic regression analyses, which included maternal age, BMI, educational status, and neonatal sex. We were interested in studying VEGF polymorphisms in the Korean population because of its relatively homogenous ethnic origin, which stands in contrast with the more heterogeneous characteristics of the European women who were examined in previous studies. To our knowledge, no previous study has examined specifically the association between VEGF polymorphisms and preeclampsia in an Asian population.

It is difficult to separate maternal from fetoplacental genetic mechanisms in the development of preeclampsia. However, population-based studies from Norway and Sweden indicate that the maternal genetic effect has a greater role than the fetal effect in the risk of preeclampsia and that fetal transmission of a paternal gene contributes to the risk of preeclampsia. Some studies have reported associations between preeclampsia and other maternal genetic polymorphisms, which include angiotensinogen, factor V Leiden, methylene tetrahydrofolate reductase, and tumor necrosis factor-α genes.

Some study limitations have to be considered when the results of our study are evaluated. First, in our case-control study, approximately one-third of the study group were preeclamptic individuals, the remaining two-thirds were control subjects, because of the relatively low incidence of preeclampsia. Second, we did not analyze the association between the polymorphism and disease severity. Only preeclamptic patients who visited our in-patient clinic were enrolled. In addition, because of the sample size, it is difficult to ensure that subgroup analyses have adequate power. Finally, the homogeneity of the study populations may represent a disadvantage in the generalization of the results in terms of ethnicity.

There is now compelling evidence that VEGF production is controlled by polymorphisms that have been identified within the VEGF genes. Functional polymorphisms may result in altered transcription factor recognition sites, which may affect transcriptional activation and alter levels of protein production.
As with many environmental risks, the relative risk or OR might be small because of genetic variation. Haplotype analysis, which is currently the focus of intense genetic research effort, will enable more specific risk estimates than single locus analyses because it may reduce the dimension of association tests and may increase statistical power. Identification of associations between candidate genes and disease will be of the main objectives in the development of personalized and interactive medicine.

In conclusion, our findings indicate that VEGF polymorphisms may be useful as indicators of susceptibility to pre eclampsia. Further large-scale genetic studies, which include haplotype analyses, may be needed to improve statistical power and to investigate the functional relevance of VEGF polymorphisms. This area should be a focus of future activity.

REFERENCES

Cande V. Ananth, PhD, MPH; Morgan R. Peltier, PhD; Wendy L. Kinzler, MD; John C. Smulian, MD, MPH; Anthony M. Vintzileos, MD

OBJECTIVE: The purpose of this study was to evaluate whether the increased risk of placental abruption among women with chronic hypertension is modified by ischemic placental disease, specifically pregnancy-induced hypertension (PIH) and fetal growth restriction (FGR).

STUDY DESIGN: We used the US linked natality and fetal death data files (1995-2002) and restricted the analysis to women who had a singleton birth at ≳22 weeks of gestation and to fetuses who weighed ≥500 g (n = 30,189,949). Fetal growth was defined both on a continuum (<1, 1-2, 3-4, 5-9, 10-19, . . . , 90) and as birthweight of <10th percentile for gestational age (FGR) or birthweight of >90th percentile (large for gestational age [LGA]). All analyses were adjusted for potential confounding factors through multivariable logistic regression.

RESULTS: Rates of abruption among women with and without chronic hypertension were 15.6 and 5.8 per 1000 pregnancies, respectively (relative risk [RR], 2.4; 95% CI, 2.3, 2.5). In comparison with normotensive women with appropriately grown babies (ie, 10th-90th percentile), the association between chronic hypertension and abruption was modified in the presence of FGR (RR, 3.8; 95% CI, 3.6, 4.1) and PIH (RR, 7.7; 95% CI, 6.6, 8.9). However, the highest risk was seen among women with chronic hypertension, PIH, and LGA (RR, 9.0; 95% CI, 7.2, 11.3). A dose-response relationship was observed between the risk of abruption and fetal growth (assessed on a continuum), with the risk being lowest among LGA babies.

CONCLUSION: The association between chronic hypertension and abruption is strong; ischemic placental disease (PIH and FGR) modified this relationship. These findings suggest an etiologic relationship between abruption and chronic placental disease. Chronic hypertension, if associated with LGA, is not associated with abruption; however, chronic hypertension with superimposed PIH accompanied by LGA is associated with significantly increased risk.

Key words: chronic hypertension, fetal growth, ischemic placental disease, placental abruption


Chronic or preexisting hypertension complicates approximately 3-8 per 1000 pregnancies.1,2 The condition confers increased risks for an array of reproductive and perinatal outcomes that include stillbirth,3,4 preterm birth,5-7 and restricted fetal growth.3,5,8,9 The most common maternal risks that are associated with chronic hypertension include preeclampsia, pregnancy-induced hypertension (PIH), insulin resistance, and placental abruption.10 The causes of hypertensive diseases, fetal growth restriction (FGR), and placental abruption are heterogeneous, yet speculative. Nevertheless, hypoxia-induced changes in the maternal-fetal circulation,11,12 uteroplacental vascular insufficiency,13 and placental ischemia are believed to be the chief predisposing pathophysiologic mechanisms that are common to all these pregnancy complications.14-16 Although women with chronic hypertension are at increased risk of placental abruption,17-19 it remains unclear whether this is a direct association or whether the association is modified by complications that accompany chronic hypertension, namely, PIH and/or FGR. Such knowledge may provide important clues to understanding the pathophysiologic mechanisms to placental abruption. Because preterm PIH and preterm FGR represent more severe forms of the underlying disease states, we stratified the association between chronic hypertension and placental abruption by gestational age at delivery.

MATERIALS AND METHODS

Data source and cohort composition

We used the National Center for Health Statistics (linked birth and infant death)
### TABLE 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total births (n)</th>
<th>Chronic hypertension</th>
<th>Rate per 1000 pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>30,189,949</td>
<td>221,404</td>
<td>7.3</td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>3,728,270</td>
<td>9557</td>
<td>2.6</td>
</tr>
<tr>
<td>20-24</td>
<td>7,553,149</td>
<td>32,830</td>
<td>4.3</td>
</tr>
<tr>
<td>25-29</td>
<td>8,178,160</td>
<td>53,649</td>
<td>6.6</td>
</tr>
<tr>
<td>30-34</td>
<td>6,891,465</td>
<td>62,796</td>
<td>9.1</td>
</tr>
<tr>
<td>35-39</td>
<td>3,196,547</td>
<td>46,636</td>
<td>14.6</td>
</tr>
<tr>
<td>≥40</td>
<td>642,358</td>
<td>15,936</td>
<td>25.1</td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10,065,100</td>
<td>63,174</td>
<td>6.3</td>
</tr>
<tr>
<td>2</td>
<td>8,804,044</td>
<td>59,739</td>
<td>6.8</td>
</tr>
<tr>
<td>≥3</td>
<td>11,320,805</td>
<td>98,491</td>
<td>8.7</td>
</tr>
<tr>
<td>Maternal race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>23,879,305</td>
<td>15,567</td>
<td>6.4</td>
</tr>
<tr>
<td>Black</td>
<td>4,633,272</td>
<td>61,133</td>
<td>13.4</td>
</tr>
<tr>
<td>Other</td>
<td>1,677,372</td>
<td>8704</td>
<td>5.2</td>
</tr>
<tr>
<td>Maternal education (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>6,590,795</td>
<td>29,884</td>
<td>4.5</td>
</tr>
<tr>
<td>12</td>
<td>9,690,753</td>
<td>77,696</td>
<td>8.0</td>
</tr>
<tr>
<td>13-15</td>
<td>6,511,350</td>
<td>58,639</td>
<td>9.0</td>
</tr>
<tr>
<td>≥16</td>
<td>6,981,470</td>
<td>51,801</td>
<td>7.4</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>9,989,368</td>
<td>66,752</td>
<td>6.7</td>
</tr>
<tr>
<td>Married</td>
<td>20,200,581</td>
<td>154,679</td>
<td>7.7</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>22,005,904</td>
<td>176,439</td>
<td>8.0</td>
</tr>
<tr>
<td>1-10 cigarettes/d</td>
<td>2,047,370</td>
<td>14,598</td>
<td>7.1</td>
</tr>
<tr>
<td>11-20 cigarettes/d</td>
<td>792,315</td>
<td>6115</td>
<td>7.7</td>
</tr>
<tr>
<td>≥21 cigarettes/d</td>
<td>106,302</td>
<td>1135</td>
<td>10.7</td>
</tr>
<tr>
<td>Missing</td>
<td>5,238,058</td>
<td>23,117</td>
<td>4.4</td>
</tr>
<tr>
<td>Infant sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15,463,653</td>
<td>114,278</td>
<td>7.4</td>
</tr>
<tr>
<td>Female</td>
<td>14,726,296</td>
<td>107,126</td>
<td>7.3</td>
</tr>
</tbody>
</table>

All comparisons are statistically significant ($P < .0001$).

Statistical analysis

Multiple logistic regression models were fitted to evaluate the independent associa-

---

tion between chronic hypertension and abortion after adjustment for potential confounders. The variables that were considered to be potential confounders included maternal age (grouped in 5-year intervals as <20, 20-24, 25-29, 30-34, 35-39, and ≥40 years), gravidity (gravida 1, gravida 2, and gravida ≥3), maternal race/ethnicity (white, black, and other races), education (<12, 12, 13-15, and ≥16 years of completed schooling), marital status (single or married), smoking during pregnancy (nonsmoker; 1-10, 11-20, and ≥21 cigarettes smoked per day), and infant sex. All analyses were also further adjusted for birth year (1995-2002).

We evaluated whether the association between chronic hypertension and abortion was modified in the presence of either fetal growth abnormalities (FGR or LGA) and/or PIH. For this analysis, we created the following exposure: (1) women with chronic hypertension as an isolated condition; (2) women with chronic hypertension with FGR, but no PIH; (3) women with chronic hypertension with LGA, but no PIH; (4) women with chronic hypertension and PIH, but no FGR or LGA babies; (5) women with chronic hypertension, FGR, and PIH; and (6) women with chronic hypertension, LGA, and PIH. Risks of abortion in each of these groups were compared with women who were normotensive before and during pregnancy who delivered fetuses with birthweight between 10th and 90th percentiles for gestational age. We also examined the association among chronic hypertension, PIH and fetal growth, and abortion, with fetal growth defined on a continuum to evaluate dose-response relationships. Finally, we stratified these analyses on gestational age at delivery, which were classified as 22-31, 32-36, and ≥37 weeks of gestation.

The study was approved by the Institutional Review Board of the UMDNJ–Robert Wood Johnson Medical School,

### TABLE 2: Association among chronic hypertension, fetal growth, PIH, and abortion: US singleton births, 1995-2002

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total births (n)</th>
<th>No. of cases of placental abruption (rate per 1000 pregnancies)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td></td>
<td></td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Absent</td>
<td>29,968,545</td>
<td>173,865 (5.8)</td>
<td>2.7 (2.62, 2.80)</td>
</tr>
<tr>
<td>Present</td>
<td>221,404</td>
<td>3445 (15.6)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>FGR</td>
<td></td>
<td></td>
<td>2.1 (2.0, 2.2)</td>
</tr>
<tr>
<td>Absent</td>
<td>24,142,117</td>
<td>135,543 (5.6)</td>
<td>0.5 (0.4, 0.6)</td>
</tr>
<tr>
<td>Present</td>
<td>2,900,136</td>
<td>33,637 (11.6)</td>
<td>2.5 (2.4, 2.6)</td>
</tr>
<tr>
<td>LGA</td>
<td></td>
<td></td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Absent</td>
<td>24,142,117</td>
<td>135,543 (5.6)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>3147,696</td>
<td>8131 (2.6)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>PIH</td>
<td></td>
<td></td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Absent</td>
<td>29,036,188</td>
<td>161,699 (5.6)</td>
<td>4.7 (4.4, 5.0)</td>
</tr>
<tr>
<td>Present</td>
<td>1,153,761</td>
<td>15,611 (13.6)</td>
<td>2.6 (2.5, 2.8)</td>
</tr>
</tbody>
</table>

* Adjusted for period of birth, maternal age, gravidity, maternal race, education, marital status, smoking during pregnancy, and infant sex.

### TABLE 3: Association among chronic hypertension, PIH, FGR, and placental abruption: US singleton births, 1995-2002

<table>
<thead>
<tr>
<th>Ischemic placental disease</th>
<th>Total births (n)</th>
<th>No. of cases of placental abruption (rate per 1000 pregnancies)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>No complication</td>
<td>23,136,270</td>
<td>123,203 (5.3)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Chronic hypertension only</td>
<td>159,205</td>
<td>2222 (14.0)</td>
<td>2.6 (2.5, 2.8)</td>
</tr>
<tr>
<td>Chronic hypertension with FGR only</td>
<td>34,367</td>
<td>842 (24.5)</td>
<td>4.7 (4.4, 5.0)</td>
</tr>
<tr>
<td>Chronic hypertension with LGA only</td>
<td>22,020</td>
<td>112 (5.1)</td>
<td>1.0 (0.8, 1.2)</td>
</tr>
<tr>
<td>Chronic hypertension with PIH only</td>
<td>4003</td>
<td>180 (45.0)</td>
<td>8.8 (7.6, 10.2)</td>
</tr>
<tr>
<td>Chronic hypertension with FGR and PIH</td>
<td>372</td>
<td>10 (26.9)</td>
<td>5.2 (2.8, 9.7)</td>
</tr>
<tr>
<td>Chronic hypertension with LGA and PIH</td>
<td>1437</td>
<td>79 (55.0)</td>
<td>10.9 (8.7, 13.6)</td>
</tr>
</tbody>
</table>

* Adjusted for the confounding effects of period of birth, maternal age, gravidity, maternal race, maternal education, marital status, smoking during pregnancy, and infant sex.

* Includes normotensive women with no fetal growth abnormalities.
New Brunswick, NJ. Statistical analyses were performed with SAS software (version 9.1; SAS Institute, Cary, NC).

**RESULTS**
Between 1995 and 2002, 7.3 per 1000 (n = 221,090) singleton pregnancies were complicated by chronic hypertension (Table 1). Increasing maternal age and gravidity were associated with increased risk of chronic hypertension. Compared with white women, black women were associated with 2-fold increased risk of chronic hypertension, as were women who smoked over 1 pack of cigarettes per day.

Associations between chronic hypertension, fetal growth abnormalities, PIH, and abruption are shown in Table 2. The rates of abruption among women with and without chronic hypertension were 15.6 and 5.8 per 1000 singleton pregnancies, respectively. After adjustment for potential confounders, women with chronic hypertension were at a 2.4-fold (95% CI, 2.3, 2.5) increased risk of abruption. Similar increased risks for abruption were observed with PIH and FGR, and a decreased risk was observed in relation to LGA status.

Rates of abruption among women with chronic hypertension, fetal growth, and PIH are shown in Table 3. In comparison with normotensive women with appropriately grown babies (ie, 10th-90th percentile), the risk for abruption was 2.4-fold higher (95% CI, 2.3, 2.5) among women with chronic hypertension as an isolated condition. However, if women experienced either FGR and/or PIH, the risks were substantially higher. Importantly, the risk was high among women who had chronic hypertension, PIH, and FGR (relative risk, 4.5; 95% CI, 2.4, 8.5), and highest among women who had chronic hypertension, PIH, and LGA (relative risk, 9.0; 95% CI, 7.2, 11.3).

Placental abruption rates (per 1000 singleton pregnancies) among women with chronic hypertension, fetal growth abnormalities, and PIH at 22-31, 32-36, and ≥37 weeks’ gestational age are shown in Figure 1. Although the association among these 3 conditions and abruption had similar overall patterns (Table 3), the associations were generally

### TABLE 4
**Association among chronic hypertension, FGR, PIH, and placental abruption—associations stratified on gestational age at delivery: US singleton births, 1995-2002**

<table>
<thead>
<tr>
<th>Ischemic placental disease</th>
<th>22-31 wks</th>
<th>32-36 wks</th>
<th>≥37 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>No complications(^*)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Chronic hypertension only</td>
<td>1.1 (1.0, 1.2)</td>
<td>1.5 (1.4, 1.6)</td>
<td>1.8 (1.7, 2.0)</td>
</tr>
<tr>
<td>Chronic hypertension with FGR only</td>
<td>0.8 (0.6, 0.9)</td>
<td>2.4 (2.2, 2.7)</td>
<td>4.8 (4.3, 5.3)</td>
</tr>
<tr>
<td>Chronic hypertension with LGA only</td>
<td>0.8 (0.5, 1.1)</td>
<td>0.4 (0.2, 0.6)</td>
<td>1.0 (0.8, 1.3)</td>
</tr>
<tr>
<td>Chronic hypertension with PIH only</td>
<td>1.5 (1.2, 1.8)</td>
<td>2.4 (1.8, 3.1)</td>
<td>3.7 (2.4, 5.8)</td>
</tr>
<tr>
<td>Chronic hypertension with PIH and FGR</td>
<td>2.6 (1.2, 6.0)</td>
<td>0.8 (0.1, 5.5)</td>
<td>2.4 (0.6, 9.6)</td>
</tr>
<tr>
<td>Chronic hypertension with PIH and LGA</td>
<td>1.3 (0.7, 2.2)</td>
<td>3.5 (2.5, 4.8)</td>
<td>13.0 (8.8, 19.1)</td>
</tr>
</tbody>
</table>

\(^*\) Adjusted for the confounding effects of period of birth, maternal age, gravidity, maternal race, maternal education, marital status, smoking during pregnancy, and infant sex.

\(^\dagger\) Includes normotensive women with no fetal growth abnormalities.

These rates are stratified on the basis of gestational age at delivery (22-31 weeks of gestation, 32-36 weeks of gestation, and ≥37 weeks of gestation).

stronger among term births, followed by births at 32-36 weeks (Table 4).

Rates and relative risks of abruption among women with chronic hypertension and, in addition, fetal growth abnormalities and/or PIH are shown in Figures 2 and 3, respectively. Regardless of fetal growth, the rate of abruption was highest among women with chronic hypertension with superimposed PIH and lowest among normotensive women, with women having either chronic hypertension or PIH at intermediate risk. The greater the fetal growth, the lower the abruption rates across all chronic hypertension and PIH groups.

**COMMENT**

This large, population-based study supports our hypothesis that the association between chronic hypertension and placental abruption is mediated largely through both PIH and fetal growth. We observed that the association between chronic hypertension and abruption is strong, but is further strengthened if FGR and/or PIH are present. This finding corroborates data from our earlier studies that abruption is probably a long-standing chronic condition, with origins early in pregnancy. It provides further support to the concept that conditions that are associated with ischemic placental disease (which includes preeclampsia, small for gestational age, and abruption) may be linked through a unified pathophysiologic mechanism.

An interesting finding of our study was the association between the risk of abruption and fetal growth abnormalities that were assessed on a continuum. These data demonstrate a clear dose-response association between abruption and birthweight percentiles within all groups of chronic hypertension and PIH and among normotensive women. In particular, the rate of abruption was lowest among large babies (ie, LGA) across all chronic hypertension and PIH categories. Although this observation corroborates previous studies, taken together, these findings suggest that chronic hypertension, PIH, and fetal growth abnormalities occur on a continuum of less-severe-to-more-severe disease processes and that there may be an etiologic underpinning in the association between abruption and fetal growth.

Aggressive fetal monitoring with appropriate and timely clinical intervention by delivery in modern obstetrics in the scenario of impending maternal and/or fetal compromise is likely to have influenced our findings. For instance, the association between chronic hypertension and abruption at term, in the setting of PIH, was lower than among women with FGR babies (Table 4). These patterns were reversed generally at 22-31 and 32-26 weeks of gestation. Early delivery may have prompted early intervention, thereby preventing the opportunity for placental abruption to develop. Similarly, clinicians may be more likely to manage expectantly mild PIH,
especially if there is no evidence for FGR, thereby allowing more time for an abruption to occur when the fetus is larger. Therefore, abruption rates may be underestimated for FGR and closer to natural risk with LGA.

Under-ascertainment of hypertensive diseases and abruption in these data files may have led to a nondifferential misclassification that resulted in diluted effect measures of association. Although rates of chronic hypertension and abruption in our study (7.3 and 6.1 per 1000 singleton pregnancies, respectively), fell well within the range of those previously reported (5-8 and 4-10 per 1000 pregnancies, respectively), the reported rates from many of these previous studies are based on high-risk hospital populations.

Our study is also limited by the fact that women with hypertensive diseases may have been treated with antihypertensive medication or, when the disease was severe, may have been delivered early. This may be especially true in the setting of chronic hypertension accompanied by FGR that occurs at very preterm gestational ages. The data that were used for this study did not include information on body mass index or gestational diabetes mellitus; therefore, residual confounding by these and other unmeasured factors may have affected our results to some extent.

Notwithstanding the limitations, this large, population-based study is consistent with our previous work and provides important new evidence that the strong association between chronic hypertension and risk of abruption is modified largely by fetal growth abnormalities and PIH. Women with chronic hypertension may well benefit from appropriate clinical attention to evaluate impending risks of not only abruption but also complications that accompany it. Furthermore, the increased risk of abruption among small babies with progressively diminishing risk among large babies suggests a possible etiologic underpinning between placental abruption and fetal growth.

**REFERENCES**

The influence of maternal cigarette smoking on placental pathology in pregnancies complicated by abruption

Lilian M. Kaminsky, MD; Cande V. Ananth, PhD, MPH; Vinay Prasad, MD; Carl Nath, MD; Anthony M. Vintzileos, MD; for the New Jersey Placental Abruption Study Investigators

OBJECTIVE: The purpose of this study was to evaluate the effect of maternal cigarette smoking on placental histology in women with abruption.

STUDY DESIGN: Data were derived from the New Jersey Placental Abruption Study (NJ-PAS)—an ongoing, case-control study, conducted since August 2002 in 2 large hospitals in NJ. Abruption cases were identified based on a clinical diagnosis. Histologic evaluations were performed by 2 perinatal pathologists who were blinded to the abruption status. Maternal smoking during pregnancy was determined based on patient’s self-report. Among abruption cases, histologic findings were compared between smokers and nonsmokers, and the association expressed as odds ratio (OR) with 95% confidence interval (CI). All analyses were adjusted for potential confounders.

RESULTS: A total of 189 abruption cases were available for analysis, of which 10.6% (n = 20) were smokers. Intervillous thrombus was more common in women who smoked (20%) than in nonsmokers (3.0%) (OR, 17.5; 95% CI, 3.1-99.4). However, placental infarcts were seen less frequently among smokers than nonsmokers (10.0% vs 32.5%; OR, 0.2; 95% CI, 0.1-0.8).

CONCLUSION: These findings suggest that different pathologic mechanisms may be responsible for the histologic findings between smokers and nonsmokers diagnosed with placental abruption.

Key words: intervillous thrombus, microinfarcts, placental abruption, smoking, trophoblast knotting, villous fibrosis

Placental abruption complicates approximately 1% of all pregnancies,1,2 with an increase in its incidence over time.3 This condition is associated with significant maternal morbidity and perinatal morbidity and mortality.1,4 Although the etiology of abruption remains unclear, a number of risk factors have been identified. These include advanced maternal age, poor socioeconomic and single marital status, hypertension, preeclampsia, cigarette and cocaine use, grand multiparity, multiclicity, multifetal gestation, premature rupture of membranes, and prior abruption.2,4-8

The association of maternal smoking with placental abruption has been well documented with studies reporting relative risks of 1.4-2.5.9-11 Cigarette use is associated with a 2.5-fold increase in severe abruption resulting in fetal death.9 The risk of abruption increases with the number of cigarettes smoked per day,11 with a threshold effect at approximately 10 cigarettes per day after which the risk remains constant.12

Studies focusing on smoking-related placental abruption pathology are sparse. These studies allude to the mechanism of abruption in smokers that is initiated by decidual necrosis at the margin of the placenta.13,14 Maternal smoking has been shown to decrease placental blood flow.15 This effect may be mediated through changes in production of vasoactive substances, such as prostacyclin and nitric oxide,16 or endothelial cell damage.17

The sequence of events in the placentas of smokers leading to placental abruption is poorly understood. The direct effect of smoking may be mediated through vasoconstrictive effects of nicotine on uterine and umbilical arteries as well as increased carboxyhemoglobin concentrations that interfere with oxygenation. We believe that such hypoxic change can cause microinfarctions occurring at the periphery of the placenta leading to necrotic foci, separation at the necrotic foci, and eventually, an abruption.

Although the biologic mechanisms through which maternal smoking increases the risk for abruption remain unclear, we hypothesized that abruption as-
associated with smoking may have placental abnormalities that would suggest a distinct pathophysiologic pathway. Therefore, we carried out a secondary analysis of a case-control study of abruptio to compare, among abruptio cases, histologic lesions between the placentas of smokers versus those that remained nonsmokers throughout their pregnancy.

**Materials and Methods**

This study is part of the ongoing “New Jersey-Placental Abruption Study,” a multicenter, case-control study of placental abruption. It has been conducted since August 2002 at Robert Wood Johnson University Hospital and Saint Peter’s University Hospital, New Brunswick, NJ. Placental abruption cases for this study were identified in the antepartum or intrapartum period by an attending physician based on any 1 (or more) of the following clinical criteria: (1) vaginal bleeding accompanied by fetal distress or uterine hypertonicity/contractions; (2) ultrasound-based diagnosis; or (3) evidence of retroplacental clot(s) or hemorrhage on the placental surface. Data pertaining to maternal smoking, defined as smoking consistently at least 1 cigarette per day during a pregnancy, was ascertained through in-person interview of women following their delivery and before discharge from the hospital. Further details of the NJ-PAS have been published elsewhere.18

**Histopathological Lesions**

All placentas were immersed in 10% buffered formalin. After a detailed gross examination, representative sections were submitted including 1 each from the fetal and maternal surfaces, 1 from the membranes and 1 from the umbilical cord. Any unusual areas and areas of infarcts and intervillous thrombi were sampled. These blocks were embedded in paraffin and cut at 4-6 μm thickness. The slides were put into a stainer that put the slides through several stages including deparaffinization, rehydration through alcohols and water, and finally

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**Table 1**

Distribution of maternal and pregnancy characteristics of women with placental abruption based on their smoking status

| Maternal characteristics                  | Smokers | | % | Nonsmokers | | % | | P value |
|-------------------------------------------|---------|---|---|------------|---|---|--------|
| Maternal age (y)                          |         |---|---|------------|---|---|--------|
| < 20                                      | 7       | 35.0 | 30 | 17.8       |
| 20-34                                     | 8       | 40.0 | 91 | 53.9       |
| ≥ 35                                      | 5       | 25.0 | 48 | 28.4       |
| Maternal race/ethnicity                   |         |---|---|------------|---|---|--------|
| Caucasian                                 | 5       | 25.0 | 28 | 16.6       |
| African American                          | 9       | 45.0 | 54 | 32.0       |
| Hispanic                                  | 6       | 30.0 | 58 | 34.3       |
| Other                                     | 0       | 0    | 29 | 17.1       |
| Parity                                    |         |---|---|------------|---|---|--------|
| Nulliparous                               | 4       | 20.0 | 63 | 37.3       |
| Primiparous                               | 9       | 45.0 | 60 | 35.5       |
| Parity ≥ 2                                 | 7       | 35.0 | 46 | 27.2       |
| High school education or higher           | 8       | 40.0 | 104| 61.5       |
| Single marital status                     | 10      | 50.0 | 50 | 29.6       |
| Chronic hypertension                      | 2       | 10.0 | 10 | 5.9        |
| Preeclampsia                              | 2       | 10.0 | 22 | 13.0       |
| Diabetes (IDDM)                           | 0       | 0    | 4  | 2.4        |
| Preterm PROM                              | 2       | 15.4 | 28 | 23.9       |
| BMI (mean ± SD)                           | 22.7 ± 6.5 | | 24.0 ± 6.1 | | .51 |
| Gestational age (mean ± SD)               | 31.9 ± 4.0 | | 32.0 ± 5.1 | | .34 |
| Birthweight (mean ± SD)                   | 2280.2 ± 701.7 | | 2090.7 ± 887.2 | | .48 |
| Placental weight (mean ± SD)              | 411.6 ± 79.3 | | 391.5 ± 137.6 | | .46 |

IDDM, insulin-dependent diabetes mellitus; PROM, premature rupture of membranes; BMI, body mass index; SD, standard deviation.
stained with hematoxylin–eosin. Histologic evaluation of placentas was performed by 1 of the 2 perinatal pathologists who were blinded to the patients’ abruption status. Microscopic examination was performed on all the cases. Fetal membranes were examined for presence of pigment (meconium or hemosiderin staining), related pigment-laden macrophages, and hemosiderin granules. The presence of chorioamnionitis was noted, and graded as mild, moderate, or severe. The umbilical cord was examined for the presence of funisitis. Placental sections were carefully studied during microscopic examination for foci of infarction (areas of ischemic villous necrosis) and microinfarctions, segmental villous fibrosis, villous morphology (edema, stromal hemorrhage, and vascularity), degree of maturation, presence of villous maldevelopment. The presence of vascular lesions such as chorangioma and features suggestive of hypoxia such as chorangiomatosis were noted. Evidence of hypoxic change in the form of increased perivillous fibrin, intervillus fibrinoid, intervillous thrombus was noted. Decidual pathology such as acute or chronic deciduitis, decidual hemosiderosis, or decidual vasculopathy (decidual vessel exhibiting muscular hypertrophy, decidual thrombosis, fibrinoid necrosis) were also recorded when present.

### Statistical analysis

We compared the distributions of maternal sociodemographic and pregnancy characteristics between women who did and did not smoke throughout their pregnancy among all abruption cases. Fisher exact probability test or the χ² test was applied for categorical data, or the Student t test for continuous variables. The associations between histologic lesions and smoking status among women diagnosed with placental abruption were based on fitting multivariable logistic regression model, from which odds ratio (OR) and 95% confidence interval (CI) were derived. A variety of confounders were used in the model for adjustment, including maternal race/ethnicity (White, African American, Hispanic, and others), parity (0, 1, 2, or more), maternal education (high school or more), and marital status (single or married). If inclusion of confounders in the regression model changed the crude OR by at least 10%, we retained the confounder for adjustment. In addition, we adjusted all analyses for parity and maternal race/ethnicity. Statistical analysis was performed using SAS software (SAS Institute, Cary, NC). All statistical tests were 2-tailed, and P < .05 was considered statistically significant.

### Results

There were 189 women diagnosed with placental abruption for whom complete histological analysis was available. Of these abruption cases, 10.6% (n = 20) women smoked during the pregnancy. The distributions of sociodemographic and pregnancy characteristics of women that did and did not smoke are shown in Table 1. There were no statistically significant differences for any of these characteristics between the groups. However, there was a tendency for smokers with abruption to be less educated and nonmarried.

Table 2 shows the comparison of histologic lesions between the 2 groups of women with placental abruption analyzed by smoking status. Intervillous thrombus was more common in women who smoked as compared to those that remained nonsmokers throughout the pregnancy (20.0% vs 3.0%, respectively; OR, 17.5; 95% CI, 3.1–99.4). Placental infarcts and microinfarcts were seen less...
frequently among smokers compared to nonsmokers (10.0% vs 32.5%, respectively; OR, 0.2; 95% CI, 0.1-0.8). There was a trend toward higher rate of villous fibrosis (25.0% vs 11.8%) in women who smoked compared to nonsmokers, although this association did not reach statistical significance ($P = .10$). Adjustment for confounding factors did not alter these associations.

**Comment**

Smoking during pregnancy is 1 of the most important and preventable risk factors for an array of adverse pregnancy outcomes, including placental abruption.\(^4\)\(^9\)-\(^21\) Despite widely known risks and large educational campaigns, up to 25% of reproductive age women continue to smoke, and an increasing percentage of them smoke heavily.\(^22\)

Although smoking is a well known risk factor for placental abruption, the etiology of this association continues to be unknown. Several hypotheses linking maternal smoking with abruption have been proposed. It has been previously reported that smoking is associated with increased frequency of placental calcifications and subchorionic fibrin deposits.\(^23\) There is higher frequency of hyperplastic cytrophoblasts and obliterator arteriopathy\(^4\) and nuclear clumps in the perivillous syncytiotrophoblasts\(^24\) in placentas of smokers. It is likely that the prominent syncytiotrophoblast knotting is caused by an abortive attempt by the villi to increase surface area by angiogenesis and neovascularization. This would increase oxygenerating capacity in order to compensate for possible chronic ischemia caused by smoking. Several researchers focused on structural changes in placentas of women who smoked and showed increased villous membrane thickness as well as decreased capillary volume.\(^25\)\(^26\) It has been speculated that placental hypoperfusion resulting from vasoconstrictive effects of smoking on maternal placental vasculature could cause decidual ischemia with subsequent necrosis and hemorrhage leading to placental separation.\(^13\)

Our results are consistent with previous studies\(^23\)\(^24\) that have shown increased rates of lesions that are essentially reflective of chronic hypoxic change in the placentas of smokers. We demonstrated higher frequency of intervillous thrombi in the placentas of smokers who had placental abruption versus nonsmokers with abruption. Intervillous thrombosis is thought to result from intraplacental hemorrhage from villous capillaries and is associated with chorionic villous hemorrhage.\(^27\) This may alter uteroplacental/fetal blood flow leading to chronic underperfusion. This chronic hypoxia is manifested by increased rate of villous fibrosis, decrease in villous capillaries, and increased trophoblast knotting.

One of the interesting but unexpected findings was the increased frequency of placental infarcts and microinfarcts among nonsmoking women with abruption. The biologic underpinnings for this association remain unclear. Perhaps the etiology of abruption in nonsmokers may be related to intrinsic maternal conditions, such as thrombophilia, which is more likely to cause ischemic villous necrosis.

The possibility of our findings being affected by type II error (ie, lack of statistical power) is likely. The NJ-PAS was not primarily designed to compare histologic lesions between smokers and nonsmokers in the setting of placental abruption. A post-hoc power analysis for many of the lesions evaluated in this study reveal that the lack of association might be driven by low statistical power. Furthermore, some of the associations may be imprecise because of fairly wide confidence intervals. Another potential limitation is that data on cigarette smoking was collected through maternal self-report, possibly resulting in an underestimation of its prevalence. Moreover, because most women would have been informed of their abruption status before the interviews, the possibility of a smoking misclassification may have been differential. If present, the nondifferential misclassification would have likely biased the effect measures (ie, OR) away from the null. Studies using biochemical markers, such as urinary cotinine, have demonstrated that women consistently underreport smoking behavior.\(^28\)\(^29\)

In summary, it appears that there might be different pathologic mechanisms responsible for the placental findings in abruption of smokers vs nonsmokers. Placental abruption caused by cigarette smoking may be associated with chorionic villous hemorrhage and intervillous thrombosis. However, the ischemic villous damage, as evidenced by the number of infarcts, is higher in the absence of smoking suggesting a distinct underlying pathophysiologic pathway.

**References**

Appendix

Investigators currently participating or who have been involved in the New Jersey—Placental Abruption Study include Cande V. Ananth, PhD, MPH (principal investigator), Darios Getahun, MD, MPH, Neela Srinivas, MD, MPH, Celeste DeMarco, RN, BSN, Denise Elsasser, MPH, Yu-Ling Lai, RN, and Shelby Pitts, RN (Division of Epidemiology and Biostatistics), John C. Smulian, MD, MPH, Wendy L. Kinzler, MD, Morgan R. Peltier, PhD, and Marian Lake, RN, MPH (Division of Maternal-Fetal Medicine), Department of Obstetrics, Gynecology, and Reproductive Sciences; Claire Philipp, MD (Department of Medicine), all at UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; and George G. Rhoads, MD, MPH (Department of Epidemiology), and Dirk F. Moore, PhD (Department of Biostatistics), UMDNJ-School of Public Health, Piscataway, NJ.

Other investigators that were involved with the study included Rima R. Rozen, PhD, and Jacques Genest, MD (McGill University, Montreal, Canada); Susan Shen-Schwarz, MD (Department of Pathology, Saint Peter’s University Hospital, New Brunswick, NJ); and Vinay Prasad, MD (Department of Pediatric Pathology, Arkansas Children’s Hospital, University of Arkansas Medical Sciences, Little Rock, AR).
Antibodies to the 70 kDa heat shock protein in midtrimester amniotic fluid and intraamniotic immunity

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OBJECTIVE: Antibodies to the 70 kDa heat shock protein (hsp70) immunoglobulin (Ig) G are markers for exposure to adverse or nonphysiological stimuli. In addition, these antibodies cross-link hsp-70 microbial antigen complexes and enhance development of antimicrobial immunity. The association between intraamniotic hsp70 IgG concentrations and intraamniotic immune responses were evaluated.

STUDY DESIGN: Midtrimester amniotic fluids from 90 women undergoing an amniocentesis were tested for hsp70 IgG, hsp70 antigen, tumor necrosis factor (TNF)-α, secretory leukocyte protease inhibitor (SLPI), and interferon (IFN)-α by enzyme-linked immunosorbent assay. Clinical outcomes were obtained after completion of all testing. Associations were analyzed by nonparametric statistics.

RESULTS: Intraamniotic hsp70 IgG concentrations, but not hsp70 antigen levels, were positively associated with levels of TNF-α (P < .0001), IFN-α (P = .0001), and SLPI (P = .0038). There were no associations between hsp70 IgG and maternal age or parity, race/ethnicity or pregnancy outcome.

CONCLUSION: The hsp70 IgG levels correlate with intraamniotic concentrations of antimicrobial immune mediators. This antibody may potentiate antimicrobial immunity during fetal development.

Key words: amniotic fluid, heat shock protein, heat shock protein antibodies, immune regulation, interferon-α, secretory leukocyte protease inhibitor, tumor necrosis factor-α


In response to environmental stresses such as infection, ischemia, free oxygen radicals, and rapid growth and differentiation, the gene coding for the in-ducible 70 kDa heat shock protein (hsp70) is rapidly activated. Within the cell, hsp70 prevents the incorrect folding and assembly of peptides, transports misfolded or degraded proteins for elimination, and inhibits the induction of apoptosis.1 Under these same nonphysiological conditions, hsp70 is also released from cells into the surrounding milieu. Extracellular hsp70 acts as an early warning danger signal.2 Cell-free hsp70 binds to Toll-like receptors3 and possibly other cell surface components4 and initiates a protective proinflammatory immune response. Extracellular hsp70 also readily binds to microbial-derived peptides. The resulting hsp70-peptide complexes bind to receptors on antigen-presenting cells and facilitate production of antibodies to both the microbial and hsp70 components.5 Antibodies to hsp70 have been identified in individuals with a variety of autoimmune,6 neurological,7 and vascular8 disorders. It has recently been demonstrated that hsp70 immunoglobulin (Ig) G markedly enhances the capacity of hsp70 to induce proinflammatory immunity. This elevated efficacy appears to be a result of the aggregation of multiple hsp70-antigen complexes by the hsp70 IgG.9 Hsp70 is present in midtrimester amniotic fluid10 as well as trophoblast cells.11 We have been characterizing midtrimester intraamniotic immune responses.10,12,13 It is our hypothesis that mechanisms operational at this gestational stage effectively preserve an ongoing pregnancy in the face of potentially adverse infectious and noninfectious stimuli. Given the association between hsp70 IgG and promotion of hsp70-mediated immune system activation and our prior detection of extracellular hsp70 in amniotic fluid,10 we examined associations between intraamniotic hsp70 IgG and intraamniotic immune responses.

MATERIALS AND METHODS

Ninety consecutive women with singleton pregnancies, age 18-45 years, who were undergoing amniocenteses between 15 and 19 weeks’ gestation and who delivered at The New York Presbyterian Hospital between December 2005 and April 2006 comprised the study population. Indications for amniocentesis were...
advanced maternal age, maternal request, abnormal values on first- or second-trimester multiple serum marker testing, family history of chromosome abnormalities, or ultrasound identification of markers of aneuploidy. All subjects were in apparent good health and were negative for cervical infections as determined by routine cultures for gonorrhea and chlamydia.

Samples were collected by transabdominal amniocentesis after disinfection of the skin, and excess whole amniotic fluids were transported to the laboratory within 2-3 hours of collection. Samples were centrifuged and supernatant fractions stored at −80°C until analyzed.

The study was approved by the Institutional Review Board at the Weill Medical College of Cornell University and written informed consent was obtained.

Pregnancy outcome data were obtained from medical records after completion of all laboratory testing. The chart reviewer was unaware of all laboratory data. Preterm birth was defined as delivery less than 37 weeks’ gestation. Spontaneous preterm birth is delivery at less than 37 weeks that was preceded by preterm premature rupture of membranes (PPROM) and/or preterm labor. Race/ethnicity was self-reported by the mother.

Amniotic fluid supernatants were assayed in duplicate by enzyme-linked immunosorbent assay (ELISA) for concentrations of hsp70 (StressGen, Victoria, British Columbia, Canada), hsp70 IgG (Assay Design, Ann Arbor, MI), tumor necrosis factor (TNF)-α (Ultrasensitive ELISA, Biosource, Camarillo, CA), interferon (IFN)-α (Invitrogen, Carlsbad, CA), and secretory leukocyte protease inhibitor (SLPI; Cell Sciences, Canton, MA). Values were converted to micrograms per milliliter or picograms per milliliter by reference to a standard curve that was assayed in parallel to the test samples. The lower limits of sensitivity were 0.5 ng/mL (hsp70), 0.007 μg/mL (hsp70 IgG), 0.09 pg/mL (TNF-α), 2.0 pg/mL (IFN-α), and 20 pg/mL (SLPI).

Associations among maternal age, race/ethnicity, pregnancy outcome, and concentrations of hsp70 IgG were analyzed by the nonparametric Mann-Whitney test. Correlations between the concentration of hsp70 IgG or hsp70 antigen and TNF-α, IFN-α, and SLPI levels were analyzed by Spearman rank correlation. A P < .05 was considered significant.

**RESULTS**

Hsp70 IgG as well as hsp70 antigen was identified in amniotic fluids. The median (range) concentration was 3.7 μg/mL (0.6-20.1) for hsp70 IgG and 3.5 ng/mL (less than 0.5 to 34.7) for hsp70 antigen. As shown in Figures 1-3, hsp70 IgG concentrations were directly proportional to intraamniotic levels of TNF-α (r = 0.627, P < 0.0001), IFN-α (r = 0.442, P = 0.0001), and SLPI (r = 0.337, P = 0.0038). In contrast to the antibody results, intraamniotic hsp70 antigen levels were not associated with TNF-α, IFN-α, or SLPI concentrations (P > .05).

Race/ethnicity data were available from 74 of the subjects (82.2%). Thirty-nine of the subjects were white, 16 were Hispanic, 10 were Asian, and 9 were black. There was no association between race/ethnicity and hsp70 IgG levels (Figure 4). The intraamniotic concentration of hsp70 IgG was also unrelated to maternal age (Figure 5). In addition, there were no associations between hsp70 IgG levels and parity, indications for amniocentesis, or gestational age at amniocentesis (data not shown).

Five of the subjects had an indicated preterm birth because of preeclampsia, 8 had a spontaneous preterm birth, and 77 delivered at term. In 2 women, spontaneous preterm birth was preceded by PPROM. None of the neonates had a birth defect. Acknowledging the limitations of the small sample size and the fact that the study was not designed to evaluate antibody levels and outcome, there were no observable differences in me-
dian hsp70 IgG levels between women in any of the pregnancy outcome categories (P > .05). The median (range) antibody levels (micrograms per milliliter) were 4.4 (0.6 to 20.2) term birth, 3.2 (1.4 to 12.2) spontaneous preterm birth, and 2.4 (1.1 to 16.4) indicated preterm birth.

**Comment**

Hsp70 IgG levels, but not hsp70 antigen concentrations, in sera from pregnant women have previously been associated with birth defects in their neonates.14 Similarly, detection of hsp70 antigen-antibody complexes in placental tissues, but not hsp70 antigen alone, was associated with preterm birth.15 In nonpregnant individuals, hsp70 IgG, but not hsp70 antigen levels, correlated with an increased risk of hypertension and cardiovascular disease.16

In the present investigation, intraamniotic hsp70 IgG levels, but not hsp70 antigen concentrations, were positively correlated with levels of TNF-α, IFN-α, and SLPI in midtrimester amniotic fluid. Hsp70 antigen-antibody complexes are known to be potent inducers of TNF-α.9 TNF-α has been shown previously to stimulate SLPI production by human amnion cells, resulting in increased amniotic fluid levels.17 This elevated release of SLPI can neutralize leukocyte-derived toxic products should these cells invade into the amniotic cavity. In addition, SLPI may protect the developing fetal immune system. It induces production of proteins that inactivate viruses and stimulates apoptosis of virus-infected cells. In addition, IFN-α modulates the extent of inflammatory reactions to limit damage to host tissues.20,21 Thus, the induction of these components in the amniotic cavity in response to elevated anti-hsp70 IgG levels provides a mechanism to prepare the fetus for defense against exposure to a potentially pregnancy-threatening event.

The amniotic fluids in the present study were collected in the midtrimester, months preceding eventual delivery, and therefore, it is not surprising to us that the measured intraamniotic hsp70 IgG concentrations were unrelated to adverse pregnancy outcome. We would further suggest that the lack of an observed association between hsp70 IgG level and adverse outcome is consistent with the function of this antibody as a component of a physiological fetal immune protection mechanism. However, given the small number of patients who delivered preterm, our observations must be regarded as tentative and need to be confirmed in larger studies.

The mechanism responsible for the ability of hsp70 IgG to markedly enhance hsp70-mediated immune activation has been demonstrated previously.9 We propose that the production of hsp70 IgG by the pregnant mother in response to a nonphysiological event and its passage into the amniotic cavity is a component of a coordinated maternal-fetal immune defense mechanism to prevent premature pregnancy termination. Hsp70 antigen is present in the amniotic cavity in midtrimester.10 Thus, the production of hsp70 IgG by the mother in response to nonphysiological conditions and its transport across the placenta in which it can interact with hsp70 antigen provides a sensitive mechanism to rapidly activate intraamniotic immunity to respond to a potential danger. Whether this response is beneficial or destructive probably depends on the extent of immune system activation. The potential for heat shock protein antibodies to possess either helpful or harmful properties has been discussed previously.22 Ideally, the response should be sufficient to provide protection against potentially pathogenic factors but below the threshold needed to trigger the sequence of events leading to premature labor.

The present study furthers the evidence for the existence of intraamniotic regulatory mechanisms operational during the midtrimester that modulate immune responses to potentially pathogenic insults. Further studies to elucidate the mechanism for controlling intraamniotic immune system activation are warranted.

**REFERENCES**

Effect of a previous pregnancy on vascular function in endothelial nitric oxide synthase 3 knockout mice

Labib M. Ghulmiyyah, MD; Esther Tamayo; Shannon M. Clark, MD; Gary D. V. Hankins, MD; Garland D. Anderson, MD; George R. Saade, MD; Monica Longo, MD, PhD

OBJECTIVE: Nitric oxide deficiency has been implicated in adverse pregnancy outcomes. Mice that lack endothelial nitric oxide synthase (NOS3) have abnormal in vitro vascular reactivity. Our objective was to assess the effect of a previous pregnancy on the abnormal vascular function of NOS3 knockout mice.

STUDY DESIGN: Carotid arteries from pregnant NOS3 knockout (NOS3−/−KO) and wild-type control mice (NOS3+/+WT) from first and second pregnancy were obtained for in vitro vascular reactivity studies. Vascular responses to cumulative concentrations of the vasoconstrictors phenylephrine, serotonin, and thromboxane and the vasorelaxants acetylcholine, sodium nitroprusside, and isoproterenol were determined.

RESULTS: In the first pregnancy, contractile responses were exaggerated in the knockout animals, compared with the wild-type animals. However, the second pregnancy in knockout animals was associated with normalization of responses to phenylephrine and serotonin and increased responses to the endothelium-independent relaxants.

CONCLUSION: The vascular function of NOS3 knockout mice improves with subsequent pregnancy becoming comparable to wild-type animals.

Key words: fetal programming, subsequent pregnancy, vascular reactivity

Pregnancy-induced hypertensive disorders that include preeclampsia are known to occur more often in first pregnancies. The mechanisms that are responsible for the protective effect of the first pregnancy are not understood clearly. One possible mechanism could be that the cardiovascular adaptations that occur during the first pregnancy persist well beyond the postpartum period and into the subsequent pregnancies, thereby lowering the risk of cardiovascular disease that includes preeclampsia in the mother. Pregnancy may result in nonreversible vasodilatation that leads to an increase in blood flow to the uterine-placental unit in future pregnancies. Gestational vascular remodeling as a mechanism for the protective effect of a first pregnancy is supported by emerging evidence. Khong et al.1 have shown that pregnancy results in permanent anatomic changes in the spiral arteries that may modify subsequent vascular remodeling in the next pregnancy. Histologically, the muscular layer in the spiral arteries from a nulliparous uterus is well-developed, compared with an absent muscular layer in the spiral arteries from a multiparous uterus. They also showed that the structural changes that are induced by a pregnancy are not obliterated totally after parturition. The changes in the muscular layer of the spiral arteries may well lead to improved uteroplacental perfusion. These adaptations appear to affect the vascular tree beyond the myometrium. In their longitudinal study, Clapp and Capeless2 found that multiparous women have a more significant decrease in systemic peripheral vascular resistance and increase in cardiac output during gestation, compared with nulliparous women.2

A possible explanation may be that residual arterial alterations after a first pregnancy allow trophoblasts to remodel spiral arteries more effectively in a subsequent pregnancy, thereby preventing superficial implantation and decreasing the risk of preeclampsia.3 An important mechanism that regulates vascular tone and is involved in gestational vascular adaptations is nitric oxide (NO). NO is produced in the vasculature by the endothelial NO synthase (NOS3).4 NO is a potent smooth muscle relaxant and 1 of the primary modulators of vascular tone.5-7 NO also plays an important role in the physiologic vascular adaptation that occurs in pregnancy, particularly in relation to uteroplacental perfusion and fetal growth. Pharmacologic inhibition of NO in pregnant rats results in hypertension, intrauterine growth restriction, and a preeclampsia-like syndrome.8,9

To circumvent the limitations of pharmacologic inhibition, transgenic mice that lack functional NOS3 were developed. This animal model allows the study of the effect of NOS3 deficiency on the uterine environment without the confounding effect of pharmacologic treatment.10,11 NOS3 knockout mice have an abnormal vascular function and are hypertensive. In the absence of functional NOS3, these knockout mice lack the endothelium-dependent vasorelax-
Thus, in the current study, we sought to test the hypothesis that the abnormal vascular function that is seen in the pregnant NOS3 knockout mouse is attenuated with a second pregnancy. Our objective was to assess the effect of a previous pregnancy on the abnormal vascular function of the NOS3 knockout mice. This study will allow us to determine differences in vascular responses between first and second pregnancy in both wild-type and knockout mice.

**Material and Methods**

**Animals**

Mature cycling female and male mice that are homozygous for disruption of the NOS3 gene (NOS3-knockout, strain B6.129P2-Nos3<sup>tm1Unc</sup>, stock no. 002684, NOS3<sup>-/-KO</sup>) and their age-matched wild-type controls (NOS3-Wild-Type, strain C57BL/6J, stock no. 000664, NOS3<sup>+/+WT</sup>) were purchased from Jackson Laboratory (Bar Harbor, ME). The mice were maintained and bred in the animal care facility at the University of Texas Medical Branch. All procedures were performed after approval by the Animal Care and Use Committee of the University of Texas Medical Branch. The mice were housed separately in temperature- and humidity-controlled quarters with constant 12-hour light:dark cycles. They were provided with food and water ad libitum. We used pregnant female homozygous NOS3 knockout (KO, C57BL/6J-NOS3) and wild-type (WT, C57BL/6J-NOS3) mice in a first pregnancy, referred to as KO-P0 and WT-P0, and similar mice in a second pregnancy, referred to as KO-P1 and WT-P1. On day 18 of gestation, the P0 and P1 mice from both groups were killed by the CO<sub>2</sub> inhalation method according to the Animal Care and Use Committee guidelines and the American Veterinary Medical Association guidelines. The carotid arteries were dissected, isolated, and mounted on a wire myograph system for vascular reactivity studies.

**In vitro experiments**

Two-millimeter segments of the carotid artery were mounted on a wire myograph system and perfused with Krebs-Henseleit buffer at 37°C at constant tension of 1 g. Phenylephrine, serotonin, and U46619 (thromboxane agonist) were added at a range of concentrations in each group to determine maximal efficacy and area under the curve. The data were analyzed using GraphPad software, and statistical significance was determined using Student’s t-test for paired samples.

**TABLE 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maximal effect</th>
<th>Area under the curve</th>
<th>Log IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenylephrine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT-P0</td>
<td>77.9 ± 12.9</td>
<td>179 ± 26.8</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>KO-P0</td>
<td>161.5 ± 20.1*</td>
<td>388.8 ± 62.0*</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>WT-P1</td>
<td>77.9 ± 8.1</td>
<td>206.5 ± 25.3</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>KO-P1</td>
<td>77.4 ± 20.6</td>
<td>174.6 ± 49.4</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td><strong>Serotonin</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>WT-P0</td>
<td>77.1 ± 2.4</td>
<td>320.4 ± 31.6</td>
<td>8.55 ± 0.33</td>
</tr>
<tr>
<td>KO-P0</td>
<td>156.7 ± 18.1*</td>
<td>325.9 ± 78.5</td>
<td>7.77 ± 0.31</td>
</tr>
<tr>
<td>WT-P1</td>
<td>79.3 ± 11.4</td>
<td>218.9 ± 77.6</td>
<td>7.45 ± 0.54</td>
</tr>
<tr>
<td>KO-P1</td>
<td>79.6 ± 17.5</td>
<td>330.4 ± 46.6</td>
<td>8.72 ± 0.36</td>
</tr>
<tr>
<td><strong>U46619 (thromboxane agonist)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT-P0</td>
<td>278.7 ± 23.0†</td>
<td>301.9 ± 52.4</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>KO-P0</td>
<td>206.3 ± 18.9</td>
<td>274.0 ± 46.1</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>WT-P1</td>
<td>162.7 ± 30.0</td>
<td>222.5 ± 73.3</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>KO-P1</td>
<td>232.4 ± 36.4</td>
<td>230.1 ± 76.8</td>
<td>6.7 ± 0.3</td>
</tr>
</tbody>
</table>

* P < .05
† P < .05 of WT-P0 vs WT-P1.
graph (model 410A; J.P. Trading I/S, Aarhus, Denmark) with 25 μm tungsten wires. The preparations were bathed in a physiologic salt solution that was maintained at 37°C, pH approximately 7.4, and bubbled continuously with a mixture of 95% O₂ and 5% CO₂. The tension was recorded continuously by an isometric force transducer. After stabilization of the tone, the vessels were contracted twice with 60 mmol/L KCl for 30 minutes to enhance reproducibility of responses. The second response to KCl was used as the reference contraction in the final calculations. After 1 hour equilibration in physiologic salt solution, contractile responses to the 1-adrenergic agonist phenylephrine (10⁻¹⁰ to 10⁻⁵ mmol/L), serotonin (10⁻¹⁰ to 10⁻⁵ mmol/L), and the thromboxane agonist (U46619; 10⁻¹⁰ to 10⁻⁶ mmol/L) were assessed. In addition, relaxant responses to the endothelium-dependent vasorelaxant acetylcholine (10⁻¹⁰ to 10⁻⁵ mmol/L), the endothelium-independent vasorelaxant sodium-nitroprusside (10⁻⁶ to 10⁻⁵ mmol/L) and the β-adrenoceptor agonist isoproterenol (10⁻¹⁰ to 10⁻⁵ mmol/L) were evaluated after precontraction of the vessels with phenylephrine (10⁻⁷ to 10⁻⁶ mmol/L) to produce matching contractions in the study groups. After each agent tested, the vessels were washed with Krebs solution and left to recover for ≥30 minutes until they returned to their basal passive tension.

Data analysis
Vascular tension was recorded continuously by an isometric transducer and then analyzed with Power laboratory data acquisition and playback software (DataQ Instruments, Inc, Akron, OH). Data were expressed as the mean ± SEM. Concentration-response curves to the agents that were tested were constructed. The maximal effect, area under the curve and log IC₅₀ (dose at which 50% of the effect is achieved; measure of sensitivity) were calculated and used for statistical analysis. The data analysis was performed with 1-way analysis of variance followed by Neuman-Keuls multiple comparisons test. A probability value of

**FIGURE 2**
Serotonin (5-HT) concentration-response curves in the carotid artery of WT-P0, WT-P1, KO-P0, and KO-P1 pregnant mice (n = 5-8/group)

Responses were normalized to 60 mmol/L KCl and used as the reference. The asterisk denotes a probability value of < .05 for KO-P0 vs WT-P0, WT-P1, and KO-P1.


**FIGURE 3**
Thromboxane (U46619) concentration-response curves in the carotid artery of WT-P0, WT-P1, KO-P0, and KO-P1 pregnant mice (n = 5-8/group)

Responses were normalized to 60 mmol/L KCl and used as the reference. The asterisk denotes a probability value of < .05 for WT-P0 vs WT-P1.


**FIGURE 4**
Acetylcholine (Ach) concentration-response curves in the carotid artery of WT-P0, WT-P1, KO-P0, and KO-P1 pregnant mice (n = 5-8/group)

Responses are presented as percent relaxation of phenylephrine contraction. The asterisk denotes a probability value of < .05 for KO-P0 vs WT-P0 and WT-P1.


**FIGURE 5**
Sodium nitroprusside (SNP) concentration-response curves in the carotid artery of WT-P0, WT-P1, KO-P0, and KO-P1 pregnant mice (n = 5-8/group)

Responses are presented as percent relaxation of phenylephrine contraction. The asterisk denotes a probability value of < .05 for KO-P0 vs WT-P0, WT-P1, and KO-P1.

WT-P0, WT-P1, and KO-P1.

notes a probability value of 279.e4 American Journal of Obstetrics

nist (U-46619) was purchased from Cay-

en Chemical (Ann Arbor, MI). Stock solutions of all of the drugs (10⁻² mol/L) were prepared in deionized water and stored at −20°C. The composition of physiologic salt solution was NaCl, 119 mmol/L; KCl, 4.7 mmol/L; NaH₂PO₄, 1.2 mmol/L; NaHCO₃, 25 mmol/L; MgCl₂, 1.2 mmol/L; CaCl₂, 2.5 mmol/L; ethylenediaminetetraacetic acid, 0.026 mmol/L; glucose, 11.5 mmol/L.

Responses are presented as percent relaxation of phenylephrine contraction. The asterisk denotes a probability value of <.05 for KO-P0 vs WT-P0, WT-P1, and KO-P1. Ghulmiyyah. Effect of a previous pregnancy on vascular function in endothelial nitric oxide synthase 3 knockout mice. AJOG 2007.

<.05 was considered statistically significant.

Drugs and solutions

The drugs that were used in the in vitro experiments were acetylcholine hydrochloride, phenylephrine hydrochloride, serotonin hydrochloride, and isoproterenol hydrochloride, which were purchased from Sigma Chemical Company (St. Louis, MO); thromboxane A2 agonist (U-46619) was purchased from Cay-

As expected, the KO-P0 mice had absent relaxation to acetylcholine, which is an effect that persisted in the KO-P1 mice and confirmed the complete dependence of acetylcholine responses on a functional NOS3 (Figure 4).

The vasorelaxations that were induced by sodium nitroprusside, an endothelium-independent vasorelaxant, and isoproterenol, a β-adrenoreceptor agonist, were increased significantly in the KO-P1 mice, compared with KO-P0 mice (Figures 5 and 6, respectively). The dose-response curves for the endothelium-independent vasorelaxant sodium nitroprusside in the KO-P1 mice was shifted significantly to the left, compared with vessels from KO-P0 mice, which indicated an increase in the sensitivity of the carotid arteries from KO-P1 mice, compared with KO-P0 mice (Table 2). In addition, the vasorelaxant response of isoproterenol was increased significantly in the WT-P1 and KO-P1 mice, compared with WT-P0 and KO-P0 mice (Table 2).

TABLE 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sodium nitroprusside</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximal effect</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>WT-P0</td>
<td>99.6 ± 3.3</td>
<td>306.6 ± 12.8</td>
</tr>
<tr>
<td>KO-P0</td>
<td>100.2 ± 2.0</td>
<td>361.9 ± 18.9</td>
</tr>
<tr>
<td>WT-P1</td>
<td>100.8 ± 2.4</td>
<td>351.9 ± 28.5</td>
</tr>
<tr>
<td>KO-P1</td>
<td>101.9 ± 2.3</td>
<td>384.1 ± 17.8</td>
</tr>
</tbody>
</table>

* P < .05 for isoproterenol area under the curve of WT-P1 vs KO-P0 and WT-P0.
† P < .05 for sodium nitroprusside Log IC₅₀ of KO-P1 vs all other groups.

**TABLE 2**

Sodium nitroprusside and isoproterenol in the carotid arteries of WT-P0, KO-P0 and WT-P1, KO-P1 (n = 5-8 mice in each group)

**RESULTS**

**In vitro reactivity of carotid artery**

In the first pregnancy, the responses to phenylephrine were significantly greater in the KO-P0 mice, compared with WT-P0 mice. However, this increased reactivity in the knockout animals was abolished in their second pregnancy (Figure 1). The maximal effect and area under the curve were significantly higher in the KO-P0 mice, compared with KO-P1, WT-P0, and WT-P1 mice (Table 1).

Similarly, responses to serotonin were greater in the KO-P0 mice, compared with KO-P1 pregnant mice. The maximal effect was significantly different between the groups (Table 1). KO-P1 contractile response to serotonin was similar to WT-P0 and WT-P1 mice (Figure 2).

The contractile response to the U46199 was not different between KO-P0 and KO-P1 mice (Table 1). However, the contractile response was decreased significantly in the WT-P1 mice, compared WT-P0 mice (Figure 3).

The carotid arteries from the WT-P0 and WT-P1 mice had a normal response to acetylcholine, which indicated a normal endothelium-dependent relaxation. As expected, the KO-P0 mice had absent relaxation to acetylcholine, which is an effect that persisted in the KO-P1 mice and confirmed the complete dependence of acetylcholine responses on a functional NOS3 (Figure 4).

The vasorelaxations that were induced by sodium nitroprusside, an endothelium-independent vasorelaxant, and isoproterenol, a β-adrenoreceptor agonist, were increased significantly in the KO-P1 mice, compared with KO-P0 mice (Figures 5 and 6, respectively). The dose-response curves for the endothelium-independent vasorelaxant sodium nitroprusside in the KO-P1 mice was shifted significantly to the left, compared with vessels from KO-P0 mice, which indicated an increase in the sensitivity of the carotid arteries from KO-P1 mice, compared with KO-P0 mice (Table 2). In addition, the vasorelaxant response of isoproterenol was increased significantly in the WT-P1 and KO-P1 mice, compared with WT-P0 and KO-P0 mice (Table 2).

**COMMENT**

We found that the contractile and relaxant responses in the wild-type mice did not differ between first and second pregnancy. As would be expected in the absence of NOS3, contractile responses in the knockout animals were exaggerated in the first pregnancy. In contrast, a second pregnancy in knockout animals was associated with normalization of the responses to phenylephrine and serotonin and an increase in the responses to the
endothelium-independent relaxants sodium nitroprusside and isoproterenol.

The findings of this study support the hypothesis that the abnormal vascular function of the NOS3 knockout mice improves with the second pregnancy and becomes comparable with the wild-type mice. A second pregnancy in this animal model of hypertension was associated with a more physiologic vascular adaptation, compared with the first pregnancy. The improvement in in vitro vascular function that was seen here in the second pregnancy is consistent with the more significant decrease in systemic vascular resistance in multiparous vs primiparous women reported by Clapp et al. The beneficial effects of a previous pregnancy on vascular function in subsequent pregnancy may also explain our previously reported improvement in vascular function in offspring of NOS3 knockout mice as parity increases. More importantly, the improvement in vascular function with subsequent pregnancy may underlie the mechanisms that are responsible for the protective effect of a first pregnancy on the risk of preeclampsia in future pregnancies. Understanding these protective mechanisms may shed light on the pathogenesis of preeclampsia and lead to strategies to improve pregnancy outcomes.

The compensatory vascular changes that these mice experience during sequential pregnancies may overcome the altered production of NO because of the NOS3 deficiency and explain the amelioration that was observed. This compensatory mechanism could be due to the upregulation of other NOS isoforms (such as the inducible and the neuronal), which can lead to an increased production of NO in the vasculature of the knockout pregnant mice. A more likely mechanism, however, is a compensatory increase in responses to relaxant agents, including NO, rather than an increased production of NO. The latter possibility is reinforced by our findings of increased responses to sodium nitroprusside, a NO donor, and isoproterenol, a beta adrenergic receptor agonist that is independent of NO.

In the absence of NOS3 expression in the maternal/uterine environment, alternate pathways such as prostacyclins that are produced through the activation of the cyclooxygenase cascade or the recently discovered vasodilator endothelium-derived hyperpolarizing factor may be involved. Another possible mechanism could be the activation of the second messenger pathway cyclic guanosine monophosphate through which NO functions. This molecular pathway should be further investigated.

To assess the other possible pathways, further in vivo and in vitro studies that will use NO, cyclooxygenase, and endothelium-derived hyperpolarizing factor inhibitors should be conducted. In addition, it would be important to repeat these experiments in other vessels such as the mesenteric arteries, which represent a more resist vessel than the carotid artery; to assess the vascular function in pregnancies beyond the second; and to determine whether the improvement in vascular function is persistent. Moreover, it would be interesting to investigate the effect of the interval period between pregnancies on vascular function changes.

REFERENCES
Objective: In utero exposure to repeated doses of antenatal corticosteroids (ACSS) has been shown to reduce fetal growth. Our goal was to evaluate whether weekly betamethasone (R-ACS) alters placental growth and histologic findings.

Study design: In a multicenter randomized controlled trial of R-ACS vs a single course of ACS followed by weekly placebo (S-ACS), placentas were weighed after removal of the membranes and umbilical cord. A single pathologist who was masked to study group and pregnancy outcomes performed histologic evaluation for placental calcifications, infarction, fibrin deposition, and hemorrhage or thrombus formation, acute and chronic chorioamnionitis, fibromuscular vascular hyperplasia, nucleated red blood cells, and villous crowding, edema, fibrosis, or fibrinoid necrosis. Findings were compared between study groups and according to the number of courses of ACS.

Results: One hundred ninety-four placentas were available for evaluation. Univariable analyses revealed no differences between study groups in any of the 19 evaluated histologic parameters between R-ACS and S-ACS groups overall or in analyses that were restricted to deliveries at \(<32\) or \(\geq 32\) weeks of gestation. Calcifications were more common \((P = .045)\) in the R-ACS group after controlling for other factors. Multivariable analysis revealed increasing gestational age at delivery, but not increasing ACS courses, to be associated with decreasing chorionic inflammation, villous edema, and fibrosis and with increasing villus crowding, fibrin deposition, and calcifications. Ninety-three placentas were weighed before formalin fixation. After controlling for delivery gestation and infant gender, placental weight was significantly lower in the R-ACS group \((P = .017)\) and was related inversely to the number of ACS courses \((P = .037)\). This finding was confirmed only for deliveries at \(\geq 32\) weeks of gestation \((525 \text{ vs } 441 \text{ g for R-ACS and S-ACS group, respectively; } P = .036)\).

Conclusion: Repeated antenatal corticosteroid treatments in pregnancy are associated with decreased placental growth in a dose-dependent fashion, but not with evident differences in histologic markers of placental inflammation, ischemia, or infarction. Histologic placental abnormalities should not be attributed to repeated courses of corticosteroids.

Support from the National Institute of Child Health and Human Development Beneficial Effects of Antenatal Repeated Steroids study: impact of repeated doses of antenatal corticosteroids on placental growth and histologic findings

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Antenatal corticosteroid administration is perhaps one of the most effective prenatal interventions for prevention of complications that are related to preterm birth, which include respiratory distress syndrome, intraventricular hemorrhage, and neonatal death.\(^1\)\(^-\)\(^4\) Because the effects of antenatal steroid administration are believed to be limited in duration and because efforts to identify those fetuses that are destined to deliver preterm within a brief period of time have been of limited success, many practitioners in the United States adopted the practice of weekly treatment of women who are at risk for preterm birth. Accumulating evidence suggests that, despite some potential benefits regarding enhanced fetal pulmonary maturation, repeated doses of antenatal steroids have potentially negative effects on fetal growth and adverse effects on brain growth and development in animals and humans.\(^5\)\(^-\)\(^10\)

In animal studies, corticosteroid exposure results in a significant lag in placental growth and alterations in expression of various placental transport proteins (eg, GLUT 1 and GLUT 3) and protein kinase C isoforms that are associated with apoptosis, among others.\(^11\)\(^-\)\(^13\) In human placentas, glucocorticoid exposure has been associated with decreased placental aromatase activity and steroid production, decreased cytotrophoblast expression of cellular adhesion molecules (alpha2 integrin), and decreased amnion epithelial cell expression of extracellular matrix proteins.\(^14\)\(^-\)\(^16\) In retrospective analyses of human placentas that were derived from preterm births, Ghidini et al\(^17\)\(^,\)\(^18\) found increasing antenatal corticosteroid exposure to be associated with villous fibrosis, stromal mineralization, and less frequent villous infarction and that repeated corticosteroid courses were associated with less frequent histologic evidence of placental inflammation. However, prospective evaluations of the impact of antenatal corticosteroid exposure in humans on placental histologic findings are lacking.

We have reported the results of a multicenter randomized clinical trial in which we found repeated antenatal corticosteroid administration to reduce specific neonatal morbidities significantly, including neonatal surfactant administration, mechanical ventilation, CPAP, and pneumothoraces.\(^19\) However, such treatment did not improve the predefined composite neonatal outcome. Although repeated antenatal steroids did not lower mean birthweight or head circumference, such treatment was associated with more frequent birthweight at <10th percentile, and infants exposed to ≥4 courses were significantly smaller.

The purpose of this ancillary study was to evaluate whether repeated antenatal corticosteroid administration alters placental growth and/or histopathologic findings within the context of this randomized clinical trial.

**Materials and Methods**

This is an ancillary study of placental specimens that were collected during the conduct of a randomized, double-masked, placebo-controlled, multicenter clinical trial that evaluated the potential Beneficial Effects of Antenatal Repeated Steroids (BEARS Study) and was performed by 18 centers of the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. The original trial was approved by the institutional review boards at all centers, and informed consent was obtained from all participants. This analysis was approved by the institutional review board of MetroHealth Medical Center, Cleveland, OH. The primary study has been described previously.\(^19\) Briefly, pregnant women with intact membranes between 23 weeks 0 days and 31 weeks 6 days of gestation were eligible between 7 and 10 days after a single full course of betamethasone or dexamethasone, if they remained at high risk for spontaneous preterm birth. Major exclusion criteria were preterm premature rupture of the membranes, confirmed fetal lung maturity, chorioamnionitis, major fetal anomaly, nonreassuring fetal status, systemic corticosteroid use in the current pregnancy, and insulin-dependent diabetes mellitus. Twin pregnancies were excluded from this evaluation. Women were assigned randomly to receive either weekly courses of betamethasone (2 injections of betamethasone 12 mg repeated once in 24 hours) or matching placebo. Initially, patients received courses until 33 weeks 6 days of gestation, unless delivery was sooner. After 67 patients had been enrolled, the number of study courses was limited to 4.

At delivery, the placentas from the centers that were participating in the ancillary study were weighed after removal of the membranes and umbilical cord at the clinical center. The placentas were placed in a solution of 10% formalin and shipped to a central facility for processing, or they were processed at the clinical center and shipped for storage. Only placental weights that were obtained before formalin fixation are included in this analysis. From each placenta, 3 representative samples were cut and embedded in paraffin blocks. These included samples of the fetal and maternal surfaces that were taken from the central portion of the placental disc. Paraffin blocks were processed with standard hematoxylin and eosin staining techniques.

A fellowship-trained and experienced perinatal pathologist (J.S.), who was masked to study group assignment and pregnancy outcomes, performed microscopic evaluation of the slides that were
TABLE 1
Demographic characteristics of participating patients by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single course of antenatal steroids followed by weekly placebo group (n = 93)</th>
<th>Weekly betamethasone group (n = 101)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication for initial course of steroids (%)</td>
<td></td>
<td></td>
<td>---------</td>
</tr>
<tr>
<td>Previous spontaneous preterm birth</td>
<td>57.0</td>
<td>60.4</td>
<td>.630</td>
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<tr>
<td>Preterm labor</td>
<td>74.2</td>
<td>68.3</td>
<td>.367</td>
</tr>
<tr>
<td>Cervical cerclage</td>
<td>20.4</td>
<td>15.8</td>
<td>.406</td>
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<tr>
<td>Placenta previa or abruption</td>
<td>8.6</td>
<td>14.9</td>
<td>.179</td>
</tr>
<tr>
<td>Weeks of gestation at randomization</td>
<td>28.2 ± 2.3</td>
<td>28.0 ± 2.4</td>
<td>.620</td>
</tr>
<tr>
<td>Maternal age (y)*</td>
<td>25.7 ± 5.8</td>
<td>25.8 ± 5.8</td>
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<tr>
<td>Predominant race (%)</td>
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<tr>
<td>African American</td>
<td>40.9</td>
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<td>.833</td>
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<tr>
<td>White</td>
<td>34.4</td>
<td>36.6</td>
<td></td>
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<tr>
<td>Other</td>
<td>24.7</td>
<td>26.7</td>
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</tr>
<tr>
<td>Marital status (%)</td>
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<tr>
<td>Married</td>
<td>61.3</td>
<td>58.4</td>
<td>.155</td>
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<tr>
<td>Divorced</td>
<td>6.5</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>32.3</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>Total years of school*</td>
<td>12.1 ± 2.7</td>
<td>11.8 ± 2.5</td>
<td>.281</td>
</tr>
<tr>
<td>Smoked during this pregnancy (%)</td>
<td>24.7</td>
<td>27.7</td>
<td>.636</td>
</tr>
<tr>
<td>Nulliparity (%)</td>
<td>9.7</td>
<td>9.9</td>
<td>.958</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>26.4 ± 7.0</td>
<td>25.9 ± 6.4</td>
<td>.709</td>
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<tr>
<td>Maternal genital tract infection (%)†</td>
<td>19.4</td>
<td>25.7</td>
<td>.289</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
† Includes a history of Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, herpes simplex virus, syphilis, or bacterial vaginosis.

derived from each placenta at ×10 and ×40 magnification and across multiple microscopic fields. Because placental findings that are evident on routine histologic examination have not been well-defined in relation to antenatal corticosteroid exposure, we elected to evaluate a broad range of commonly assessed characteristics and criteria that have been associated previously with placental maturation, inflammation, infection, ischemia, infarction, and fetal hypoxia.20-25 Each of these characteristics is the subject of basic training in histopathologic evaluation and is evaluated routinely in clinical practice. For each placenta, a determination was made regarding the presence or absence of the following characteristics: villous crowding or prominent syncytiot knots, placental infarction, placental thrombus, villous edema (hydrops placentalis), villous fibrosis, placental villitis (acute/chronic), villous fibrinoid necrosis, fibrin hyperplasia of stem vili blood vessels, nucleated red blood cells, fibrin deposition (subchorionic/intervillous/predecidual), maternal floor infarction, calcifications, abruption (acute/chronic), chorionic plate inflammation (chorioamnionitis), and intraplacental hemorrhage. For those characteristics that occur as a spectrum, the findings were graded (none = 0; grade 1 [<10%] = 1; grade 2 [10%-50%] = 2; grade 3 [>50%] = 3). Because placental membranes were excised before transportation, these were not available for evaluation.

Histopathologic findings were categorized as present or absent and were compared between study groups and according to number of courses of antenatal corticosteroids. Statistical analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC). Categoric variables were compared with the use of the chi-square or Fisher’s exact tests, where appropriate. Continuous variables were compared with the use of the Wilcoxon rank-sum test or Kruskal Wallis test for continuous data. Multivariable logistic regression analyses included gestational age at delivery, infant gender, and study group assignment or total number of courses of antenatal corticosteroids administered, as independent variables. Nominal 2-sided probability values are reported with no adjustments made for multiple comparisons. A probability value of <.05 was considered statistically significant.
RESULTS

A total of 194 placental specimens that met our inclusion criteria were obtained from 14 of 18 participating centers. Clinical characteristics were similar between the placebo and repeated corticosteroid groups (Table 1). Pregnancy outcomes among the two study groups are delineated in Table 2. Latency from randomization to delivery, number of study drug courses, gestational age at delivery, and infant birthweights were similar between groups. Both study groups had a high incidence of preterm birth at <37 weeks of gestation and early preterm birth at <32 weeks of gestation.

Regarding histologic findings, univariable analysis revealed no significant differences between the repeated corticosteroid and placebo groups for the presence of any of the evaluated markers of placental maturation, infarction, infection, abruptio, or necrosis or the presence of nucleated red blood cells (Table 3). Similar findings were seen for those deliveries at <32 weeks of gestation and those deliveries thereafter. Multivariable analysis revealed placental calcifications to be more common in the repeated steroids group ($P = .045$) but not with an increasing number of steroid courses ($P = .174$). Increasing gestational age at delivery was associated with decreasing chorionic inflammation ($P = .008$), villous edema ($P = .009$), and fibrinosis ($P = .006$) and with increasing villus crowding ($P = .003$), fibrin deposition ($P = .025$), and calcifications ($P = .008$) being controlling for infant gender and number of steroid courses. However, an increasing number of corticosteroid courses was not associated significantly with any of these placental findings.

Placental weights were obtained before formalin fixation for 93 patients. Infant birthweights and placental weights were similar between the placebo and active groups for this subset (Table 4). However, mean placental weights were 13.0% smaller in the repeated corticosteroid group, and birthweights were 9.0% smaller. Because differences in fetal and placental growth with steroid exposure could be anticipated to take time to become evident after exposure, we performed subgroup analysis for those deliveries that were remote from (<32 weeks of gestation) and near or at term (≥32 weeks of gestation). Among the deliveries ≥32 weeks of gestation (n = 69; betamethasone group, 38; placebo group, 31), the median number of study courses was 4 for both the repeated steroid group and the placebo group. Within this subset, the repeated steroid group had significantly smaller placentas than the placebo group (441 ± 142 g vs 525 ± 162 g; $P = .036$), which was a 16.1% discrepancy between groups. Similarly, birthweights in the repeated steroid group were 14.3% smaller for those who delivered ≥32 weeks of gestation (2410 vs 2811 g; $P = .011$). Although significant differences in placental weight and birthweight were not identified among those deliveries at <32 weeks of gestation (n = 24), placental weights were 13.1% smaller; birthweights were only 2.9% smaller in the repeated steroids group. Placental weight and birthweight were correlated highly overall and within both subgroups (correlation coefficient, 0.77-0.82; $P < .0001$ for each). Multivariable analysis controlling for gestational age at delivery and infant sex confirmed the repeated steroid group to

### Table 2

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Weekly placebo group (n = 93)</th>
<th>Weekly betamethasone group (n = 101)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study courses of steroids/placebo (median)</td>
<td>4.0</td>
<td></td>
<td>.798</td>
</tr>
<tr>
<td>Study courses received (n)</td>
<td></td>
<td></td>
<td>.695</td>
</tr>
<tr>
<td>$1$</td>
<td>9 (9.7%)</td>
<td>8 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>$2$</td>
<td>10 (10.8%)</td>
<td>7 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>$3$</td>
<td>13 (14.0%)</td>
<td>18 (17.8%)</td>
<td></td>
</tr>
<tr>
<td>$≥4$</td>
<td>61 (65.6%)</td>
<td>68 (67.3%)</td>
<td></td>
</tr>
<tr>
<td>Latency to delivery (d)*</td>
<td>47.9 ± 26.4</td>
<td>49.2 ± 28.4</td>
<td>.862</td>
</tr>
<tr>
<td>Gestation at delivery (wks)*</td>
<td>35.0 ± 4.2</td>
<td>35.0 ± 3.8</td>
<td>.924</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>2457.7 ± 858.5</td>
<td>2351.3 ± 769.8</td>
<td>.354</td>
</tr>
<tr>
<td>Preterm premature rupture of membranes (%)</td>
<td>15.1</td>
<td></td>
<td>.969</td>
</tr>
<tr>
<td>Preterm delivery &lt;37 wks (%)</td>
<td>60.2</td>
<td></td>
<td>.693</td>
</tr>
<tr>
<td>Preterm delivery &lt;32 wks (%)</td>
<td>20.4</td>
<td></td>
<td>.913</td>
</tr>
<tr>
<td>Clinical chorioamnionitis (%)</td>
<td>3.2</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Preeclampsia/gestational hypertension (%)</td>
<td>1.1</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Cesarean delivery (%)</td>
<td>29.0</td>
<td></td>
<td>.688</td>
</tr>
<tr>
<td>Postpartum endometritis (%)</td>
<td>1.1</td>
<td></td>
<td>.622</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD.
be associated with lower placental weight ($P = .017$). Similarly, placental weight was related inversely to the number of courses of antenatal steroids after controlling for delivery gestation and infant sex ($P = .037$).

**COMMENT**

In this masked evaluation of placental specimens that were collected during a randomized placebo-controlled study, we found repeated doses of antenatal corticosteroids to lead to smaller placentas but no significant pathologic histologic findings. The lack of impact on placental histologic findings was evident for those deliveries at <32 weeks of gestation and for those deliveries thereafter. Placental weight was reduced significantly in the repeated steroid group after controlling for potentially confounding factors (delivery gestation, infant sex), and was related inversely to the number of steroid courses that were received. After controlling for other factors, the only histologic finding that was associated significantly with repeated antenatal corticosteroids was the more common occurrence of placental calcifications. However, no dose-dependent relationship was identified. Increasing gestational age at delivery, but not increasing number of antenatal corticosteroid courses, was associated with decreasing chorioamnionitis, villous edema, and fibrosis and with increasing villus crowding, fibrin deposition, and calcifications.

Placental calcifications are common and nonspecific findings that are associated with placental maturation that may be present before term or absent at term.
in uncomplicated pregnancies. It is plausible that this finding could result from accelerated placental maturation because antenatal corticosteroids act on the fetus and placenta to induce biochemical changes at the expense of decreased placental growth.11-16 Although placental calcifications have also been associated with pathologic states (eg, fetal infections and remote placental infarction), we believe it unlikely that this finding is of pathologic significance, given the absence of any other findings that suggest increased placental ischemia, infarction, thrombosis, or inflammation and particularly given the high prevalence of placental calcifications in both study groups. The common occurrence of this finding in both the corticosteroid and placebo groups (51% vs 37%) and the lack of a dose-dependent effect of antenatal steroids highlight the nonspecific nature of placental calcifications, that only a small fraction of cases with calcifications could be attributed to corticosteroid treatment, or that this finding could represent a type I error. It has been speculated that antenatal corticosteroids might predispose mothers and infants to infection as a result of their immunosuppressive effects. However, we found no increase in histologic evidence of chorioamnionitis or villitis that would suggest such a predisposition. In a retrospective analysis of placenta from women who delivered at 22-32 weeks of gestation, Ghidini et al17 found increased antenatal corticosteroid exposure to be related to increased severity of villous fibrosis and stromal mineralization and to fewer villous infarcts. Despite the larger number of subjects in our cohort who were exposed to ≥ 4 course of antenatal steroids, we were unable to confirm this finding. It is possible that the more advanced gestational age at delivery in our cohort allowed time for resolution of differences between groups after discontinuation of steroid treatment, but it is unlikely that evidence of histologic infarction and fibrosis would resolve over time. In a separate study of repeated antenatal steroids after preterm premature rupture of membranes, Ghidini et al18 found less frequent histologic amnionitis among those women who were exposed to multiple courses. We recognize that our analysis has less ability to confirm this association by virtue of the lack of availability of fetal membranes in many cases. Further, the incidence of chorionamnionitis is explicitly less frequent in our study, because the focus in the study of Ghidini et al was on women with premature rupture of membranes and preterm birth. However, it is also plausible that those women who remained pregnant long enough after premature rupture of membranes to receive multiple courses of antenatal steroids in the study of Ghidini et al were less likely to have intrauterine infection than were the women who delivered soon after premature rupture of membranes, regardless of steroid treatment.

Repeated steroid administration has been shown to delay fetal and neonatal growth in animals and humans.5,6,9-11,19 Similarly, animal studies have suggested antenatal corticosteroid administration to result in apoptosis and delayed placental growth.11-13 Our study confirms that repeated antenatal corticosteroids delay placental growth among women who deliver long enough after initial steroid exposure to allow manifestation of this finding. Although not statistically significant, the discrepancy in placental weights between control and repeated steroid groups for those deliveries at <32 weeks of gestation appears greater than that seen for newborn infant weight. We speculate that the negative impact on placental growth might occur before such findings become apparent in the fetus. However, we are cognizant of the small number of patients who deliver at <32 weeks of gestation and of the need for further evaluation of this hypothesis.

One of this study’s limitations is that placental weights before formalin fixation were available only for a fraction of participants. Although this resulted predominantly from differing processing practices at individual participating clinical centers, it remains plausible that there was a bias in which placentas were more likely to be weighed for smaller infants. Our evaluation is limited by the lack of bio-

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Weekly placebo group (n = 44)*</th>
<th>Weekly betamethasone group (n = 49)*</th>
<th>Discrepancy between groups (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks)</td>
<td>34.3 ± 4.5</td>
<td>34.2 ± 3.9</td>
<td></td>
<td>.854</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2366.4 ± 896.6</td>
<td>2153.3 ± 698.8</td>
<td>9.0</td>
<td>.239</td>
</tr>
<tr>
<td>Birthweight at &lt;32 weeks of gestation</td>
<td>1306.2 ± 391.8</td>
<td>1266.1 ± 247.3</td>
<td>2.9</td>
<td>.772</td>
</tr>
<tr>
<td>Birthweight at ≥32 weeks of gestation</td>
<td>2811.1 ± 632.1</td>
<td>2409.5 ± 562.7</td>
<td>14.3</td>
<td>.011</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>464.6 ± 171.2</td>
<td>404.3 ± 147.5</td>
<td>13.0</td>
<td>.104</td>
</tr>
<tr>
<td>Placental weight at &lt;32 weeks of gestation (n = 24)</td>
<td>320.9 ± 85.9</td>
<td>278.9 ± 84.4</td>
<td>13.1</td>
<td>.310</td>
</tr>
<tr>
<td>Placental weight at ≥32 weeks of gestation (n = 69)</td>
<td>524.9 ± 162.3</td>
<td>440.6 ± 142.3</td>
<td>16.1</td>
<td>.036</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
chemical evaluations and other potential ancillary tests that might have further elucidated the impact of repeated corticosteroid administration on placental function. Histopathologic evaluation is a subjective interpretation and previous studies have shown limited agreement between pathologists. We elected to evaluate common histologic characteristics that are assessed routinely by pathologists, are part of routine training and experience, and can be anticipated to be evaluated consistently by any given pathologist. Although the use of multiple observers can sometimes enhance study results, we do not believe that the addition of 1 or 2 pathologists would alter our findings significantly. Finally, it is possible that we were unable to identify subtle differences for several of the uncommon findings. This study is powered adequately to identify a 2-fold increase in histologic findings with a prevalence of \( \geq 20\% \) or a 1.5-fold increase in findings with a prevalence of \( \geq 40\% \) (alpha = .05; beta = .20). However, for characteristics with a 1%-2% incidence, this study has adequate power to evaluate only large increases (6-12-fold increases), and many thousands would need to be studied to evaluate characteristics that were less common with repeated corticosteroid exposure.

This study provides new information that repeated antenatal corticosteroid doses do not appear to impact routinely assessed histologic placental features that are related to placentational maturation, infarction, infection, abruption, necrosis, or fetal hypoxia and appears to have a negative impact on placental growth. Because of this, placental abnormalities that are identified on routine histologic evaluation after exposure to repeated courses of antenatal corticosteroids should not be attributed to such treatment.

REFERENCES
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Betamethasone vs dexamethasone for the prevention of morbidity in very-low-birthweight neonates

Deborah M. Feldman, MD; Jeannine Carbone, MD; Laura Belden, BS; Adam F. Borgida, MD; Victor Herson, MD

OBJECTIVE: The purpose of this study was to compare neonatal outcomes in very-low-birthweight infants who were exposed to antenatal betamethasone vs dexamethasone.

STUDY DESIGN: We reviewed all inborn very-low-birthweight infants from January 1997 through February 2006. Maternal medical records were reviewed to determine the type of antenatal steroids that each patient received; neonatal outcomes were compared using chi-square and Student t tests.

RESULTS: There were 334 very-low-birthweight infants who met the criteria for evaluation: 186 infants received betamethasone, and 148 infants received dexamethasone. There were no differences in race, gestational age at delivery, or mean birthweight between the 2 groups. There were significantly lower rates of respiratory distress syndrome and bronchopulmonary dysplasia in the betamethasone group, compared with the dexamethasone group. Other neonatal outcomes were similar in both groups.

CONCLUSION: Antenatal betamethasone was associated with a significantly lower rate of pulmonary complications caused by prematurity, when compared with dexamethasone.

Key words: betamethasone, dexamethasone, neonatal outcome, prematurity

Since the introduction of antenatal corticosteroids to accelerate fetal lung maturity by Liggins and Howie in 1972,1 several studies have confirmed the protective effects that are offered by such intervention.2,3 The use of antenatal corticosteroids reduces the risk of neonatal pulmonary morbidity, intraventricular hemorrhage (IVH), necrotizing enterocolitis, and neonatal death. Betamethasone and dexamethasone are the most widely used corticosteroids for acceleration of fetal maturation because they both readily cross the placenta and have similar biologic activities. Based on a consensus panel of the National Institutes of Health in 1994,4 the American College of Obstetricians and Gynecologists has recommended that pregnant women between 24 and 34 weeks of gestation who are at risk for preterm delivery within 7 days receive a single course of betamethasone (12 mg) intramuscularly every 24 hours for 2 doses or dexamethasone (6 mg) intramuscularly every 12 hours for 4 doses.6

Because of the longer half-life, the administration of a course of betamethasone requires only 2 injections 24 hours apart, as opposed to the 4 injections recommended for a course of dexamethasone. Although there has been some data that have compared the efficacy of the 2 agents, the results have been conflicting. Both agents have been shown in meta-analyses of randomized trials to reduce the risk of respiratory distress syndrome (RDS) and other morbidities; however, only betamethasone has been shown to decrease neonatal mortality rates. To date, there has been no recommendation to use 1 agent over the other.

Although there is little question as to the beneficial effects of antenatal corticosteroid therapy for fetal maturation, the optimal corticosteroid preparation still remains unanswered. Our institution typically uses betamethasone as first-line therapy for this indication; however, the preparation was unavailable to our pharmacy for approximately 3.5 years (October 2001-April 2005). During this time, only dexamethasone was available for antenatal use. Our objective in the current study was to evaluate the morbidity and mortality rates of very-low-birthweight (VLBW) infants who were born during a 6-year period, including the period when only dexamethasone was available, and to compare the outcomes between those neonates who were exposed to betamethasone vs dexamethasone.

MATERIALS AND METHODS

We performed a retrospective cohort study of all VLBW neonates (≤1500 g) who were born at our institution from January 1997 through February 2006. Maternal medical records were reviewed for exposure and type of antenatal steroids. Patients were excluded for the following reasons: no steroid exposure, multiple steroid courses, fetal congenital or chromosomal abnormality, gesta-
tional age <23 weeks, birthweight <500 g, and/or incomplete medical records.

Maternal demographics, pregnancy complications, gestational age at delivery, and neonatal outcomes were compared. Our primary outcome was the overall incidence of neonatal IVH as defined by intracranial ultrasound evaluation during the neonatal intensive care unit (NICU) admission. Respiratory morbidity was compared by evaluating the rates of RDS and bronchopulmonary dysplasia (BPD) and the number of days on a ventilator. RDS was defined as clinical findings of respiratory distress and chest radiographic findings of reticulogranular appearance of the lungs. BPD was defined as respiratory symptoms, compatible chest radiograph findings and oxygen requirement at 28 days after delivery. Other secondary outcomes included the rates of severe (grade 3 or 4) IVH, periventricular leukomalacia, RDS, BPD, necrotizing enterocolitis, retinopathy of prematurity, patent ductus arteriosus ligation, and neonatal death. The NICU length of stay was also evaluated in each group.

Data were analyzed with descriptive statistics, analysis of variance, and the Student t test for continuous variables; chi-square test with Fisher exact test was used, where appropriate, for categoric variables and multivariate regression analysis. A probability value of <.05 was considered significant. Based on previously published data, we assumed an overall rate of IVH of 20% in this population. To detect a 30% reduction in the rate of IVH among neonates who were exposed to betamethasone compared with dexamethasone, a power analysis revealed that we would need a sample size of 220 patients (110 in each group), with a 2-tailed \( \alpha \) of 5% and a power of 80%. Statistical analysis was performed with Statistical Analysis System software (version 8.2; SAS Institute, Cary, NC) and JMP software (version 4; SAS Institute). The study was approved by our institutional review board.

**Results**

During the study period, there were 614 VLBW infants who were admitted to our NICU. Of these, 35 patients were excluded because of gestational age <23 weeks or birthweight <500 g. Four infants were excluded for congenital or chromosomal abnormalities. Forty-eight patients received >1 course of steroids, and 3 patients received both dexamethasone and betamethasone, who were excluded from analysis. Eighty-eight patients did not receive steroids and were not analyzed in this study; 102 patients were excluded for incomplete maternal records. After exclusions, 334 neonates from 190 pregnancies were left for analysis; 186 of these neonates had been exposed to betamethasone, and 148 the neonates had been exposed to dexamethasone.

Maternal characteristics and birthweight for both groups are depicted in Table 1. Patients who received betamethasone were slightly younger than the dexamethasone group (28.6 ± 6.8 years vs 30.4 ± 6.7 years; \( P = .03 \)). The maternal age was similar between the 2 groups, as was gestational age at delivery.

There was a similar number of multiple gestations between the groups (22.6% in the dexamethasone group vs 30.4% in the betamethasone group; \( P = .13 \)). Overall, there was a higher rate of cesarean delivery in the dexamethasone group (80.4% vs 62.9%; \( P < .001 \)). The mean birthweight was slightly higher in the dexamethasone group (1098 ± 247 g vs 1048 ± 279 g); however, the difference did not meet statistical significance (\( P = .09 \)).

Pregnancy complications in both groups are depicted in the Figure. The incidence of preterm premature rupture of membranes was higher in the betamethasone group (39.7% vs 46.3%; \( P = .007 \)); the rate of fetal growth restriction was lower in this group (14.2% vs 26.9%; \( P = .007 \)). Rates of other pregnancy complications were similar in both groups, and the number of patients who underwent tocolysis with magnesium sulfate was also similar in each group (44.6% vs 41.2%; \( P = .53 \)). Multivariate regression was used to evaluate the effect of multiple gestation, cesarean delivery, preterm premature rupture of membranes, intrauterine growth restriction, and type of steroid on the rates of RDS and BPD. Of the variables tested, only intrauterine growth restriction and type of steroid had a significant effect on RDS (\( P < .001 \) and \( P = .001 \), respectively). Only type of steroid had a significant effect on BPD (\( P = .03 \)).

Neonatal outcomes are summarized in Table 2. There were significantly lower rates of RDS and BPD in the betamethasone group, compared with the dexamethasone group. The mean number of
ventilatory days was similar for both groups (5.5 ± 9.1 days vs 6.3 ± 11.6 days; \( P = .50 \)). The rates of all IVH and severe IVH were similar for both groups, although there was a trend toward increased severe IVH in the betamethasone group. Other neonatal outcomes were similar in each group, including the rate of neonatal death and mean NICU length of stay.

**Comment**

Although a large number of controlled, observational studies and metaanalyses have supported the use of antenatal steroids to prevent neonatal complications of prematurity, there is no consensus as to the type of corticosteroid that should be favored.\(^\text{7}\) In 1999, Baud et al\(^\text{8}\) showed a higher rate of cystic periventricular leukomalacia among neonates who were exposed to dexamethasone, as opposed to betamethasone. More recently, Lee et al\(^\text{9}\) compared the use of betamethasone and dexamethasone in VLBW infants and found no significant differences in the rates of IVH or periventricular leukomalacia between the groups. However, they did note a significantly lower risk of neonatal death among those who were exposed to betamethasone, which is a finding that had also been demonstrated previously in a metaanalysis.\(^\text{4}\)

Both animal\(^\text{10,11}\) and human studies\(^\text{12}\) have suggested that dexamethasone may be associated with a higher risk of neonatal neurologic morbidity, when compared with betamethasone, although these data are also conflicting.\(^\text{13}\) In addition, there are pediatric data that implicate a higher rate of neurologic morbidity with the neonatal use of dexamethasone to decrease BPD; however, these studies did not evaluate the use of antenatal steroids.\(^\text{14}\)

Few previous published studies have compared betamethasone directly with dexamethasone for antenatal use. A recently published randomized trial that compared betamethasone and dexamethasone in 299 women showed no difference in respiratory morbidity between the 2 groups.\(^\text{15}\) Interestingly, this study demonstrated a lower rate of IVH in the dexamethasone group, which is contradictory to previous published literature and suggests a higher rate of IVH with dexamethasone.\(^\text{8,9}\) Although our data showed a nonsignificant trend toward a higher rate of grade 3 and 4 IVH in the betamethasone group, the incidence was low in both groups.

The most pronounced finding in our study is the lower rate of RDS and BPD among neonates who were exposed to betamethasone. Although most studies have shown a lower rate of respiratory complications with the use of either corticosteroid when compared with placebo, no studies have demonstrated such a benefit to using betamethasone in reducing RDS, the most common neonatal morbidity.

Our study was limited by its retrospective design and associated known and unknown confounding variables. We attempted to address some of these using multivariate analysis; however, there were still factors for which we were unable to control. For example, we were unable to compare rates of chorioamnionitis between the groups because this information was difficult to discern in the data that we reviewed. Our rate of preterm premature rupture of membranes was higher in the betamethasone group, which may correlate to a higher rate of chorioamnionitis. Given the association between chorioamnionitis and neurologic morbidity, this may explain the reason that the rate of severe IVH was higher in the betamethasone group. In addition, the number of multiple gestations was too small to make any valid conclusions as a separate analysis; when the multiples were excluded from the data, the results were not different than in the entire cohort.

Betamethasone has a longer half-life than dexamethasone, and the interval from treatment to delivery may have impacted efficacy of the steroid. Because of limitations of our retrospective design, we were not able to address this in the study adequately. In addition, our power analysis was based on previously published data that showed a higher rate of IVH than we found in our population of neonates, which suggests that perhaps we would need to evaluate more patients to detect a statistical difference in IVH between the groups. Finally, our data are limited to short-term outcomes in the neonatal period. Therefore, we cannot ascertain the long-term effects of neurodevelopment or respiratory complications in childhood.

The current study provides evidence that betamethasone may be the preferred corticosteroid for pregnant women who are at risk for premature delivery, because it was associated with significantly lower rates of both RDS and BPD in this retrospective cohort. Although the study design is somewhat limiting, data from

**TABLE 2**

Neonatal morbidities

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Betamethasone (n = 186)</th>
<th>Dexamethasone (n = 148)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDS (%)</td>
<td>62.9</td>
<td>81.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (%)</td>
<td>43.0</td>
<td>54.7</td>
<td>.03</td>
</tr>
<tr>
<td>IVH (%)</td>
<td>8.6</td>
<td>6.1</td>
<td>.38</td>
</tr>
<tr>
<td>Grade 3 or 4 IVH (%)</td>
<td>4.8</td>
<td>1.4</td>
<td>.08</td>
</tr>
<tr>
<td>Periventricular leukomalacia (%)</td>
<td>1.2</td>
<td>1.6</td>
<td>.63</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (%)</td>
<td>7.5</td>
<td>7.4</td>
<td>.97</td>
</tr>
<tr>
<td>Retinopathy of prematurity (%)</td>
<td>22</td>
<td>15.5</td>
<td>.13</td>
</tr>
<tr>
<td>Persistent ductus arteriosus ligation (%)</td>
<td>3.8</td>
<td>2.0</td>
<td>.36</td>
</tr>
<tr>
<td>Length of stay (d)*</td>
<td>62 ± 34.7</td>
<td>57 ± 29.4</td>
<td>.20</td>
</tr>
<tr>
<td>Neonatal death (%)</td>
<td>5.4</td>
<td>4.7</td>
<td>.79</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
this study and other studies that support the use of betamethasone over dexamethasone justify further randomized trials that will compare them.

REFERENCES
OBJECTIVE: Nucleated red blood cells (NRBC) are fetal hematologic
markers for placental dysfunction, hypoxemia, and asphyxia. NRBC
count elevation at birth or persistence is linked statistically to adverse
outcome, but clinical predictive value is variable. We studied novel
count elevation at birth or persistence is linked statistically to adverse
markers for placental dysfunction, hypoxemia, and asphyxia. NRBC
count parameters were related to major morbidity (broncho-
ciliation enterocolitis included) and neonatal death (NND).

RESULTS: Twenty-two of 176 patients (12.5%) had acidosis. Complica-
tions included bronchopulmonary dysplasia (n = 36; 20.5%), intra-
ventricular hemorrhage (n = 18; 10.2%), necrotizing enterocolitis (n
= 18; 10.2%), NND (n = 18; 10.2%). NRBC-AUC and NRBC-mean
correlated most strongly with pH, birthweight, and gestational age
(Pearson coefficient, r = -0.45 to -0.18; all P < .001). NRBC-AUC var-
ied most between nonmorbid and morbid; NRBC-mean varied most
between survivors and NND (all P < .001). NRBC persistence strongly
predicted NND: clearance by day 4 was achieved by 80% of survivors
and only 35% of NNDs. Logistic regression identified prematurity and
NRBC counts as primary morbidity determinants (r² = 0.56; P < .01).
Although the importance of individual NRBC counts varied,
day-4 NRBC counts of >70 predicted morbidity best (sensitivity, 82%;
specificity, 96%). Presence of morbidity and birthweight were prime
determinants of death (r² = 0.42; P < .01).

CONCLUSION: Simple daily NRBC counts provide clinical information
that is equivalent to more complicated methods. The importance of
prematurity and growth are emphasized, but elevated NRBC counts
beyond day 3 are relevant independent predictors of adverse outcome.

Key words: fetus, growth restriction, nucleated red blood cells,
perinatal outcome

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T
e the identification of prognostic
markers for adverse perinatal out-
come is a major focus of modern fetal
and neonatal medicine. Among the
markers that have been studied, those
that reflect compromised metabolic sta-
tus during the transition from fetal to
neonatal life have received special atten-
tion. Obstetric intervention and neonatal
resuscitation in this important period
are especially critical in the management
of fetal growth restriction. The perinatal
and long-term liabilities of fetal growth
restriction are due to the combination of
adverse intrauterine environment, peripertum events, and postdelivery
complications that are manifested in
many organ systems.1-4 In this context,
fetal hematologic responses have re-
ceived great interest because they reflect
the chronic nature of the fetal condition
and the acutely superimposed distur-
bances in the transition to extraterine
life.5-10

Fetal hypoxemia can trigger erythro-
poietin release that causes stimulation of
red blood cells, both at intramedullary
and extramedullary sites. In the human
fetus, erythropoiesis typically progresses
from erythroid commitment of colony-
forming stem cells to extrusion of nu-
clear material with concomitant reduc-
tion in cell size.11,12 This process yields a
mature red blood cell without a nucleus
that contains the highest concentration
of hemoglobin. Early stages of mature
erythropoiesis typically are confined to
the bone marrow, where capillary fos-
enations limit the passage of large nucle-
ated red blood cell (NRBC) precursors
into the peripheral circulation.13 Con-
versely, extramedullary sites are believed
to have larger capillary fenestrations that
permit the release of large NRBCs. During periods of high extramedullary production, NRBC counts of up to 30/100 white blood cells (WBC) are physiologic at <30 weeks of gestation, although levels of 5-10/100 WBC are normal thereafter. Isolated polycythemia in intrauterine growth-restricted fetuses suggests enhanced intramedullary erythropoiesis; polycythemia with elevated NRBC counts is suggestive of chronic extramedullary hematopoiesis.

The NRBC count in the peripheral circulation of growth-restricted neonates has received special interest as a surrogate marker for the severity and chronicity of fetal acid-base disturbance and a prognostic factor for adverse outcome. Peak NRBC count at delivery and persistence of NRBC count elevation after delivery have been studied, but prognostic value is limited because of their wide variability and the overriding effect of gestational age. Another limitation of such an approach is that it emphasizes 2 isolated time points without accounting for the pattern of NRBC decline. The impacts of disease in the intensive care setting on NRBC counts and the associated prognostic value have been documented in critically ill adults. In these patients, worsening of the clinical condition is associated with increasing NRBC counts. Similarly, growth-restricted neonates may react to adverse events in the neonatal intensive care unit with their large extramedullary potential for NRBC release. Therefore, neonatal NRBC counts may show a rapid drop, sustained elevation, or a delayed decline on the basis of the clinical condition. Evaluating the NRBC response by the initial count at birth and the persistence in days therefore may be inadequate to define this response accurately. This study was designed to quantify the NRBC response with more comprehensive parameters. It was our hypothesis that these parameters describe the NRBC response more accurately and provide an accurate prediction of outcome in preterm growth-restricted neonates.

**Patients and Methods**

We performed a prospective observational study at 2 tertiary referral centers for fetal medicine from 2000-2005. Patients carrying a fetus with a sonographically measured abdominal circumference of ≤5th percentile for gestational age and an elevated umbilical artery Doppler index as evidence of placental dysfunction were asked to participate. The study was approved by the institutional review boards, and all patients gave informed written consent at enrollment. Only patients with accurate gestational dating were enrolled. The final analysis was restricted to neonates delivered at <34.0 weeks of gestation. Exclusion criteria were maternal diabetes mellitus, fetal anomalies, abnormal karyotype, chorioamnionitis, and twin gestation.

Birthweight, gestational age at delivery, Apgar scores at 1 and 5 minutes, and arterial cord blood gas were recorded. A peripheral blood sample from the neonate was obtained in an EDTA-tube within 2 hours of delivery. A manual count of NRBCs per 100 WBC after Pappenheim staining of the blood smear was performed in the hospital laboratory; this constituted the delivery NRBC count. Peripheral blood samples were analyzed daily until day 7 of life.

Several NRBC parameters were ascertained for the final analysis. These included the first NRBC count (y1) on the day of delivery (x1), persistence of NRBC count elevation, the percentage of days with NRBC count elevation corrected for days alive, the mean NRBC count in the first week of life, the area under the curve (AUC) for the pattern of NRBC decline and the calculated slope of the NRBC count decline. Persistence was defined as the number of days the NRBC count was >30/100 WBC, with y2 being the first value below 30 and x2 being the day of life that this occurred. For neonates with several daily blood draws, mean NRBC count for each individual day was used in the analysis. In addition to the persistence in days, the percentage of days with elevated NRBC count was calculated for the first week. This was done to account for early neonatal deaths. For example, if a neonate had elevated NRBC counts for 2 days but died on the second day of life, then the persistence would be only 2 days. However, the NRBC count was elevated 100% of the days that the neonate was alive. The AUC was calculated with the trapezoid formula as the average of the daily product of NRBC count times the day of life. The slope was calculated as = y2 - y1/x2 - x1. For neonates who died within the first week of life, the number of days alive was taken as the denominator. The parameters used in the calculation are summarized in Table 1.

On completion of the neonatal course development of bronchopulmonary dysplasia (BPD), severe intraventricular hemorrhage (>3 grade 2, according to Papile et al8), and necrotizing enterocolitis were noted. The diagnosis of BPD was based on radiologic criteria. Intraventricular hemorrhage was diagnosed by cranial ultrasound scan that was performed routinely on postpartum days 2 and 7, or more often if clinically indicated. Presences of any of the neonatal

<table>
<thead>
<tr>
<th>NRBC parameter</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak NRBC count</td>
<td>First NRBC count/100 WBC at birth</td>
</tr>
<tr>
<td>NRBC count persistence</td>
<td>Persistence of NRBC count elevation of &gt;30 NRBC/100 WBC in days</td>
</tr>
<tr>
<td>Percentage NRBC count elevation</td>
<td>No. of days with NRBC count of &gt;30 NRBC/100 WBC/ days alive during week 1</td>
</tr>
<tr>
<td>Mean NRBC count</td>
<td>Sum of daily NRBC counts/7</td>
</tr>
<tr>
<td>NRBC AUC</td>
<td>Sum (NRBC day 1-7 × day 1-7)/7</td>
</tr>
<tr>
<td>NRBC slope of decline</td>
<td>(First NRBC count &lt;30/100 WBC − NRBC count at birth)/days of NRBC count of &gt;30/100 WBC</td>
</tr>
</tbody>
</table>

**TABLE 1**

Calculation of various NRBC parameters
complications and/or neonatal death were grouped as the composite variable “poor outcome.”

We restricted our study population to very preterm growth-restricted neonates for several reasons. This population is at highest risk for adverse outcomes and is treated in the neonatal intensive care setting. Daily blood counts typically are performed as part of neonatal monitoring and do not constitute a significant deviation from the standard of care. Conversely, term- or near-term growth-restricted neonates are at relatively low risk for major morbidities and typically are treated in the nursery. Examination of this subset of patients was not at the core of our research question, and the institution of daily blood draws that would have been nesessitated by study inclusion appeared unjustified.

The results were analyzed with SPSS software (version 10.0; SPSS Inc, Chicago, IL). Distribution of gestational age, birthweight, cord artery blood gases, and NRBC parameters were related to individual complications and neonatal death with the use of the Mann-Whitney U test after the results failed tests of normality. Proportional distribution of Apgar score <7 at 5 minutes and cord artery pH <7.20 were also related to these outcome variables with the use of the chi-square test. Parameters significantly different among neonates with individual adverse outcomes were selected for further analysis by logistic regression. In this regression analysis, the individual outcomes were selected as dependent variables. A probability value of <.05 was considered statistically significant.

**Results**

A total of 186 patients agreed to participate in the study. Following 10 stillbirths, 176 neonates remained for final analysis. Placental dysfunction was documented in all cases by abnormal umbilical artery Doppler findings in the form of Doppler index elevation with preserved end-diastolic velocity (n = 98; 55.7%), absent (n = 27; 15.3%), or reversed end-diastolic velocity (n = 51; 29%). One hundred sixty-four (93.2%) fetuses received a complete course of betamethasone before delivery. In the remaining patients, the timing of the delivery decision allowed for only the administration of a single course of steroids.

In this predominantly white population, delivery at a median of 29.6 weeks of gestation was mainly by cesarean section for fetal indications (Table 2). Umbilical cord artery pH was <7.20 in 12.5%, and difficult resuscitation with a low 5-minute Apgar score occurred in 10.8% (Table 2). BPD was the most frequently observed complication (36; 20.5%). Major morbidities were observed in 50 neonates (28.4%). Although the neonatal mortality rate was 10.2% overall, it rose to 26% (13/50) in the presence of major neonatal morbidities (chi-square, P < .001). Five of the neonates who died within the first week of life did not have any of the aforementioned complications but experienced severe cardiorespiratory instability that required high-frequency ventilation and pressor support.

A wide range of distribution in NRBC counts was observed from the day of delivery through the end of the first week of life. Although a decline in NRBC counts after delivery was observed generally, some neonates had a rise reflected in a positive slope (Table 3). All NRBC parameters correlated most strongly with the umbilical artery, pulsatility index, umbilical artery base excess, birthweight, and gestational age at delivery (Table 4). More marked elevations of the Doppler index were associated with higher NRBC parameters; earlier gestational ages, smaller birthweights, and more decline in the base excess were related to an increase in NRBC parameters. These relationships remained significant throughout the first week of life. Overall correlations were strongest for the AUC and mean NRBC count elevation in the first week of life (Table 4). Similarly, an abnormal biophysical profile score, delivery for nonreassuring fetal status, a cord artery pH <7.20, and a 5-minute Apgar score of <7 were all associated with significantly higher daily NRBC counts, longer persistence of NRBC count elevation, and higher calculated NRBC parameters (Mann-Whitney U, all P < .005).

All NRBC parameters were elevated significantly in neonates who experienced BPD and severe intraventricular hemorrhage, who died, and who had composite poor outcome. (Table 5). Necrotizing enterocolitis was the only complication that was not associated consistently with in elevation of NRBC parameters. Differences in the magnitude of NRBC response in neonates with postpartum complications were best illustrated by the AUC (Figure 1). Mean NRBC count was the variable that best separated neonatal survivors from those who died (Table 5; Figure 2). Although >80% of survivors cleared their NRBC count elevation by the fourth day of life, 65% of neonates who died had a persistent elevation beyond this point.

Next, the contribution of NRBC parameters to adverse outcome was analyzed by logistic regression analysis. Despite significantly altered distributions of NRBC parameters with individual complications, none of these were selected by the regression model as independent predictors of adverse outcomes. Overall, gestational age at delivery was the primary determinant for the development of BPD (P = .005; r² = 0.54) and intraventricular hemorrhage (P = .014; r² = 0.21). Birthweight was identified as the primary determinant for necrotizing enterocolitis (P = .05; r² = 0.17). Gestational age and birthweight were significant independent predictors of neonatal death (P < .005; r² = 0.53). Receiver operator curve statistics identified gestational age of 28 weeks and birth weight of 600 g as the best combination of sensitivity and specificity for the prediction of death. Accordingly, a subanalysis was performed to identify the contribution of NRBC parameters to the mortality risk after these cutoffs. This subanalysis showed that, in neonates delivered at <28 completed weeks of gestation with a birthweight of <600 g, the gestational age at delivery remained the primary determinant of death, although the slope of NRBC decline was an independent predictor (P < .05; r² = 0.35). After this gestational age, persistence of NRBC count elevation beyond day 3 of life was the...
TABLE 2
Perinatal characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Mean ± SD (range)</th>
</tr>
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<tbody>
<tr>
<td>Maternal age (y)</td>
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</tr>
<tr>
<td>Maternal race</td>
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</tr>
<tr>
<td>White</td>
<td>127 (72.2)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>49 (27.8)</td>
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</tr>
<tr>
<td>Parity</td>
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<tr>
<td>0</td>
<td>124 (70.5)</td>
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</tr>
<tr>
<td>1</td>
<td>36 (20.5)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13 (7.4)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>29.6 (24.0-33.6)</td>
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</tr>
<tr>
<td>Birthweight (g)</td>
<td>918 (360-1520)</td>
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</tr>
<tr>
<td>Mode of delivery</td>
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</tr>
<tr>
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<tr>
<td>Cesarean section</td>
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<tr>
<td>Delivery indication</td>
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<tr>
<td>Nonreassuring fetal heart rate</td>
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</tr>
<tr>
<td>Nonreassuring Doppler scan</td>
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<tr>
<td>Nonreassuring biophysical profile score of &lt;6</td>
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<tr>
<td>Fetal distress*</td>
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<tr>
<td>Placental abruption</td>
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</tr>
<tr>
<td>Severe preeclampsia/ hemolysis, elevated liver enzymes, and low platelet count syndrome (HELLP)</td>
<td>46 (26.1)</td>
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<tr>
<td>Spontaneous onset of labor</td>
<td>5 (2.9)</td>
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</tr>
<tr>
<td>Other</td>
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<td></td>
</tr>
<tr>
<td>Cord artery blood gas (mm Hg)</td>
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<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.23 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>pO2</td>
<td>16.1 ± 5.5</td>
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</tr>
<tr>
<td>pCO2</td>
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</tr>
<tr>
<td>HCO3</td>
<td>22.3 ± 2.0</td>
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</tr>
<tr>
<td>Base excess</td>
<td>−5.1 ± 2.6</td>
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<tr>
<td>pH &lt; 7.20</td>
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<tr>
<td>5-Minute Apgar &lt;7</td>
<td>19 (10.8)</td>
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</tr>
<tr>
<td>BPD</td>
<td>36 (20.5)</td>
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<tr>
<td>Necrotizing enterocolitis</td>
<td>18 (10.2)</td>
<td></td>
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<tr>
<td>Severe intraventricular hemorrhage</td>
<td>13 (7.4)</td>
<td></td>
</tr>
<tr>
<td>Major morbidity</td>
<td>50 (28.4)</td>
<td></td>
</tr>
<tr>
<td>Postnatal death</td>
<td>18 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Poor outcome</td>
<td>55 (31.3)</td>
<td></td>
</tr>
</tbody>
</table>

* Bradycardia of the fetal heart rate or spontaneous repetitive late decelerations.

Only NRBC parameter that remained as an independent predictor in addition to gestational age ($r^2 = 0.26$). On day 4 of life, an NRBC count of >70/100 WBC predicted major morbidity, with a sensitivity of 82% and a specificity of 96% (odds ratio, 7.33; 95% CI, 2.4-22.4; chi-square, $P < .001$).

**Comment**

NRBC counts at birth and persistence of NRBC count elevation have been studied as prognostic indicators for poor neonatal outcome and risk factors for poor neurodevelopment. This interest is based on the assumption that the expansion of hematopoiesis to extramedullary sites is a prerequisite for the appreciable peripheral release of these cells. Because stimulation of extramedullary sites and release of their less mature cells requires a period of time, an increase in peripheral NRBC counts implies chronic acid-base disturbance. Such chronic deterioration of fetal acid-base status is considered an important antecedent for poor neurodevelopment. Cord artery pH at birth is an acute marker; it does not provide a good estimate of the chronicity of acid-base derangement. On the other hand, fetal/neonatal NRBC responses reflect both chronicity and severity of acid-base disturbance and may hold promise as an independent prognostic marker for short-and-long-term outcome. Previous studies have used the NRBC count at birth and the persistence of NRBC count elevation with variable results. Because neonatal events may have additional impacts on the complicated dynamics of the NRBC response, this study used several novel indices to evaluate relationships with short-term outcome in preterm growth-restricted neonates.

We studied a selected population of preterm neonates who had growth restriction because of early onset placental dysfunction. Metabolic acidemia, deterioration of biophysical parameters, elevation in placental blood flow resistance, birthweight, and gestational age at delivery were the primary factors that determined the magnitude of NRBC response in this group of otherwise normal
Necrotizing enterocolitis was the only complication that has reported associations between elevated NRBC count and adverse perinatal outcome and neurologic impairment, the magnitude of the neonatal NRBC response has not been studied in much detail. Buoncure et al. reported that the NRBC count was significantly higher in infants with abnormal cerebral artery Doppler scans at 48-72 hours after birth, compared with healthy neonates. Similar observations were made for 6-month-old infants with sequelae of hypoxic-ischemic encephalopathy and in 3-year-old children with abnormal developmental status. The authors concluded that the NRBC count at birth reflects not only a response of the infant to growth-restricted neonates. Although all NRBC parameters showed wide variability their primary correlation with acid-base balance, placental blood flow resistance, birthweight, and gestational age at delivery were maintained over the first week of life. Composite neonatal morbidity and death were associated with higher absolute NRBC counts and the derived parameters. Necrotizing enterocolitis was the only complication that was not associated consistently with elevated NRBC parameters.

Although multiple previous studies have reported associations between elevated NRBC count and adverse perinatal outcome and neurologic impairment, the magnitude of the neonatal NRBC response has not been studied in much detail. Buoncure et al. reported that the NRBC count was significantly higher in infants with abnormal cerebral artery Doppler scans at 48-72 hours after birth, compared with healthy neonates. Similar observations were made for 6-month-old infants with sequelae of hypoxic-ischemic encephalopathy and in 3-year-old children with abnormal developmental status. The authors concluded that the NRBC count at birth reflects not only a response of the infant to growth-restricted neonates. Although all NRBC parameters showed wide variability their primary correlation with acid-base balance, placental blood flow resistance, birthweight, and gestational age at delivery were maintained over the first week of life. Composite neonatal morbidity and death were associated with higher absolute NRBC counts and the derived parameters. Necrotizing enterocolitis was the only complication that was not associated consistently with elevated NRBC parameters.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NRBC parameters</strong></td>
</tr>
<tr>
<td>NRBC parameter</td>
</tr>
<tr>
<td>NRBC count</td>
</tr>
<tr>
<td>At birth</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Day 2</td>
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<tr>
<td>Day 3</td>
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<td>Day 4</td>
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<td>Day 5</td>
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<tr>
<td>Day 6</td>
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<tr>
<td>Day 7</td>
</tr>
<tr>
<td>Days of NRBC persistence &gt; 30/100 WBC (n)</td>
</tr>
<tr>
<td>Percentage of days with NRBC persistence (%)</td>
</tr>
<tr>
<td>Mean first week NRBC count</td>
</tr>
<tr>
<td>NRBC-AUC</td>
</tr>
<tr>
<td>NRBC slope of decline</td>
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<table>
<thead>
<tr>
<th>TABLE 4</th>
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<tr>
<td><strong>Correlations between NRBC and perinatal parameters</strong></td>
</tr>
<tr>
<td>NRBC parameter</td>
</tr>
<tr>
<td>NRBC count</td>
</tr>
<tr>
<td>At birth</td>
</tr>
<tr>
<td>Day 1</td>
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<tr>
<td>Day 2</td>
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<td>Day 4</td>
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<td>Day 5</td>
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<tr>
<td>Day 6</td>
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<tr>
<td>Day 7</td>
</tr>
<tr>
<td>Days of NRBC persistence &gt; 10/100 WBC</td>
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<tr>
<td>Percentage of days with NRBC persistence</td>
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<tr>
<td>Mean first week NRBC count</td>
</tr>
<tr>
<td>NRBC-AUC</td>
</tr>
<tr>
<td>NRBC slope of decline</td>
</tr>
</tbody>
</table>

- Data are presented with the Pearson correlation coefficients for NRBC and perinatal parameters. UA PI: umbilical artery Doppler index z-score, pH, pCO₂, pO₂, HCO₃, and BE are components of the umbilical artery blood gas at birth.
- Two-tailed, *P* < 0.01.
- Two-tailed, †P* < 0.05.
<table>
<thead>
<tr>
<th>Variable</th>
<th>No BPD</th>
<th>BPD</th>
<th>No intraventricular hemorrhage</th>
<th>Intraventricular hemorrhage</th>
<th>No necrotizing enterocolitis</th>
<th>Necrotizing enterocolitis</th>
<th>No morbidity</th>
<th>Neonatal morbidity</th>
<th>Alive</th>
<th>Neonatal death</th>
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</thead>
<tbody>
<tr>
<td><strong>NRBC count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>At birth</td>
<td>54 (0-1600)</td>
<td>184 (0-2930)*</td>
<td>66 (0-2930)</td>
<td>269 (0-1660)*</td>
<td>69 (0-1660)</td>
<td>99 (2-2930)</td>
<td>49 (0-1600)</td>
<td>182 (0-2980)*</td>
<td>57 (0-1660)</td>
<td>265 (0-2930)*</td>
</tr>
<tr>
<td>Day 1</td>
<td>21 (0-2380)</td>
<td>95 (0-1800)*†</td>
<td>24 (0-1400)</td>
<td>247 (2-2380)*</td>
<td>25 (0-2380)</td>
<td>52 (0-1394)</td>
<td>19 (0-1400)</td>
<td>102 (0-2380)*</td>
<td>22 (0-1800)</td>
<td>247 (0-2380)*</td>
</tr>
<tr>
<td>Day 2</td>
<td>8 (0-1500)</td>
<td>79 (0-1723)*</td>
<td>9 (0-1723)</td>
<td>135 (0-1452)*</td>
<td>10 (0-1723)</td>
<td>121 (0-1022)</td>
<td>5 (0-1500)</td>
<td>90 (0-1723)*</td>
<td>6 (0-1723)</td>
<td>144 (4-1452)*</td>
</tr>
<tr>
<td>Day 3</td>
<td>1 (0-1730)</td>
<td>17 (0-1502)*</td>
<td>2 (0-1502)</td>
<td>16 (0-1730)*</td>
<td>2 (0-1730)</td>
<td>21 (0-701)*</td>
<td>0 (0-1389)</td>
<td>17 (0-1730)*</td>
<td>2 (0-1502)</td>
<td>110 (3-1730)*</td>
</tr>
<tr>
<td>Day 4</td>
<td>0 (0-851)</td>
<td>5 (0-779)*</td>
<td>0 (0-850)</td>
<td>4 (0-851)*†</td>
<td>0 (0-851)</td>
<td>10 (0-107)*</td>
<td>0 (0-850)</td>
<td>9 (0-851)*</td>
<td>0 (0-850)</td>
<td>51 (0-851)*</td>
</tr>
<tr>
<td>Day 5</td>
<td>0 (0-980)</td>
<td>3 (0-628)*</td>
<td>0 (0-648)</td>
<td>4 (0-980)*†</td>
<td>0 (0-980)</td>
<td>6 (0-150)*</td>
<td>0 (0-648)</td>
<td>4 (0-980)*</td>
<td>0 (0-648)</td>
<td>10 (0-980)*</td>
</tr>
<tr>
<td>Day 6</td>
<td>0 (0-372)</td>
<td>1 (0-300)*</td>
<td>0 (0-372)</td>
<td>2 (0-300)*</td>
<td>0 (0-372)</td>
<td>2 (0-23)*</td>
<td>0 (0-372)</td>
<td>2 (0-300)*</td>
<td>0 (0-372)</td>
<td>7 (0-300)*</td>
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<td>Day 7</td>
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<td>0 (0-69)*</td>
<td>0 (0-87)</td>
<td>2 (0-69)*</td>
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<td>0 (0-10)</td>
<td>0 (0-87)</td>
<td>0 (0-69)*</td>
<td>0 (0-87)</td>
<td>2 (0-69)*</td>
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<tr>
<td>NRBC persistence (%)</td>
<td>0 (0-7)</td>
<td>2 (0-7)*</td>
<td>0 (0-7)</td>
<td>2 (0-7)*†</td>
<td>0 (0-7)</td>
<td>2 (0-5)</td>
<td>0 (0-7)</td>
<td>2 (0-7)*</td>
<td>0 (0-7)</td>
<td>4 (0-7)*</td>
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<tr>
<td>NRBC persistence (%)</td>
<td>0 (0-100)</td>
<td>29 (0-100)*</td>
<td>0 (0-100)</td>
<td>29 (0-100)*</td>
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<td>29 (0-71)</td>
<td>0 (0-100)</td>
<td>43 (0-100)*</td>
<td>0 (0-100)</td>
<td>100 (0-100)*</td>
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<tr>
<td>Mean first week NRBC</td>
<td>13 (0-2245)</td>
<td>64 (43-994)*</td>
<td>16 (0-995)</td>
<td>117 (43-2245)*</td>
<td>17 (0-2245)</td>
<td>59 (29-570)</td>
<td>11 (0-896)</td>
<td>107 (1-2245)*</td>
<td>14 (0-995)</td>
<td>211 (8-2245)*</td>
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<tr>
<td>NRBC-AUC</td>
<td>60 (1-6148)</td>
<td>357 (2-6167)*</td>
<td>76 (1-6167)</td>
<td>658 (29-6148)*</td>
<td>63 (1-6167)</td>
<td>331 (1-3586)</td>
<td>50 (1-5683)</td>
<td>595 (1-6167)*</td>
<td>70 (1-6167)</td>
<td>679 (38-6148)*</td>
</tr>
<tr>
<td>NRBC slope of decline</td>
<td>–30.5 (–618 to 186)</td>
<td>–71 (–624 to 19)*</td>
<td>–31 (–743 to 102)</td>
<td>–61 (–824 to 186)</td>
<td>–33 (–824 to 186)</td>
<td>–37 (–743 to 0)</td>
<td>–27 (–618 to 103)</td>
<td>–61 (–824 to 186)</td>
<td>–32 (–824 to 0)</td>
<td>–48.7 (–743 to 186)</td>
</tr>
</tbody>
</table>

Data are presented as median and range (in brackets); all are Mann Whitney U tests.

* P < .001, compared with neonates without the respective complication.
† P < .05, compared with neonates without the respective complication.
NRBC counts and NRBC persistence also correlated with short- and long-term outcomes. The variability in these parameters has precluded the identification of a discrete prognostic cutoff. Although this variability could be explained by variations in the severity of the fetal condition, there is evidence to suggest that neonatal factors may modulate NRBC dynamics equally.

Studies on adults in the intensive care unit have shown that development of complications is associated with an increase in NRBC counts in the peripheral circulation. Although individual triggers for NRBC release have not been elucidated fully, these findings suggest that postpartum events may be capable of triggering additional NRBC release. Therefore, the wide variability of NRBC count and persistence in previous neonatal studies is more likely to be due to the combination of fetal and neonatal factors. In this setting, the magnitude of NRBC release will depend on the severity of insult and the extent of extramedullary hematopoietic sites. Because these are already stimulated in growth-restricted fetuses, significant NRBC release with prolonged persistence of NRBC count elevation follows. This is the first study that evaluates these NRBC dynamics in greater detail. We have shown that the AUC gives a better mathematical description of the NRBC response than delivery count and persistence alone. This technique emphasizes failure to clear their NRBC counts <70/100 WBC as a significant risk for adverse outcome. Our analysis does not allow us to conclude whether this prolonged persistence is due to delayed clearance or new release of NRBCs. If prolonged persistence is due to delayed clearance or new release of NRBCs, it remains to be determined whether continued effects of fetal metabolic disturbance or neonatal neonatal complications are the principal triggers.

The strengths of this study are a large population of carefully defined neonates who were closely monitored with multiple antenatal and postnatal parameters. The limitations of our findings arise from the complexity of red blood cell production. The various previous observations on NRBC parameters and short- and long-term sequelae offer no clear explanation. This study clarifies the best way to depict NRBC characteristics, but further investigations are needed to define the dynamics of NRBC regulation as neonatal life progresses. It may well be that both NRBC peak at delivery (as a marker of chronic fetal impact) and NRBC persistence (as a marker of continued and/or added neonatal impacts) are separate predictors. When NRBCs persist at >70 beyond day 3 of life, new attention may need to be focused on measures that are specific to growth-restricted neonates, and a diagnostic workup for subclinical or clinical deterioration may be initiated. Wide variability and the complexity of the interactions mean that NRBC count cannot be used not only in isolation but also as a part of a consortium of hypoxemia markers to optimize predictive accuracy. Previously identified risk factors for postpartum complications play a predominant role, particularly in preterm growth-restricted neonates. Further studies that will expand to other hypoxemia markers are needed to clarify the associations with perinatal outcome in growth-restricted and appropriately grown neonates.

**REFERENCES**


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**FIGURE 1**

NRBC area under the curve and major morbidity

A display of the AUC of the NRBC response in the first week of life in relationship to major morbidity.


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**FIGURE 2**

Mean NRBC counts and morbidity

A display of the distribution of mean NRBC counts in survivors and neonates that died in the first 28 days of life.


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perinatal hypoxia but also is the risk for perinatal brain damage. Growth-restricted neonates have significantly higher NRBC counts than adequately grown counterparts, but elevated NRBC counts in the first week of life: a critical appraisal of relationships with perinatal outcome in preterm growth-restricted neonates. AJOG 2007
Differential expression of microRNAs with progression of gestation and inflammation in the human chorioamniotic membranes

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OBJECTIVE: The aim of this study was to identify differential expression of microRNAs (miRNAs) in chorioamniotic membranes with advancing gestation, labor, and inflammation.

STUDY DESIGN: Expression profiles of 157 miRNAs in the chorioamniotic membranes were obtained from patients in the following groups: 1) term not in labor (n = 10); 2) term in labor (n = 10); 3) preterm labor with histologic chorioamnionitis (n = 9); and 4) without histologic chorioamnionitis (n = 10).

RESULTS: More than 95% of the miRNAs screened were expressed. Gestational age-dependent changes in expression were observed for 13 miRNAs. No differences in miRNA expression were observed between women without labor and women in labor. Membranes with chorioamnionitis displayed increased expression of miR-223 and miR-338. Gene Ontology analysis of genes targeted by differentially expressed miRNAs revealed enrichment for specific biological process categories.

CONCLUSION: Chorioamniotic membranes with advancing gestational age and chorioamnionitis are associated with the differential expression of a subset of miRNAs.

Key words: chorioamniotic membranes, gestation, inflammation, labor, microRNA, real-time qRT-PCR


The human chorioamniotic membranes are involved in a variety of physiological and pathological processes during pregnancy, such as accommodation and protection of the developing fetus, parturition, and response to intraamniotic infection. Infection and inflammation of the amniotic cavity are often accompanied by histologic chorioamnionitis, which is associated with both preterm delivery and adverse perinatal outcome. Although the structure of the chorioamniotic membranes does not substantially change after the first trimester, gestational age-dependent changes in gene expression have been reported. Functional genomic studies of the chorioamniotic membranes have shown that spontaneous labor at term and intraamniotic infection are both characterized by unique gene expression signatures. Little is known, however, about the mechanisms involved in the regulation of gene expression in the chorioamniotic membranes.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that are critical for the posttranscriptional regulation of gene expression. Originally discovered in the nematode, *Caenorhabditis elegans*, miRNAs inhibit translation or initiate degradation of target messenger RNA (mRNA) transcripts via complementary base-pairing. Conservation of miRNAs within and across species, as well as coevolution with mRNAs, suggests that miRNAs have had a significant impact on the evolution of protein-coding genes. Over 400 miRNAs have been identified in humans, and bioinformatics studies predict over one-third of human genes are regulated by miRNAs. miRNAs are being studied extensively, particularly in embryonic development. Mice and zebrafish lacking the miRNA...
processing enzyme Dicer are not viable past the early embryonic period.\textsuperscript{13,14} Moreover, lack of Dicer in mice disrupts hair germ invagination, suggesting that miRNAs are critical for hair follicle morphogenesis.\textsuperscript{15} A role for miRNAs in mammalian immunity has also recently been proposed.\textsuperscript{16,17} miR-155 expression by macrophages is induced by the ligands of multiple Toll-like receptors.\textsuperscript{16}

In neutrophils, miR-146a and miR-146b target adapter molecules TRAF6 and IRAK1, which are essential for the downstream signaling of both cytokine receptors and Toll-like receptors.\textsuperscript{17}

Because of the evidence supporting miRNA involvement in normal developmental processes as well as immune responses, we sought to determine whether miRNA expression in the human chorioamnionic membranes varied with advancing gestational age, spontaneous labor at term, and histologic chorioamnionitis.

**Materials and Methods**

**Study design**

A cross-sectional study was conducted to examine the patterns of miRNA expression in chorioamnionic membranes obtained from patients in the following groups: 1) normal pregnancy at term not in labor and delivered by elective cesarean section (\([\text{TNL}], n = 10\)); 2) normal pregnancy with spontaneous labor at term (\([\text{TL}], n = 10\)); 3) spontaneous preterm labor and delivery without histologic chorioamnionitis (\([\text{PTL}], n = 10\)); and 4) spontaneous preterm labor and delivery with histologic chorioamnionitis (\([\text{PTL-HC}], n = 9\)). Normal pregnancy was defined by the absence of medical, surgical, or obstetrical complications. Preterm labor was defined by the presence of regular uterine contractions (at a frequency of at least 2 every 10 minutes) associated with cervical changes and leading to preterm delivery before 37 completed weeks of gestation. Histologic chorioamnionitis was diagnosed by the presence of neutrophilic infiltration on the chorioamnionic membranes. All neonates were appropriate for gestational age (birthweights 10th–90th percentile),\textsuperscript{18} and none of the individuals who delivered at term had evidence of histologic chorioamnionitis. Patients with premature rupture of membranes, multiple gestation, stillbirth, or fetal anomalies were excluded. All women provided written informed consent for the collection of clinical data and tissue samples under protocols approved by the Institutional Review Boards of both Wayne State University (Detroit, MI) and the National Institute of Child Health and Human Development of the National Institute of Health (NIH/ DHHS).

**RNA isolation**

Details of the materials and methods used in this study were previously reported\textsuperscript{19} and will be briefly described. Snap-frozen chorioamnionic membranes stored at \(-80^\circ\text{C}\) were used. Small RNAs (<200 nucleotides) were obtained using the mirVana RNA Isolation kit (Ambion, Austin, TX). Small RNA integrity was determined by polyacrylamide gel electrophoresis. Small RNA concentration was determined with the RNA 6000 Nano Assay on the 2100 Bioanalyzer (Agilent Technologies, Inc, Palo Alto, CA).

**Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for miRNA assay**

miRNA expression profiling of 157 human miRNAs was determined with the TaqMan MicroRNA Assays Human Panel–Early Access Kit (Applied Biosystems, Foster City, CA). A custom-designed TaqMan assay for 5S ribosomal RNA was used to normalize miRNA expression.

**Statistical analysis**

miRNA expression profiles were measured to evaluate 3 processes: 1) gestational age (\([\text{PTL}], n = 10\)); 2) labor (\([\text{TL}], n = 10\) vs \([\text{TIL}], n = 10\)); and 3) histologic chorioamnionitis (\([\text{PTL-HC}], n = 9\) vs \([\text{PTL}], n = 9\)). For the analysis of histologic chorioamnionitis, 9 cases from the PTL group were selected with gestational ages matched to cases in the PTL-HC group.

**Results**

Demographic characteristics of the study population are summarized in Table 1. Term chorioamnionic membranes expressed 153 out of 157 miRNAs, and preterm chorioamnionic membranes expressed 152 out of 157 miRNAs (expression detected in at least 50% of samples).

The effect of gestational age on miRNA expression was evaluated in the chorioamnionic membranes of 10
patients with no evidence of histological chorioamnionitis who delivered between 26.3-35.9 weeks of gestation. The expression of 13 miRNAs decreased with advancing gestational age (Figure 1A; Table 2), and varied between a 4.8-fold (miR-199b) and a 1.6-fold (miR-330) change per month. No significant differences were observed between the miRNA expression profiles of women at term not in labor and those in spontaneous labor after adjustment for multiple comparisons. Evaluation of preterm membranes with and without chorioamnionitis identified differential expression of 2 miRNAs (Figure 1B). The expression of miR-223 and miR-338 was increased (37-fold and 24-fold, respectively) in the presence of chorioamnionitis.

GO analysis performed on gene target lists of miRNAs differentially expressed with chorioamnionitis revealed enrichment for biological process categories. Processes with significantly higher than expected representation in the gene target lists included transcription from RNA polymerase II promoter for miR-223 (Table 3) and electron transport for miR-338 (Table 4). Genes involved in parturition were enriched in the target list of miR-338 (group IVB phospholipase A2 and corticotropin-releasing hormone receptor 1).

### TABLE 1
Patient demographics and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Term not in labor (n = 10)</th>
<th>Term in labor (n = 10)</th>
<th>Preterm labor without chorioamnionitis (n = 10)</th>
<th>Preterm labor with chorioamnionitis (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>24 (19-34)</td>
<td>24 (20-34)</td>
<td>25 (18-36)</td>
<td>24 (19-32)</td>
</tr>
<tr>
<td>Parity</td>
<td>2 (0-4)</td>
<td>2 (0-5)</td>
<td>1 (0-7)</td>
<td>1 (0-5)</td>
</tr>
<tr>
<td>Black ethnicity</td>
<td>9 (90)</td>
<td>9 (90)</td>
<td>8 (80)</td>
<td>8 (89)</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>39.4 (37.0-41.7)</td>
<td>39.3 (37.0-41.9)</td>
<td>32.6 (26.3-35.9)</td>
<td>33.7 (25.3-35.9)</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3225 (2940-3915)</td>
<td>3150 (2800-3595)</td>
<td>1848 (870-2810)</td>
<td>1785 (690-2605)</td>
</tr>
</tbody>
</table>

Values expressed as median (range) or n (%).

### FIGURE 1
Differential expression of microRNAs with advancing gestational age and histologic chorioamnionitis

A. Changes in miR-214 and miR-338 expression as a function of gestational age analyzed within the group of 10 cases with preterm labor without histologic chorioamnionitis. 13 microRNAs displayed decreasing expression with advancing gestation. This figure illustrates 2 examples. Multiple adjustment for the testing of 157 miRNAs was performed (all P < .05). The y-axis represents units of delta Ct (Ct5S – CtmiRNA), with an arbitrary zero point, so that each unit measures a 2-fold change. The x-axis represents gestational age in weeks. B. Differential expression of microRNAs with histologic chorioamnionitis. Box-and-whisker plots of microRNAs differentially expressed between preterm labor cases with and without histologic chorioamnionitis after multiple adjustment for the testing of 157 miRNAs (all P < .05 and >2-fold change). The y-axis represents units of delta Ct (Ct5S – CtmiRNA), with an arbitrary zero point, so that each unit measures a 2-fold change. The fold change is displayed below each microRNA name.


### TABLE 2
MicroRNAs demonstrating changes in expression with advancing gestational age

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Fold change per month</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-199b</td>
<td>4.8</td>
<td>0.56</td>
</tr>
<tr>
<td>miR-373</td>
<td>3.5</td>
<td>0.60</td>
</tr>
<tr>
<td>miR-218</td>
<td>3.4</td>
<td>0.50</td>
</tr>
<tr>
<td>miR-154</td>
<td>3</td>
<td>0.51</td>
</tr>
<tr>
<td>miR-338</td>
<td>2.7</td>
<td>0.62</td>
</tr>
<tr>
<td>miR-198</td>
<td>2.6</td>
<td>0.44</td>
</tr>
<tr>
<td>miR-214</td>
<td>2.4</td>
<td>0.64</td>
</tr>
<tr>
<td>miR-370</td>
<td>2.4</td>
<td>0.55</td>
</tr>
<tr>
<td>miR-213</td>
<td>2.4</td>
<td>0.63</td>
</tr>
<tr>
<td>miR-107</td>
<td>2.3</td>
<td>0.51</td>
</tr>
<tr>
<td>miR-199a</td>
<td>2.1</td>
<td>0.41</td>
</tr>
<tr>
<td>miR-222</td>
<td>1.9</td>
<td>0.49</td>
</tr>
<tr>
<td>miR-330</td>
<td>1.6</td>
<td>0.40</td>
</tr>
</tbody>
</table>

All P < .05
The principal findings of this study are: 1) miRNAs are abundantly expressed in the chorioamniotic membranes; 2) distinct miRNA expression patterns are associated with advancing gestation; and 3) specific miRNAs are differentially expressed with histologic chorioamnionitis.

The temporal regulation of miRNA expression during animal development has been closely investigated. For example, the expression of let-7a, a member of the let-7 miRNA family, is critical for developmental timing in the model organisms nematode and mouse. In this study, let-7 miRNAs did not demonstrate differential expression with advancing gestation. However, 13 miRNAs displayed decreased expression with advancing gestation, which suggests a functional involvement of miRNAs in chorioamniotic membranes by reducing the translational inhibition of multiple mRNA targets.

GO analysis of genes targeted by miRNAs differentially expressed with chorioamnionitis (miR-223 and miR-338) revealed enrichment for specific biological process categories. Interestingly, miR-338 was also found to decrease with advancing gestation, and is predicted to

### TABLE 3

<table>
<thead>
<tr>
<th>Biological process category</th>
<th>Genes targeted by miR-223</th>
<th>Genes targeted by all miRNAs*</th>
<th>Predicted targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription from RNA polymerase II promoter</td>
<td>15</td>
<td>155</td>
<td>BTF3, MEF2C, HLX1, TCFL5, CBF1, TCERG1, NFE2L3, LM04, FOXO3A, PARP1, ATBF1, ECD, GTF2H4, RFXANK, CREB3</td>
</tr>
<tr>
<td>Protein modification</td>
<td>9</td>
<td>70</td>
<td>RP2N, BAG1, C16orf33, PIGH, TTL9, PAM, SUMO1, PCMT1, DDI</td>
</tr>
<tr>
<td>Protein targeting</td>
<td>7</td>
<td>29</td>
<td>SRP54, SEC61B, SRP19, GABARAP, SEC61G, SIL1, RRB1</td>
</tr>
<tr>
<td>Transcription from RNA polymerase III promoter</td>
<td>4</td>
<td>14</td>
<td>SSB, TR0VE2, GTF3C2, GTF3C1</td>
</tr>
<tr>
<td>Negative regulation of immune response</td>
<td>2</td>
<td>3</td>
<td>SPINK5, TGF82</td>
</tr>
<tr>
<td>Superoxide release</td>
<td>2</td>
<td>3</td>
<td>DUOX1, DUOX2</td>
</tr>
<tr>
<td>Antinflammatory response</td>
<td>2</td>
<td>5</td>
<td>SPINK5, INF81</td>
</tr>
<tr>
<td>Hydrogen peroxide biosynthesis</td>
<td>2</td>
<td>2</td>
<td>DUOX1, DUOX2</td>
</tr>
<tr>
<td>Hydrogen peroxide catabolism</td>
<td>2</td>
<td>7</td>
<td>DUOX1, DUOX2</td>
</tr>
</tbody>
</table>

All P < .05.
* miRNAs studied in this analysis.

### TABLE 4

<table>
<thead>
<tr>
<th>Biological process category</th>
<th>Genes targeted by miR-338</th>
<th>Genes targeted by all miRNAs*</th>
<th>Predicted targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transport</td>
<td>22</td>
<td>253</td>
<td>CYP28B1, DUOX, HIF1A, BLRVA, NNT, FTSJ3, PYCR2, NFA, CYP3A5, MICAL3, NDUF1, FDXR, DHX34, SURF1, TXNRD2, COX411, PDIA3, GCIT, P4HB, PADOX, CYP3A7, GPD2</td>
</tr>
<tr>
<td>Protein catabolism</td>
<td>4</td>
<td>17</td>
<td>FBXO17, PSMB6, AFG3L2, PRSS16</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>3</td>
<td>7</td>
<td>TMPRSS6, F12, PLG</td>
</tr>
<tr>
<td>Protein secretion</td>
<td>3</td>
<td>11</td>
<td>ARFGAP3, GN5, SEC61A2</td>
</tr>
<tr>
<td>Carbohydrate transport</td>
<td>3</td>
<td>12</td>
<td>SLC2A14, SLC2A10, SLC2A3</td>
</tr>
<tr>
<td>L-cystine transport</td>
<td>2</td>
<td>2</td>
<td>SLC3A1, CTNS</td>
</tr>
<tr>
<td>Tissue regeneration</td>
<td>2</td>
<td>4</td>
<td>NINJ2, APOA5</td>
</tr>
<tr>
<td>Homeostasis</td>
<td>2</td>
<td>5</td>
<td>PKHD1, HIF1A</td>
</tr>
<tr>
<td>Parturition</td>
<td>2</td>
<td>7</td>
<td>CRHR1, PLA2G4B</td>
</tr>
</tbody>
</table>

All P < .05.
* miRNAs studied in this analysis.
target genes involved in the GO biological process of parturition (group IVB phospholipase A2 and corticotropin-releasing hormone receptor 1 [CRHR1]). Because CRH increases exponentially throughout gestation, mainly through placental production, it has been proposed as a critical factor in determining the timing of parturition. CRHR1, the primary receptor for corticotropin-releasing hormone (CRH), is expressed by amnion, chorion, and decidua, as well as reproductive tissues such as the cervix. Locally, CRH is a potent vasodilator that can induce prostaglandin and matrix metalloproteinase production by the chorioamniotic membranes. Since miR-338 may interact with CRHR1 in the membranes, additional studies are needed to evaluate the impact of miR-338 on CRHR1 function.

The target list of miR-223 was enriched for genes involved in fundamental biological processes, as well as several immunity-related processes. Chorioamniotic membranes play a complex dual role of protecting the fetus from both foreign pathogens and the maternal “host” immune response. Several studies have shown that chorioamniotic membranes are involved in the recognition and response to infection. For example, membranes with chorioamnionitis display increased expression of Toll-like receptors-2 and 4, which respond to Gram-positive and Gram-negative bacteria, respectively. Selected genes in the target list of miR-223 (SPINK5, TGFβ2, and IFNB1) are involved in the GO biological processes of negative regulation of immune response and anti-inflammatory response. The over-representation of immune-related biological process categories in the target list of miR-223 suggests a function for this miRNA in the regulation of chorioamnionitis-related inflammation.

A strength of this study is that the differential expression of 157 human miRNAs was measured using real-time qRT-PCR, which is considered the most sensitive and specific RNA quantification method. In addition, a correction for the multiple testing of 157 miRNAs was performed by employing the false discovery rate, which contributed to a robust and reliable assessment of differential miRNA expression. A constraint of this study, on the other hand, is that the real-time qRT-PCR method did not allow for a more comprehensive screening of all human miRNAs currently identified. This limitation may partly explain why no differences in expression were observed between women at term in labor and without labor.

This study reports, for the first time, the expression profiles of miRNA in human chorioamniotic membranes with advancing gestation and chorioamnionitis. The miRNAs differentially expressed in these conditions provide relevant targets for further investigation.

REFERENCES


A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes

Kun Woo Kim, MD; Roberto Romero, MD; Hyun Soo Park, MD; Chan-Wook Park, MD; Soon-Sup Shim, MD; Jong Kwan Jun, MD, PhD; Bo Hyun Yoon, MD, PhD

OBJECTIVE: To examine whether the MMP-8 PTD Check (SK Pharma Co., Ltd, Kyunggi-do, Korea), a rapid bedside test that can be performed in 15 minutes, is of value in the identification of intraamniotic infection and/or inflammation and in the assessment of the likelihood of adverse pregnancy outcome in patients with preterm premature rupture of membranes (PPROM).

STUDY DESIGN: Amniotic fluid was retrieved by transabdominal amniocentesis in 141 women with PPROM (<35 weeks' gestation). Fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas; the remaining amniotic fluid was stored. The stored amniotic fluid was analyzed for interleukin-6 and MMP-8 PTD Check test. Intraamniotic infection/inflammation was defined as a positive amniotic fluid culture and/or elevated amniotic fluid interleukin-6 concentration (>2.6 ng/mL). Nonparametric and survival analysis were used.

RESULTS: The prevalence of intraamniotic infection/inflammation was 43% (60/141 women) and that of proven amniotic fluid infection was 18% (25/141 women). Patients with a positive MMP-8 PTD Check test result had a significantly higher rate of intraamniotic infection/inflammation (77% [54/70 women] vs 9% [6/71 women]; P < .001); proven amniotic fluid infection (33% [23/70 women] vs 3% [2/71 women]; P < .001), and adverse outcome than those with a negative MMP-8 PTD Check test result. Adverse outcome included shorter interval to delivery and higher rate of preterm delivery, histologic chorioamnionitis, funisitis, low Apgar scores, and significant neonatal morbidity. A positive MMP-8 PTD Check test result had a sensitivity of 90%, a specificity of 80%, a positive predictive value of 77%, and a negative predictive value of 92% in the identification of intraamniotic infection/inflammation, and was an independent predictor of interval to delivery (hazards ratio, 3.7; 95% CI, 2.4-5.9) and significant neonatal morbidity (odds ratio, 3.1; 95% CI, 1.2-7.9).

CONCLUSION: The MMP-8 PTD Check test is a rapid, simple, and sensitive bedside test to detect intraamniotic infection/inflammation and to predict adverse outcome that includes short latency, chorioamnionitis, and significant neonatal morbidity in patients with PPROM. The results of this study bring the rapid detection of intraamniotic infection/inflammation to the bedside in clinical obstetrics.

Key words: bedside test, interleukin-6, intraamniotic infection/inflammation, MMP-8 PTD check, preterm premature rupture of membranes


Matrix metalloproteinase-8 (MMP-8) is an enzyme that is detected at the site of inflammation and is found in the amniotic fluid (AF) of patients with microbial invasion of the amniotic cavity. An elevated concentration of MMP-8 in AF is a sensitive and powerful predictor of intraamniotic infection and/or inflammation. Intraamniotic infection/inflammation is a risk factor for impending spontaneous delivery and adverse perinatal outcome and is present in approximately 50% of patients with preterm premature rupture of membranes (PPROM). The classic methods for the detection of intraamniotic infection/inflammation are the identification of microorganisms from AF or laboratory test (eg, an AF white blood cell count, cytokine determinations, and MMP determinations). However, the results of AF culture may take days and are not available in time for some clinical decisions. Recently, a bedside test was developed to detect intraamniotic inflammation based on the detection of an elevated concentration of MMP-8 in AF. Although it had been reported that the MMP-8 rapid test can identify patients at risk for preterm delivery within 7 days and 14 days among patients with preterm labor and intact membranes, the usefulness of the MMP-8 rapid test in patients with PPROM remains to be determined. The objective of this study was to examine whether the MMP-8 PTD test...
MATERIALS AND METHODS

Study population

The study population consisted of patients who were admitted to Seoul National University Hospital with the diagnosis of PPROM (<35 weeks’ of gestation) and singleton gestation who underwent amniocentesis for the assessment of microbiologic status of the amniotic cavity and fetal lung maturity between June 1995-June 2002.

Transabdominal amniocentesis is offered routinely to all patients who are admitted with the diagnosis of PPROM at our institution. This procedure was performed after written informed consent was obtained. The institutional review board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protections of the Department of Health and Human Services of the United States.

AF

AF was cultured for aerobic and anaerobic bacteria and for genital mycoplasmas (Ureaplasma urealyticum and Mycoplasma hominis). The remaining fluid was stored at -70°C until assayed. The stored AF was analyzed for interleukin-6 (IL-6) and the MMP-8 PTD Check test. IL-6 concentrations were measured with a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) with a sensitivity of <1.0 pg/mL. Intra- and interassay coefficients of variation were <10%. Intraamniotic inflammation was defined as an elevated AF IL-6 concentration (>2.6 ng/mL), as previously reported.

MMP-8 rapid test

In 2006, the MMP-8 PTD Check test was performed with stored AF by one of the authors (K.W.K.) who was blinded to the results of AF studies (ie, culture results, white blood cell count, and MMP-8 and IL-6 concentrations) and pregnancy and neonatal outcome. Of 157 AF samples that met the entry criteria for this study, a MMP-8 PTD rapid test was not performed in 16 cases because of the limited amount of the remaining AF sample. The MMP-8 PTD Check is a qualitative immunochromatographic test that detects the presence of MMP-8 in human AF with a threshold of 10 ng/mL. To accomplish this, the test manual proposed the use of the mixture of 25 µL of AF and 100 µL of the buffer provided in the kit (1:4 mixture). In the current study, we mixed 15 µL of AF and 120 µL of buffer (1:8 mixture). The cut-off value of the modified test is 20 ng/mL of MMP-8, which is similar to the cut-off value of intraamniotic inflammation that was identified and used in our previous reports (23 ng/mL).6,7 The results are available within 15 minutes without any laboratory equipment other than a pipette at the bedside. Details about the test were described in a previous report.26

Diagnosis of chorioamnionitis, funisitis, and neonatal morbidity

Histologic chorioamnionitis was defined in the presence of acute inflammatory changes on examination of a membrane roll and chorionic plate of the placenta; funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton’s jelly with the use of criteria previously published.17 Clinical chorioamnionitis and neonatal morbidity were diagnosed according to the definitions previously described in detail.28 Congenital neonatal sepsis was diagnosed in the presence of a positive blood culture result within 72 hours of delivery. The diagnosis of respiratory distress syndrome required the presence of respiratory grunting and retracting, an increased oxygen requirement (inspired oxygen fraction, >0.4), and diagnostic radiographic and laboratory findings in the absence of evidence of other causes of respiratory disease. Pneumonia was diagnosed in the presence of definite clinical and radiologic findings, with or without a positive culture result from tracheal aspirate or chest tube specimen within 7 days of birth. Bronchopulmonary dysplasia was diagnosed according to the following criteria proposed by Bancalari et al29: (1) intermittent positive pressure ventilation was required during the first week of life and for ≥3 days; (2) clinical signs of chronic respiratory disease developed that were characterized by tachypnea, intercostal and subcostal retractions, and tales on auscultation, all of which were persistent for >28 days; (3) supplemental oxygen was required for >28 days to maintain a PaO2 level of ≥50 mm Hg; and (4) a chest radiograph showed persistent strands of densities in both lungs that alternated with areas of normal or increased lucency. In some infants, these areas became coalescent into larger structures that resembled bullae. Intraventricular hemorrhage was graded according to the system proposed by McMenamin et al.30 Significant neonatal morbidity was defined as the presence of any of the following conditions: respiratory distress syndrome, proven neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade ≥II), and necrotizing enterocolitis.

Statistical analysis

Mann–Whitney U test and the Student t test were used for comparison of continuous variables. Comparisons of proportions were performed with a χ2 test or Fisher’s exact test. Logistic regression analysis was used to explore the effect of gestational age at amniocentesis on pregnancy and neonatal outcome. Survival analysis was used to compare the amniocentesis-to-delivery interval between groups. Cox proportional hazard model was used to control covariates. The intervals-to-delivery of women who did not go into labor spontaneously (because they were delivered for maternal or fetal indications) was treated as a censored observation, with a censoring time equal to the amniocentesis-to-delivery interval. Statistical significance was defined as a probability value of <.05.
TABLE 1
Clinical characteristics of the study population, according to results of the MMP-8 PTD Check test

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive MMP-8 PTD Check test result (n = 70)</th>
<th>Negative MMP-8 PTD Check test result (n = 71)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>30.5 ± 3.9</td>
<td>30.2 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (wk)*</td>
<td>29.7 (21.6-34.7)</td>
<td>33.0 (20.4-34.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nulliparity†</td>
<td>40 (28/70)</td>
<td>49 (35/71)</td>
<td>NS</td>
</tr>
<tr>
<td>Antibiotics‡</td>
<td>97 (68/70)</td>
<td>93 (66/71)</td>
<td>NS</td>
</tr>
<tr>
<td>Corticosteroid‡</td>
<td>74 (52/70)</td>
<td>66 (47/71)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.
* Data given as mean ± SD.
† Data given as median (range).
‡ Data given as percentage (n/N).

RESULTS
Characteristics of study population
The prevalence of intraamniotic infection/inflammation was 43% (60/141); the prevalence of proven AF infection was 18% (25/141). Microorganisms that were isolated by culture included Urealyticum (n = 19), M hominis (n=2), Streptococcus anigenous (n = 1), Staphylococcus epidermidis (n = 1), Peptostreptococcus (n = 1), Burkholderia cepacia (n = 1), Torulopsis glabrata (n = 1), Escherichia coli (n = 1), and mixed microorganisms (n = 2). The MMP-8 PTD Check test was positive in 50% (70/141) of cases. Table 1 compares the characteristics of study population according to the results of MMP-8 PTD Check test. Patients with a positive MMP-8 PTD Check test result had significant lower mean gestational age at amniocentesis than those with a negative result (P < .001).

AF culture and inflammation
Table 2 describes the diagnostic indices and predictive values of the MMP-8 PTD Check test for the identification of intraamniotic infection/inflammation and AF infection.

Pregnancy outcome
Table 3 compares the pregnancy and neonatal outcome of the study population according to the results of MMP-8 PTD Check test. Patients with a positive result had a significantly lower gestational age at birth and birthweight and higher rates of spontaneous preterm delivery, histologic chorioamnionitis, funnisisis, admission to neonatal intensive care unit, and significant neonatal morbidity than did those with a negative test result.

Interval to delivery
The Figure compares amniocentesis-to-delivery interval. In 37 patients who were delivered for maternal or fetal indications, this interval was censored. Patients with a positive MMP-8 rapid test result had a significantly shorter median amniocentesis-to-delivery interval than did those patients with a negative test result.

COMMENT
Principal findings of the study
Our principal findings were: (1) the MMP-8 PTD Check test is a sensitive and
Patients with a positive MMP-8 PTD Check test results had a significantly shorter amniocentesis-to-delivery interval than patients with negative MMP-8 PTD Check test results (median, 5 days [95% CI, 4–6 days] vs 20 days [95% CI, 10–31 days]; log rank, \( P < .001 \)), indicating a specific test for the identification of intraamniotic infection/inflammation among patients with PPROM; (2) a positive MMP-8 PTD Check test result in patients with PPROM is an independent risk factor for impending preterm delivery and adverse neonatal outcome; and (3) the prevalence of a positive test result among patients with PPROM is 50% (70/171), but the prevalence of a positive test result among patients with preterm labor and intact membranes is only 11%.26

### MMP-8 rapid test protocol

We used a cutoff of 23 ng/mL of AF MMP-8 concentration (determined by enzyme-linked immunosorbent assay) as the definition of intraamniotic inflammation, based on previous studies.6,7 The MMP-8 PTD Check test, however, detects the presence of MMP-8 in human AF with a threshold of 10 ng/mL, according to the original test manual. We modified the rapid test procedure to detect 20 ng/mL of MMP-8.

#### A point-of-care test for the detection of intraamniotic infection/inflammation

The rapid MMP-8 test that was used in this study is an immunoassay that uses monoclonal antibodies that were developed to determine the clinical value of qualitative AF MMP-8 assessment. It has long been recognized that intraamniotic infection/inflammation is associated with adverse pregnancy and neonatal outcome.6,8-11 We found that the MMP-8 rapid test is sensitive and specific for the identification of intraamniotic infection/inflammation in PPROM.

### TABLE 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>MMP-8 PTD Check test result</th>
<th>( P ) value</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (wk)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n = 70)</td>
<td>30.3 ( \pm ) 3.5</td>
<td></td>
<td>(&lt; .001)</td>
<td>( — )</td>
</tr>
<tr>
<td>Negative (n = 71)</td>
<td>35.0 ( \pm ) 2.6</td>
<td></td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td></td>
<td></td>
<td>(&lt; .001)</td>
<td>( — )</td>
</tr>
<tr>
<td>Positive (n = 70)</td>
<td>1591 ( \pm ) 599</td>
<td></td>
<td>(&lt; .001)</td>
<td>( — )</td>
</tr>
<tr>
<td>Negative (n = 71)</td>
<td>2363 ( \pm ) 528</td>
<td></td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
<td>1-minute Apgar score &lt;7 (%)</td>
<td>61.4</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .05)</td>
</tr>
<tr>
<td>5-minute Apgar score &lt;7 (%)</td>
<td>35.7</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .05)</td>
</tr>
<tr>
<td>Admission to neonatal intensive care unit (%)</td>
<td>84.3</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>Significant neonatal morbidity†§</td>
<td>34.9 (22/63)</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .05)</td>
</tr>
<tr>
<td>Proven congenital neonatal sepsis†</td>
<td>3.2 (2/63)</td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Amniocentesis to delivery interval (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤48 hr</td>
<td>34.3</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>≤7 d</td>
<td>70.0</td>
<td></td>
<td>(&lt; .05)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>Preterm delivery ≤32 weeks’ gestation (%)</td>
<td>62.9</td>
<td></td>
<td>(&lt; .001)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>Preterm delivery &gt;35 weeks’ gestation (%)</td>
<td>98.6</td>
<td></td>
<td>(&lt; .001)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>Histologic chorioamnionitis‡</td>
<td>86.8 (46/53)</td>
<td></td>
<td>(&lt; .001)</td>
<td>(&lt; .001)</td>
</tr>
<tr>
<td>Funisitis‡</td>
<td>64.2 (34/53)</td>
<td></td>
<td>(&lt; .001)</td>
<td>(&lt; .001)</td>
</tr>
</tbody>
</table>

NS, not significant.

* Adjusted for gestational age at amniocentesis (logistic regression analysis).

† Date are given as mean \( \pm \) SD.

‡ Data are given as percentage (n/N).

§ Significant neonatal morbidity was defined as the presence of any of the following conditions: respiratory distress syndrome, proven neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade \( \geq \)II), and necrotizing enterocolitis.

† Ten infants were excluded from the analysis because they died in utero or shortly after delivery as a result of extreme prematurity or anomaly or were delivered at another institution and thus could not be evaluated with respect to the presence or absence of neonatal complications.
The test is comparable with a rapid pregnancy test in simplicity of performance. Moreover, this test is inexpensive to set up and operator independent. As previously reported, an MMP-8 rapid test has many of the optimal properties of a point-of-care test and may be used at the bedside to assess the likelihood of inflammation when clinically indicated.

**Strengths and weaknesses of this study**

Strengths of this study are that the test was not used in the treatment of the cases and that the study was restricted to patients with PPROM and not contaminated with patients who have a different phenotype (ie, preterm labor with intact membranes) and therefore different frequency of intraamniotic infection/inflammation. It could be argued that a weakness of this study is that it was conducted with AF that was stored at -70°C. However, we previously indicated that the test underwent validation by the biotechnology company and that there was substantial agreement between results of fresh and freeze-thawed AF. A rapid bedside test is available to identify intraamniotic infection/inflammation in patients with PPROM. The next step is to determine whether treatment with either antibiotics and/or biologic response modifiers (eg, IL-10, or antiinflammatory agents) can improve pregnancy outcome.

**REFERENCES**

The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity

Si Eun Lee, MD; Roberto Romero, MD; Hanna Jung, MT; Chan-Wook Park, MD; Joong Shin Park, MD, PhD; Bo Hyun Yoon, MD, PhD

OBJECTIVE: Intraamniotic inflammation is a risk factor for adverse pregnancy and neonatal outcome, regardless of the presence or absence of a positive amniotic fluid (AF) culture. The purpose of this study was to determine whether the intensity of a fetal inflammatory response (FIR) differs between cases of intraamniotic inflammation with microbiologically proven infection and cases with negative AF cultures.

STUDY DESIGN: The FIR was examined in 89 cases of women with preterm premature rupture of membranes who delivered singleton preterm newborn infants within 48 hours of amniocentesis. AF was cultured for aerobic and anaerobic bacteria and for genital mycoplasmas. AF white blood cell (WBC) count and matrix metalloproteinase-8 (MMP-8) determinations were performed to assess the presence of intraamniotic inflammation. Intraamniotic inflammation was defined as an elevated AF MMP-8 concentration (>23 ng/mL). The intensity of the FIR was determined by the umbilical cord plasma concentrations of C-reactive protein (CRP). Patients were divided into 3 groups according to the presence or absence of intraamniotic inflammation and AF culture results: group 1, without intraamniotic inflammation and with a negative AF culture (n = 28); group 2, with intraamniotic inflammation and with a negative AF culture (n = 26); group 3, with a positive AF culture (n = 35).

RESULTS: Neonates who were born to mothers with intraamniotic inflammation and negative AF cultures had a significantly higher median umbilical cord plasma CRP concentration than did those without intraamniotic inflammation and a negative AF culture (P < .005) but a significantly lower median cord plasma CRP concentration than did those with proven AF infection (P < .05). Patients with intraamniotic inflammation and a negative AF culture had significantly higher median AF MMP-8 concentrations and WBC count than did those without intraamniotic inflammation and a negative AF culture (P < .001). However, there was no significant difference in the median AF MMP-8 and WBC count between patients with intraamniotic inflammation and a negative AF culture and those with proven AF infection (MMP-8, P > .1, and WBC, P = .09).

CONCLUSION: Intraamniotic inflammation without documented AF infection is a risk factor for a systemic FIR. However, the magnitude of the FIR in those cases was lower than in those with documented AF infection.

Key words: amniotic fluid, C-reactive protein, cord blood, fetal inflammatory response syndrome, intraamniotic inflammation

Substantial evidence indicates that fetuses who are exposed to intraamniotic inflammation are at increased risk for short-term morbidity, cerebral palsy, and bronchopulmonary dysplasia.1-6 Intraamniotic inflammation is found in approximately 40% of patients with preterm premature rupture of membranes and is a risk factor for impending preterm delivery and adverse pregnancy and neonatal outcome, regardless of whether microbial infection can be proved in the amniotic fluid (AF) with cultivation techniques.7

The fetal inflammatory response syndrome (FIRS) is a systemic inflammatory response that was diagnosed originally in the presence of an elevated fetal plasma interleukin-6 (IL-6) concentration. FIRS is characterized by multisystemic involvement1,8 and is associated with impending preterm delivery and adverse perinatal outcome.1,2,8 However, there is a paucity of information regarding the fetal inflammatory response in fetuses who are exposed to intraamniotic inflammation in the absence of proven infection. This information is clinically relevant because a greater fetal inflammatory response is associated with an increased risk for serious neonatal morbidity and death.9 The purpose of this study was to examine whether intraamniotic inflammation is a risk factor for a fetal inflammatory response and whether the intensity of the fetal inflam-
inflammatory response in cases of intraamniotic inflammation with proven infection is different from that in cases of intraamniotic inflammation with a negative AF culture.

**Material and Methods**

**Study design**

The relationship between the fetal inflammatory response and intraamniotic inflammation was examined in 89 patients who were admitted to Seoul National University Hospital between May 1993 and July 2005 with the diagnosis of preterm premature rupture of membranes who met the following criteria: (1) singleton pregnancy, (2) preterm pregnancy (gestational age between 21 and 35 weeks), (3) AF obtained for microbiologic studies by transabdominal amniocentesis or at the time of cesarean delivery, (4) umbilical cord plasma that was obtained at birth, and (5) delivery within 48 hours of amniocentesis. The last criterion was used to preserve a meaningful temporal relationship between the results of AF studies and those of the umbilical cord plasma obtained at birth. The intensity of the fetal inflammatory response was evaluated by the umbilical cord plasma concentrations of C-reactive protein (CRP), because a previous study indicated that the plasma concentration of this acute phase reactant correlated well with AF infection, congenital neonatal sepsis, and funisitis, which is a histologic hallmark of FIRS.10

Patients were divided into 3 groups according to the presence or absence of intraamniotic inflammation and AF culture results: group 1, cases without inflammation and with a negative AF culture (n = 28); group 2, cases with inflammation and with a negative AF culture (n = 26); group 3, with a positive AF culture (n = 35). Retrieval of AF and cord blood were performed after written informed consent was obtained. The institutional review board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

**AF studies**

AF was cultured for aerobic and anaerobic bacteria and for genital mycoplasmas (*Mycoplasma hominis* and *Ureaplasma urealyticum*). An aliquot of AF was transported to the laboratory and examined in a hemocytometer chamber to determine the white blood cell count. The remaining fluid was centrifuged and stored in polypropylene tubes at −70°C. Matrix metalloproteinase-8 (MMP-8) concentration was measured with a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc, Bucks, UK). The sensitivity of the test was 0.3 ng/mL. Intra- and interassay coefficients of variation were 3.1% and 9.5%, respectively. MMP-8 was used to assess the presence of intraamniotic inflammation, because previous studies indicate that it is a sensitive and specific index of inflammation.11,12

**Intraamniotic inflammation** was defined as an elevated AF MMP-8 concentration (>23 ng/mL), as previously reported.12

**Cord plasma CRP**

Umbilical cord blood was collected into ethylenediaminetetraacetic acid–containing tubes by venipuncture of the umbilical vein at birth. Samples were centrifuged, and supernatants were stored in polypropylene tubes at −70°C until assayed. CRP concentration in cord plasma was measured with a highly sensitive enzyme-linked immunoassay (Immunoagnostik AG, Bensheim, Germany). The sensitivity of the assay was 0.3 ng/mL. Intra- and interassay coefficients of variation were 5.1% and 9.6%, respectively.

**Diagnosis of funisitis and neonatal morbidity**

Funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or into Wharton’s jelly. Acute histologic chorioamnionitis was diagnosed if acute inflammatory changes were present on examination of the extraplacental membranes or the chorionic plate of the placenta, according to the criteria previously published.13 Significant neonatal morbidity was defined as the presence of any of the following conditions: congenital neonatal sepsis (proven or suspected), respiratory distress syndrome, congenital pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage (grade ≥II), or necrotizing enterocolitis. These conditions were diagnosed according to definitions previously reported in detail.13

**Statistical analysis**

Proportions were compared with the Fisher’s exact test. The Kruskal-Wallis analysis of variance test was used for comparison of continuous variables among groups. Multiple comparisons between groups were performed with the Mann-Whitney U test. A probability value of <.05 was considered significant.

**Results**

Microorganisms were isolated from the AF in 39% of cases (35/89). Microorganisms that were isolated from the amniotic cavity included *Ureaplasma urealyticum* (n = 23), *Candida* species (n = 4), *Escherichia coli* (n = 3), *Streptococcus anginosus* (n = 2), *Staphylococcus epidermidis* (n = 2), *Mycoplasma* spp (n = 2), and 1 each of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Torulopsis glabrata*, *S. mitis*, *S. agalactiae*, and *Streptococcus* spp. Six patients had ≥2 organisms that were isolated from AF.

The Table shows the clinical characteristics and pregnancy outcomes of the study population according to the groups. Patients with intraamniotic inflammation and a negative AF culture (group 2) had a significantly lower median gestational age at amniocentesis and at delivery, a lower median birthweight, a higher median white blood cell count in AF, a higher rate of Apgar score of <7 at 1 and 5 minutes, significant neonatal morbidity, bronchopulmonary dysplasia, histologic chorioamnionitis, and funisitis than did those without intraamniotic inflammation and a negative AF culture (group 1). However, there were no significant differences in the clinical characteristics and pregnancy outcomes between
cases with intraamniotic inflammation and a negative AF culture (group 2) and those with proven AF infection (group 3).

Figure 1 shows that patients with a negative AF culture and with intraamniotic inflammation (group 2) had a significantly higher median AF MMP-8 concentration than did those patients with a negative AF culture and without intraamniotic inflammation (group 1) (median, 316 ng/mL [range, 32-2875 ng/mL] vs 2 ng/mL [range, 0-23 ng/mL]; P < .001). However, there was no difference in median AF MMP-8 concentration between patients with intraamniotic inflammation and a negative AF culture (group 2) and those patients with proven

<table>
<thead>
<tr>
<th>Variable</th>
<th>AF culture</th>
<th>P value</th>
<th>AF culture</th>
<th>P value</th>
<th>AF culture</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low MMP-8*</td>
<td>(group 1, n = 28)</td>
<td></td>
<td></td>
<td>high MMP-8*</td>
<td>(group 2, n = 26)</td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>29 (21–36)</td>
<td>NS</td>
<td>31 (22–43)</td>
<td>NS</td>
<td>29 (21–37)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (wk)</td>
<td>34.2 (31.9–34.7)</td>
<td>&lt;.001</td>
<td>31.2 (24.1–35.0)</td>
<td>NS</td>
<td>31.4 (21.6–35.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>34.3 (32.0–34.9)</td>
<td>&lt;.001</td>
<td>31.4 (24.3–35.0)</td>
<td>NS</td>
<td>31.6 (21.6–35.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Amniocentesis-to-delivery interval (hr)</td>
<td>12.3 (0–43.6)</td>
<td>NS</td>
<td>10.3 (0–47.7)</td>
<td>NS</td>
<td>10.8 (0–46.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Antenatal antibiotics use (n)</td>
<td>25 (89%)</td>
<td>NS</td>
<td>24 (92%)</td>
<td>NS</td>
<td>32 (91%)</td>
<td>NS</td>
</tr>
<tr>
<td>Antenatal corticosteroid use (n)</td>
<td>10 (36%)</td>
<td>&lt;.05</td>
<td>18 (69%)</td>
<td>NS</td>
<td>17 (49%)</td>
<td>NS</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2245 (1780–3520)</td>
<td>&lt;.001</td>
<td>1690 (538–2610)</td>
<td>NS</td>
<td>1710 (360–2420)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1-Min Apgar score &lt; 7 (n)</td>
<td>7 (25%)</td>
<td>&lt;.005</td>
<td>17 (65%)</td>
<td>NS</td>
<td>23 (66%)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>5-Min Apgar score &lt; 7 (n)</td>
<td>2 (7%)</td>
<td>&lt;.005</td>
<td>11 (42%)</td>
<td>NS</td>
<td>9 (26%)</td>
<td>.09</td>
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<td>Histologic chorioamnionitis (n/N)</td>
<td>2/25 (8%)</td>
<td>&lt;.001</td>
<td>17/21 (81%)</td>
<td>NS</td>
<td>29/32 (91%)</td>
<td>&lt;.001</td>
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<tr>
<td>Funisitis (n/N)</td>
<td>1/26 (4%)</td>
<td>&lt;.001</td>
<td>12/21 (57%)</td>
<td>NS</td>
<td>23/32 (72%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Significant neonatal morbidity (n/N)</td>
<td>6/27 (22%)</td>
<td>&lt;.005</td>
<td>15/23 (65%)</td>
<td>NS</td>
<td>20/31 (65%)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Congenital sepsis: proven or suspected (n/N)</td>
<td>1/27 (4%)</td>
<td>.08</td>
<td>5/23 (22%)</td>
<td>NS</td>
<td>6/31 (19%)</td>
<td>NS</td>
</tr>
<tr>
<td>Congenital pneumonia (n/N)</td>
<td>0/27 (0%)</td>
<td>NS</td>
<td>1/23 (4%)</td>
<td>NS</td>
<td>1/31 (3%)</td>
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<td>Respiratory distress syndrome (n/N)</td>
<td>2/27 (7%)</td>
<td>NS</td>
<td>4/23 (17%)</td>
<td>NS</td>
<td>3/31 (8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (n/N)</td>
<td>0/26 (0%)</td>
<td>&lt;.01</td>
<td>6/23 (26%)</td>
<td>NS</td>
<td>4/29 (14%)</td>
<td>NS</td>
</tr>
<tr>
<td>Intraventricular hemorrhage: ≥ grade II (n/N)</td>
<td>3/27 (11%)</td>
<td>NS</td>
<td>5/23 (22%)</td>
<td>NS</td>
<td>10/31 (32%)</td>
<td>.07</td>
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<tr>
<td>Necrotizing enterocolitis (n/N)</td>
<td>0/27 (0%)</td>
<td>NS</td>
<td>1/23 (4%)</td>
<td>NS</td>
<td>1/31 (3%)</td>
<td>NS</td>
</tr>
<tr>
<td>AF white blood cell count (cells/mm³)</td>
<td>1 (0–890)</td>
<td>&lt;.001</td>
<td>100 (0–1000)</td>
<td>NS</td>
<td>&gt;1000 (0–1000)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NS, not significant.
* Low MMP-8, <23 ng/mL.
† Comparison between groups 1 and 2.
‡ High MMP-8, >23 ng/mL.
§ Comparison between groups 2 and 3.
¶ Comparison between groups 1 and 3.
Values are medians and ranges; P < .05 by Kruskal-Wallis analysis of variance test.
Eight neonates who were not actively resuscitated at birth or who died in the delivery room, despite intensive resuscitative efforts as a result of extreme prematurity or congenital anomaly, were excluded from the analysis because they could not be evaluated with respect to the presence or absence of morbidities.
AF infection (group 3; median, 284 ng/mL; range, 0-5020 ng/mL).

Figure 2 shows the CRP concentrations in umbilical cord plasma among the 3 groups. The median umbilical plasma CRP concentration in patients with a negative AF culture and intraamniotic inflammation (group 2) was significantly higher than that in those patients with a negative AF culture and without intraamniotic inflammation (group 1) (median, 43 ng/mL [range, 5-4305 ng/mL] vs 11 ng/mL [range, 2-553 ng/mL]; \( P < .005 \)). Furthermore, patients with a positive AF culture (group 3) had an even higher median umbilical cord plasma CRP concentration than those patients with a negative AF culture and intraamniotic inflammation (group 2) (median, 285 ng/mL [range, 18-4534 ng/mL] vs 43 ng/mL [range, 5-4305 ng/mL]; \( P < .05 \)).

**Comment**

Principle finding of the study

We found that (1) intraamniotic inflammation is a risk factor for the development of a fetal inflammatory response, regardless of whether microorganisms were detectable with standard cultivation techniques, and (2) microbiologically proven intraamniotic infection was associated with a greater intensity of a systemic fetal inflammatory response, as determined by plasma CRP concentrations, than intraamniotic inflammation without proven intraamniotic infection.

**Intraamniotic infection, fetal infection, and the FIRS**

Systemic fetal inflammation is often attributed to intraamniotic infection. Microorganisms that are present in the AF can gain access to the fetus by various entry points, such as respiratory tract, gastrointestinal tract, skin, and ear. Microbial products such as endotoxin or microorganisms can elicit a local inflammatory response at the site of entry (pneumonitis, enteritis, dermatitis, etc), which may progress to a systemic fetal inflammatory response. Indeed, intraamniotic infection is present in 30% of cases with preterm premature rupture of membranes, and one-third of these cases have a positive fetal blood culture. Neonates with congenital sepsis or fetuses with bacteremia have evidence of a systemic inflammatory response that is demonstrated by elevations in the concentrations of cytokines (such as IL-6 and CRP) in umbilical cord blood. Therefore, there is solid evidence that links microbial invasion of the amniotic cavity, fetal infection, and systemic fetal inflammation.

The magnitude of FIRS in patients with and without proven intraamniotic infection

We report that of all cases of funisitis, one-third had negative AF cultures before birth (Table). Because funisitis is the histologic hallmark of the FIRS, a logical question would be to evaluate the cause of the FIRS in cases with no AF infection. Two major possibilities must be considered: infection that escaped detection of traditional microbiologic methods or noninfection-related causes of fetal inflammation. Studies that used molecular microbiologic techniques indicate that culture methods underestimate the frequency of infection of the amniotic cavity.

FIRS in the absence of proven intraamniotic infection

A novel finding of this study is that, among patients with intraamniotic inflammation (defined as an elevated AF MMP-8), those patients with proven infection had a higher concentration of umbilical cord plasma CRP than did those patients with a negative AF culture. Yet, in these 2 groups, the intensity of intraamniotic inflammation was not different (Figure 1). These observations suggest that patients with positive AF cultures are more likely to have a greater degree of systemic fetal inflammation. Why? One possibility is that the microbial inoculum size of patients with a positive culture is greater than that of those patients with a negative culture and that the greater the inoculum size in the amniotic cavity, the higher the risk of fetal...
microbial invasion and the more intense FIRS. Studies are now in progress to examine this question. Alternatively, microorganisms that were recovered with culture techniques may be more virulent than those that resist cultivation in the laboratory. Such microorganisms may be more likely to invade the human fetus and elicit inflammation. It is also possible that extramniotic infection (between amnion and chorion) may elicit an inflammatory response in AF but not attack human fetus. Such an interpretation is unlikely because Andrews et al demonstrated that the magnitude of intraamniotic inflammatory response is lower in cases with infection that is confined to the extramniotic space than in those with intraamniotic infection.

Are there noninfection related causes of FIRS?
The systemic inflammatory response syndrome (SIRS) in adults frequently occurs as a consequence of a wide range of insults of noninfectious cause (eg, trauma, burns, pancreatitis). Moreover, SIRS, because of infection and noninfection-related causes, produces similar clinical features. Consequently, the possibility that fetal systemic inflammation can be caused by noninfection-related causes must be considered. We have observed that the fetal plasma concentration of IL-6 is elevated in cases of alloimmune hemolytic anemia because of Rh disease, which is a condition in which there is macrophage activation in response to antibody-coated red blood cells. The possibility that other mechanisms of disease that could lead to systemic inflammation may be operative during fetal life requires further investigation. The nature of the fetomaternal relationship in which there is bidirectional traffic of cells creates conditions that may favor immune disease and the possibility of systemic fetal inflammation.

CRP as an indicator of FIRS
Operationally, FIRS was first defined as fetal plasma IL-6 concentrations above 11 pg/mL. In the present study, we assessed the magnitude of the fetal inflammatory response using the umbilical cord plasma CRP concentration and not IL-6. CRP is an acute-phase reactant that is produced by liver cells in response to IL-6 that is released from the site of inflammation. CRP determinations are simpler to perform and more widely available than are IL-6 determinations. Moreover, there is a strong correlation between umbilical cord plasma IL-6 and CRP concentrations. Of significance is that there is a relationship between the level of plasma CRP and SIRS severity.

Unanswered questions, limitations and further considerations
Studies in adults indicate that there is disease progression that begins with SIRS and advances to sepsis, severe sepsis, and septic shock. Rangel-Frausto et al described a stepwise increase in mortality rates in these conditions (7% in SIRS, 16% in sepsis, 20% in severe sepsis, and 46% in septic shock, respectively). Of interest, positive cultures were found in 17% of cases with sepsis, in 25% of cases with severe sepsis, and in 69% of cases with septic shock. These observations may be relevant to the interpretation of our findings. Patients with positive AF cultures had a higher plasma concentration of CRP, which reflects a more severe systemic inflammatory response. However, further studies are required to explore this relationship fully. The rate of positive neonatal blood cultures in our study was very low. This may reflect treatment with antibiotics of patients with preterm premature rupture of membranes or the lack of neonatal blood cultures for genital mycoplasmas, which are the most common microorganisms that are found in the amniotic cavity. A large cohort study is required to determine whether the intensity of the fetal inflammatory response, rather than its mere presence, is associated with worse neonatal outcome.

REFERENCES


Toll-like receptors in the uterus, cervix, and placenta: is pregnancy an immunosuppressed state?

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OBJECTIVE: These studies were performed to elucidate the expression of Toll-like receptors (TLRs) in the uterus, cervix, and placenta in pregnancy and across gestation.

STUDY DESIGN: Message expressions of TLR-2, -3, -4, and -9 were investigated in nonpregnant mice and across gestation in CD-1 mice. Uterine, cervical, and placental tissues were harvested, and RNA was extracted. Quantitative polymerase chain reaction was performed.

RESULTS: Messenger RNA expression of TLRs is significantly upregulated in pregnant uterine and cervical tissues. There is differential TLR messenger RNA expression between the uterus, cervix, and placenta. In the placenta, TLR 4 is significantly downregulated.

CONCLUSION: These findings suggest that the innate immune system is a dynamic system during gestation. The concept of immunosuppression during pregnancy appears to be valid in the placenta only in regard to TLR expression. Research is warranted to determine whether the upregulation in the uterus and cervix during pregnancy is associated with an increased likelihood of responding to a pathogen or serve as a protective mechanism or both.

Key words: innate immune system, pregnancy, Toll-like receptor


The immunologic first line of defense is the innate immune system. Activation of the innate immune receptors, Toll-like receptors (TLRs), results from ligation of pathogen-associated molecular patterns. Ligation results in an inflammatory response that is generated against the invading pathogen. Activation of inflammatory pathways have been associated with adverse pregnancy outcomes that include preterm birth, intrauterine growth restriction, and preeclampsia. Studies demonstrate that TLRs are present on trophoblasts and that activation of these receptors can evoke both inflammatory and cell death pathways. Because these pathways are also implicated in preterm birth, these studies suggest a potential biologic role for TLRs in preterm birth. Mouse models of intrauterine inflammation demonstrate that the activation of TLR-4 is necessary for lipopolysaccharide or Gram-negative bacterial-induced preterm birth. Despite introductory evidence that TLRs may play a role in adverse pregnancy outcomes, there is limited information regarding the expression and regulation of TLRs in pregnancy, compared with the nonpregnant state, and whether there is differential expression of TLRs in reproductive tissues during pregnancy. These studies were preformed to elucidate the expression of TLR in the uterus and cervix in the nonpregnant, compared with pregnant, state and in the uterus, cervix, and placenta and across gestation.

Tissue collection
To determine the message expression of TLR-2, -3, -4 and -9 in the nonpregnant state and across gestation in CD-1 mice, 6 different groups, with 3-6 animals per treatment group, were used. The study groups were as follows: nonpregnant mice (not controlled by cycle), timed-pregnant mice on days 12, 16, 18, and 19 and postpartum day 0. For nonpregnant mice, the bicornuate uterus and cervix were identified and removed en bloc. All surrounding adipose tissue was removed. The fallopian tubes and ovaries were removed. The bladder and rectum were dissected off the cervix. For pregnant mice, the uterus was removed, then all gestational sacs and placentas were removed from the uterine horn. Fetal membranes were dissected off placentas before being processed. The decidua was left with the uterine specimens. The cervix was delineated and dissected from bladder and rectum. All tissues were rinsed in sterile saline solution and then flash frozen until processed for molecular experiments.

MATERIALS AND METHODS
Animals
CD-1 outbred, nonpregnant and timed-pregnant mice were purchased from Charles-Rivers Laboratories (Wilmington, MA). Animals were shipped on days 8-12 after mating. All the experiments were performed in accordance with the National Institutes of Health Guidelines on laboratory animals and with approval from the University of Pennsylvania’s Committee on Animal Use and Care.
Quantitative reverse transcriptase-polymerase chain reaction

Uterine, cervical, and placental tissues were harvested and RNA was extracted with TRIzol (Molecular Research Center, Inc, Cincinnati, OH). Complementary DNA was generated with random hexamers. Specific primers, which were conjugated to a minor groove binder probe, were purchased from Applied Biosystems Biosytems (Foster City, CA). Quantitative polymerase chain reaction (PCR) reactions were carried out with equivalent dilutions of each complementary DNA sample on the sequence detector PCR machine (Applied Model 7900; PE Applied Biosystems), as previously reported. The relative abundance of the target was normalized to the relative abundance of 18S in each sample. All samples were analyzed in duplicate.

Statistical analysis
Mean target messenger RNA (mRNA) expression between all study groups was compared by 1-way analysis of variance (ANOVA) or ANOVA on ranks, if data were nonparametric. When significance was reached (P < .05), pair-wise comparison was performed by Student-Newman-Keuls method (SNK).

Western blot analyses
To determine protein expression of TLR-2, Western blot analyses were performed with tissue homogenates from uterine and placental tissues from nonpregnant and pregnant mice (days 15 and 18). Tissues were homogenized, and the amount of crude protein that was present in each sample was determined with the Quick Start Bradford protein assay (Bio-Rad Laboratories, Hercules, CA). Twenty-five micrograms of protein were mixed with 2X sodium dodecyl sulfate sample buffer and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The separated proteins were transferred electrophoretically to nitrocellulose membranes. Membranes were blocked in tris-buffered saline solution with 5% nonfat dried milk powder for 1 hour. For TLR-2, a 1:500 dilution (#2229; Cell Signaling Technology Inc, Danvers, MA) was used. The antibody is polyclonal and cross-reacts with human, mouse, and monkey. TLR-4 (sc-10741; Santa Cruz Biotechnology Inc, Santa Cruz, CA) and TLR-9 (IMG-305A; Imgenex Corp, San Diego, CA) were used at different dilutions and were not found to be sufficiently sensitive for Western blotting in mouse samples. Vectastain Elite ABC systems from Vector Laboratories (Burlingame, CA) were used to detect the respective antigens on the nitrocellulose membrane. PMA- treated U937 and T47D cell lysate were used as a positive control for TLR expression.

Immunohistochemistry
To determine the location and expression of TLR-2 and -4 in the uterus of nonpregnant and pregnant tissues, uteri from nonpregnant (n = 3) and day 15 mice (n = 3) were harvested, flash frozen, and then prepared for immunohistochemistry studies. Frozen sections were prepared with transverse sections across the lumen. For each antibody that was investigated, 3 separate sections from 3 different dams were used. Immunohistochemistry was performed with the Vectastain Elite ABC systems (Vector Laboratories). The polyclonal antibody, TLR-2 (S-16):sc-16237, which is goat
polyclonal antibody raised against the extracellular domain of the TLR-2 of mouse origin (Santa Cruz Biotechnology, Inc). For TLR-4, we used TLR-4 (H-80; sc-10741; Santa Cruz Biotechnology, Inc), which is a rabbit polyclonal antibody raised against the amino acids 242-321 of the TLR-4 of human origin. The antibody has species cross-reactivity with mouse and rat. Immunohistochemistry was performed with these antibodies at a dilution of 1:50. A negative control was performed at the time with serum without antibody that revealed no staining.

**RESULTS**

**Quantitative reverse transcriptase-PCR**

Results from our quantitative PCR experiment demonstrate that TLRs are expressed differentially in nonpregnant, compared with pregnant, tissues (Figures 1 and 2). In the uterus, TLR-2, -3, -4, and -9 mRNA is increased significantly throughout gestation in the uterus (1-way ANOVA, \( P < 0.001 \), and .002, respectively; Figure 1). TLR-2 mRNA is increased 4.6-fold \( (P = .091, \text{SNK}) \); TLR-3 mRNA is increased 2.6-fold \( (P = .025, \text{SNK}) \); TLR-4 is increased 7.0-fold \( (P < .001, \text{SNK}) \), and TLR-9 is increased 4.2-fold \( (P = .012, \text{SNK}) \) on day 19 (term), compared with nonpregnant tissue.

In the cervix, TLR-2, -3, and -4 were expressed differentially between the study groups (1-way ANOVA, \( P = .014, .002, \) and .005, respectively). TLR-9 mRNA expression was not statistically significant between the groups \( (P = .06) \). With pair-wise comparison (Student-Newman-Keuls method), TLR-2 expression in the nonpregnant state was significantly lower from days 16 and 18 \( (P = .013 \) and .039, respectively). TLR-3 expression in the nonpregnant state and days 12, 18, and 19 postpartum day 0 was significantly lower from day 16 \( (P = .002, .007, .005, .006, \) and .005, respectively). TLR-4 expression in the nonpregnant state is significantly lower from days 12, 16, 18, and 19 postpartum day 0 \( (P = .017, .019, .003, .041, \) and .046, respectively).

**FIGURE 2**

Results from quantitative polymerase chain reaction experiments that demonstrate TLR expression in the cervix throughout mouse gestation

Each bar represents the mean ± SD of messenger RNA from 3-6 specimens for each gestational time point. TLR-2, -3, and -4 were expressed differentially between the study groups (1-way ANOVA, \( P = .014, .002, \) and .005, respectively). TLR-9 mRNA expression was not statistically significant between the groups \( (P = .06) \). With pair-wise comparison (Student-Newman-Keuls method), TLR-2 expression in the nonpregnant state was significantly lower from days 16 and 18 \( (P = .013 \) and .039, respectively). TLR-3 expression in the nonpregnant state and days 12, 18, and 19 postpartum day 0 was significantly lower from day 16 \( (P = .002, .007, .005, .006, \) and .005, respectively). TLR-4 expression in the nonpregnant state is significantly lower from days 12, 16, 18, and 19 postpartum day 0 \( (P = .017, .019, .003, .041, \) and .046, respectively).


**FIGURE 3**

Results from quantitative PCR experiments that demonstrate TLR expression in the placenta throughout mouse gestation

Each bar represents the mean ± SD of messenger RNA from 3-6 specimens for each gestational time point. TLR-2, -3, and -9 mRNA were not expressed differentially through gestation (ANOVA on ranks, \( P = .41, .40, \) and .55, respectively). TLR-4 mRNA was regulated differentially through gestation (1-way ANOVA, \( P = .015 \)). With pair-wise comparison (Student-Newman-Keuls method), TLR-4 expression in day 18 was significantly lower than on day 12 \( (P = .025) \).

ential through gestation (1-way ANOVA, \( P = .015 \)). TLR-4 mRNA expression on day 19 was decreased significantly, compared with day 12 (\( P = .021 \), SNK).

**Western blots**

Western blotting confirmed the presence of TLR-2 in the uterine tissue in the nonpregnant mouse and pregnant uterus on days 15 and 18 (Figure 4). In addition, Western blotting confirms the presence of TLR-2 in the placenta on days 15 and 18 (Figure 5). The specificity of the TLR-2 antibody is noted in Figure 5, with the expected molecular weight between 80-85 kD as observed in control cell line U937 (monocyte/macrophage). The same band can be observed in the uterus (E15) and placenta. Although protein loading was equal, actin blotting demonstrates differences in transferred protein. Understanding this, there does appear to be an increase in TLR-2 protein expression compared with nonpregnant tissue (Figure 4).

**Immunohistochemistry**

Immunohistochemistry studies demonstrated the expression of TLR-2 and TLR-4 in the uterus in both the nonpregnant and pregnant state. Localization and intensity of TLR-2 and TLR-4 staining in uterine tissues can be observed in representative sections of nonpregnant and pregnant (E15) tissues (Figures 6 and 7). During pregnancy, increased staining is observed in the smooth muscle compared with nonpregnant muscle. There also appears to be an increase in intensity of staining within the glandular tissue. These findings are consistent with quantitative PCR experiments.

**COMMENT**

The maternal–fetal interface is a unique site, as the role of the immune system is dichotomous. The immune system must promote tolerance of the allogenic fetus, while protecting the host from infections. The observations in our experiments could contribute to understanding this dichotomous phenomenon. Pregnancy resulted in a consistent up-regulation of TLRs in the uterus and cer-
vix. The observed changes in the regulation of the female reproductive tract during pregnancy could correspond to an enhanced pathogen clearance or to create an inflammatory response, depending on the TLR that is activated. TLR ligation leads to activation of NF-κB, which is a transcription factor that is involved in the expression of proinflammatory cytokines, chemokines, and antimicrobial peptides. TLRs in epithelial cells of the female reproductive tract and the maternal-fetal interface suggest that these cells can recognize and respond to the presence of organisms and coordinate the immune response. The upregulation of TLR mRNA that was observed in our experiments does not appear to be nonspecific because (1) there was no universal upregulation of TLRs as demonstrated in the placenta, (2) a different pattern mRNA expression for different TLRs occurred within the same tissue, and (3) a differential mRNA expression of the same TLR was observed in the different tissues that were studied.

These studies have limitations. Our study is unable to determine whether the dramatic changes in TLR mRNA expression have a functional significance. The lack of commercially available antibodies that are useful for Western blotting limits our ability to demonstrate a correlation in the upregulation of protein that is consistent with observed mRNA changes. Another limitation is that these studies are performed in the mouse. Although the mouse is a well-tested species for human immunologic conditions, future studies are required to correlate these findings in human pregnancy.

These studies do demonstrate that the increase in TLR mRNA expression may be due, in part, to an upregulation of TLRs in the smooth muscle of the uterus, as suggested by our immunohistochemistry results. However, these studies cannot rule out whether the increase in TLR mRNA expression in the uterus and cervix is also the result of the influx of other cells (ie, leukocytes) into these tissues with pregnancy. However, if this observed increase in TLR does indeed have a functional effect, then the cell of origin becomes less clinically significant. Pregnancy has long been considered a state of immunosuppression on the basis of the premise that the host (mother) must tolerate an antigenic different organism (fetus). In regard to the key modulators of the innate immune response, our data suggest that this may be a valid concept in regard to the placenta. However, our data demonstrate that, in regard to the uterus and cervix, the innate immune response is dramatically upregulated.

Expression of TLRs at the maternal-fetal interface has been hypothesized to play a crucial role in the pathogenesis of adverse pregnancy outcomes. These theories suggest that infections during pregnancy can activate TLR on the trophoblast and, depending on which TLR is activated, dictates trophoblast survival and may contribute to adverse pregnancy outcome. Elevated trophoblast apoptosis is observed during the first trimester of human pregnancies that are complicated with intrauterine growth restriction and preeclampsia. In the problem of preterm birth, TLRs appear to be obligatory mediators, considering the overwhelming evidence that demonstrates an association of inflammation with this adverse outcome. If TLRs in the uterus, cervix, and/or placenta mediate these effects, then our findings of differential TLR expression could play a role in the adverse outcomes. Known genetic differences in TLRs and/or downstream mediators of TLR activation may explain variability in maternal responses to infection and could contribute to adverse pregnancy outcomes.

Our findings suggest the innate immune system is an active and dynamic system during gestation. There are limited data about the role of TLRs in pregnancy. Understanding the role of the innate immune response in normal pregnancy is necessary if we are to understand how disruptions and/or alterations in the host immune response result in adverse pregnancy outcomes. Because there is known modulation of the immune response, this line of research has the potential to create new therapeutic and preventive strategies to decrease adverse pregnancy outcomes that are associated with inflammation.

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A French randomized controlled trial of ST-segment analysis in a population with abnormal cardiotocograms during labor

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OBJECTIVE: The purpose of this study was to assess whether knowledge of ST-segment analysis was associated with a reduction in operative deliveries for nonreassuring fetal status (NRFS) or with a need for at least 1 scalp pH during labor.

STUDY DESIGN: Seven hundred ninety-nine women at term with abnormal cardiotocography or meconium-stained amniotic fluid (7%) were assigned randomly to the intervention group (cardiotocography + STAN) or the control group (cardiotocography) in 2 university hospitals in Strasbourg, France. Scalp pH testing was optional in both groups. Abnormal neonatal outcome was pH <7.05 or umbilical cord blood artery base deficit of >12 or a 5-min Apgar score of <7 or neonatal intensive care unit admission or convulsions or neonatal death. Study power was 80% for the detection of a prespecified reduction from 50%-40% in operative delivery for NRFS.

RESULTS: The operative delivery (cesarean or instrumental) rate for NRFS did not differ between the 2 groups: 33.6% (134/399) in the cardiotocography + STAN analysis group vs 37% (148/400) in the cardiotocography group (relative risk, 0.91; 95% CI, 0.75-1.10). The rate of operative delivery for dystocia was also similar in both groups. The percentage of women whose fetus had at least 1 scalp pH measurement during labor was substantially lower in the group with ST-segment analysis: 27% compared with 62% (relative risk, 0.44; 95% CI, 0.36-0.52). Neonatal outcomes did not differ significantly between groups.

CONCLUSION: In a population with abnormal cardiotocography in labor, cardiotocography combined with ST-segment analysis was not associated with a reduction in operative deliveries for NRFS. The proportion of infants without scalp pH sampling during labor increased substantially, however.

Key words: cardiotocography, intrapartum monitoring, scalp pH, ST-segment analysis


Supplementary fetal surveillance in cases of abnormal cardiotocography has been a priority topic for research in recent years. Combining another continuous monitoring method with cardiotocography during labor might help to improve the diagnostic value of acidosis. Pulse oximetry was first tested during the late 1990s, and the US controlled trial by Garite et al generated substantial expectations. It reported a reduction in the rate of cesarean delivery for nonreassuring fetal status (NRFS; from 10.2%-4.5%; \( P = .007 \)). On the other hand, the overall cesarean delivery rate did not differ between the groups, because of the increased rate of cesarean delivery for dystocia in the oximetry group. More recently, an National Institute of Child Health and Human Development–sponsored multicenter trial of >5000 women demonstrated that fetal oxygen saturation was not associated with either a reduction in the rate of cesarean delivery or with improvement in the condition of the newborn infant.2

ST-segment analysis with the STAN S21 (Neoventa Medical, Göteborg, Sweden) is another continuous surveillance method. The Swedish trial included approximately 5000 women and found that this method both diagnosed metabolic acidosis better than cardiotocography alone and diminished the rate of operative delivery in a high-risk population.3 Ojala et al4 found, on the contrary, that ST-segment analysis in an unselected population was not associated with a decrease in either operative delivery or acidosis at birth (pH <7.10).

The cesarean delivery rate has increased substantially over the last decade, in France as in other developed countries.5 The purpose of this study was to examine whether ST-segment analysis leads to a decrease in the rate of operative delivery in a population with abnormal cardiotocography during labor or a decrease in the rate of scalp pH.
Materials and Methods

After review and approval of this study by the applicable institutional review board (CCPPRB), this randomized controlled trial took place from February 2004 through May 2006 in 2 French maternity wards in Strasbourg (Centre Medico-Chirurgical et Obstétrical [CMCO] with 1800 deliveries annually and Hautepierre with 2700 deliveries annually). Women received information about this study during a routine consultation during the third trimester. The population consisted of women in labor with a term (≥36 gestational weeks) singleton fetus in cephalic presentation who met the following inclusion criteria: abnormal cardiotocography or thick meconium-stained amniotic fluid (7%) during labor. Abnormal cardiotocography was defined according to the International Federation of Gynecology and Obstetrics (FIGO) classification. It included abnormal and intermediary cardiotocography, irrespective of the type of anomaly (baseline heart frequency, variability, reactivity, or deceleration) and normal cardiotocography with uncomplicated variable deceleration with a duration of <60 seconds and loss of <60 beats. The exclusion criteria were gestational age <36 weeks, normal cardiotocography without deceleration during labor, maternal infection contraindicating placement of scalp electrodes (seropositive for HIV or hepatitis B or C) cardiac malformation, severe decelerations with variability reduced immediately on entry into the delivery room, and refusal to participate.

Randomization to monitoring with cardiotocography only or cardiotocography + STAN took place after rupture of the membranes and verification of inclusion and exclusion criteria that included the provision of written informed consent. The midwife who treated the women during labor opened an opaque numbered sealed envelope at randomization. In the STAN group, fetuses were monitored continuously through a scalp electrode and recommendations for delivery were based on STAN guidelines. The current guidelines and the automatic log function have been described elsewhere.3

Scalp pH testing was optional in both groups. If scalp blood pH was <7.20, immediate delivery was recommended, in accordance with our current labor ward protocol.

As soon as possible after birth, the umbilical cord was clamped doubly, and (within 15 minutes) umbilical cord blood artery and vein gases were sampled with 3-mL preheparinized plastic syringes and analyzed with a Rapidlab 248 blood gas analyzer (Bayer Diagnostics Europe, Saint Vulbas, France). We used the Siggaard-Andersen algorithm to calculate the umbilical cord blood artery base deficit (BD_{ecf}).8 Late clamping of the cord at birth is considered unlikely to explain acidosis findings when arterial pCO_{2} (KPa) minus venous pCO_{2} (KPa) is >0.5.9,10

The delivery room staff during the trial included 109 doctors and midwives. They received training in the use of the STAN equipment from a specially trained and experienced team of 3 doctors and 3 midwives. Their training followed a protocol that was similar to the Swedish training protocol described elsewhere.3 During the trial, each new midwife and resident was trained immediately by the same team. A research resident who was not involved in the study verified data during the study. Monthly meetings were organized for the follow-up evaluation.

We used 2 STAN S21 machines in each center. For monitoring in the cardiotocography group, we used devices from Hewlett Packard (8030A; Hewlett Packard Company, Palo Alto, CA) and Philips Medical Systems (Boeblingen, Germany). Paper speed was set at 1 cm/min, as in most European countries. The principal outcome measure was rate of operative delivery (assisted vaginal birth or cesarean delivery) for NRFS. Secondary outcome criteria were total rate of operative delivery (irrespective of cause), percentage of cases with at least 1 scalp pH test during labor, and abnormal neonate outcome (pH <7.05 or BD_{ecf} >12 mmol/L or 5-minute Apgar score <7 or neonatal intensive care unit admission or convulsions or neonatal death). Finally, we examined the percentage of infants with 1 specific abnormal outcome: metabolic acidosis (pH <7.05 and BD_{ecf} >12 mmol/L).

Envelopes for randomization, stratified by center, were prepared at the Delegation of Research Unit. No intermediate analysis was planned. Calculation of sample size determined that, for a power of 80%, with alpha at .05 (bilateral test), 774 women were required to detect a reduction from 50%-40% in the number of operative deliveries for NRFS and 710 women to detect a reduction from 40%-30%.11 With 774 women, we had a power of 99% to detect a reduction from 50%-35% in the rate of deliveries with at least 1 scalp pH measurement. We planned to include 800 women to leave a margin of roughly 3% for missing data, especially because of any technical problems.

Data are described as proportions for qualitative variables and means ± SE for quantitative variables. Comparisons used Χ² test for qualitative variables and Mann-Whitney test for quantitative variables. Results are analyzed according to intention-to-treat and expressed as relative risk (RR) with 95% CI. We used mainly Bayesian methods, as described by Hornbuckle et al12 and Spiegelhalter et al.,13 and built models to predict outcome with Bayesian model averaging (BMA). The principles and technical details are described by Hoeting et al.15 BMA gives the probability of a true effect. Variables that were included were gender (cardiotocography + STAN/cardiotocography), center (CMCO/Hautepierre), maternal age (years), parity (0/≥1), previous cesarean delivery (yes/no), maternal disorder (hypertension or diabetes mellitus or intrauterine growth restriction or postterm or premature rupture of membranes), induction of labor (yes/no), reason for inclusion (cardiotocography/thick meconium-stained amniotic fluid/both), FIGO class (0/1/2), dilation at randomization (centimeters), epidural (yes/no), length of tracing (minutes), gestational age at birth (weeks), and macrosomia (yes/no). Results for these models are expressed for each variable as the posterior probability of a nonnull effect and the posterior odds.
Results

From February 2004 through May 2006, the 2 participating centers admitted 9631 pregnant women already or subsequently during that admission in labor. In all, 8745 women were excluded, and 87 women declined to participate. Finally, the study included 799 women, 399 women in the cardiotocography group (RR, 0.91 (95% CI, 0.75-1.10)). The rate of operative delivery for dystocia was also similar in both groups. The percentage of women whose fetus had at least 1 scalp blood pH measurement during labor was substantially lower in the group with STAN:

### TABLE 1
**Population characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cardiotocography + STAN (n = 399)</th>
<th>Cardiotocography (n = 400)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>29.8 ± 5.7</td>
<td>30.1 ± 5.7</td>
<td>.5</td>
</tr>
<tr>
<td>Nulliparous†</td>
<td>288 (72.2 [67.8-76.6])</td>
<td>287 (71.8 [67.3-76.2])</td>
<td>.9</td>
</tr>
<tr>
<td>Previous cesarean delivery‡</td>
<td>25/396 (6.3 [3.9-8.7])</td>
<td>24/399 (6.0 [3.7-8.3])</td>
<td>.9</td>
</tr>
<tr>
<td>Maternal disorder‡</td>
<td>168/396 (42.4 [37.6-47.3])</td>
<td>170/400 (42.5 [37.7-47.3])</td>
<td>.9</td>
</tr>
<tr>
<td>Induction of labor†</td>
<td>34 (8.5 [5.8-11.3])</td>
<td>35 (8.8 [6.0-11.5])</td>
<td>1</td>
</tr>
<tr>
<td>Inclusion for thick meconium-stained amniotic fluid†</td>
<td>26 (6.5 [4.1-9.0])</td>
<td>28 (7.0 [4.5-9.5])</td>
<td>.6</td>
</tr>
<tr>
<td>Inclusion for abnormal cardiotocography‡</td>
<td>348 (87.4 [84.2-90.7])</td>
<td>341 (85.3 [81.8-88.7])</td>
<td></td>
</tr>
<tr>
<td>Inclusion for both reasons‡</td>
<td>24 (6.0 [3.7-8.4])</td>
<td>31 (7.8 [5.1-10.4])</td>
<td></td>
</tr>
<tr>
<td>FIGO at randomization class 0†</td>
<td>98 (24.9 [20.7-29.2])</td>
<td>85 (21.9 [17.8-26.0])</td>
<td>.2</td>
</tr>
<tr>
<td>FIGO at randomization class 1‡</td>
<td>230 (58.5 [53.7-63.4])</td>
<td>252 (64.9 [60.2-69.7])</td>
<td></td>
</tr>
<tr>
<td>FIGO at randomization class 2‡</td>
<td>65 (16.5 [12.9-20.2])</td>
<td>52 (13.0 [9.9-16.7])</td>
<td></td>
</tr>
<tr>
<td>Dilation at randomization (cm)*</td>
<td>5.8 ± 2.4</td>
<td>5.5 ± 2.3</td>
<td>.07</td>
</tr>
<tr>
<td>Epidural‡</td>
<td>364 (91.2 [88.5-94.0])</td>
<td>361 (90.3 [87.3-93.2])</td>
<td>.6</td>
</tr>
<tr>
<td>Length of tracing (min)*</td>
<td>145 ± 121</td>
<td>143 ± 124</td>
<td>.8</td>
</tr>
<tr>
<td>Gestational age at birth (wk)*</td>
<td>40.1 ± 1.3</td>
<td>40.1 ± 1.3</td>
<td>1</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>3250 ± 462</td>
<td>3247 ± 491</td>
<td>1</td>
</tr>
<tr>
<td>Macrosomia†</td>
<td>21 (5.3 [3.1-7.5])</td>
<td>22 (5.5 [3.3-7.7])</td>
<td>1</td>
</tr>
</tbody>
</table>

* Data are presented as median ± SD.
† Maternal disorder included hypertension, diabetes mellitus, intrauterine growth restriction, postterm, and premature rupture of membranes.
‡ Class 0 is normal; class 1 is intermediary, and class 2 is abnormal in the FIGO classification.

### TABLE 2
**Results of maternal outcomes in each group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiotocography + ST (n = 399)</th>
<th>Cardiotocography (n = 400)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative delivery for NFRS</td>
<td>134 (33.6 [28.9-38.2])</td>
<td>148 (37.0 [32.4-41.7])</td>
<td>0.91 (0.75-1.10)</td>
</tr>
<tr>
<td>Assisted vaginal birth</td>
<td>80 (20.1 [16.1-24.0])</td>
<td>83 (20.8 [16.8-24.7])</td>
<td></td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>54 (13.5 [10.2-16.9])</td>
<td>65 (16.3 [12.6-19.9])</td>
<td></td>
</tr>
<tr>
<td>Operative delivery TOTAL</td>
<td>216 (54.1 [49.2-59.0])</td>
<td>221 (55.3 [50.4-60.1])</td>
<td>0.98 (0.86-1.11)</td>
</tr>
<tr>
<td>≥1 scalp pH during labor</td>
<td>108 (27.1 [22.7-31.4])</td>
<td>248 (62.0 [57.2-66.8])</td>
<td>0.44 (0.36-0.52)</td>
</tr>
</tbody>
</table>

Data are presented as n (% [95% CI]).

Operative delivery (cesarean or instrumental) rate for NFRS did not differ between the 2 groups: 33.6% (134/399) in the cardiotocography + STAN group vs 37% (148/400) in the cardiotocography group (RR, 0.91 (95% CI, 0.75-1.10)). The rate of operative delivery for dystocia was also similar in both groups. The percentage of women whose fetus had at least 1 scalp blood pH measurement during labor was substantially lower in the group with STAN:
levolved at full dilation of the cervix. Immediate cesarean delivery for uterine rupture could not prevent the catastrophic consequences, and the infant died on day 23 in the neonatal intensive care unit.

Multivariate analysis with BMA showed that only 4 variables were associated with a higher probability of operative delivery for NRFS: nulliparity, FIGO class, dilation at randomization, and, to a lesser extent, maternal age (Table 4). The posterior probability of a nonnull effect of STAN (study group) was only 3.0%, with a posterior odds ratio at 1.00 (95% CI, 0.92-1.07). When a previous probability of 90% or 99% was specified, the posterior probability was 30.3% and 87.6%, respectively. That is, the posterior probability of a STAN effect was smaller after the trial than before, which indicates the absence of an effect by the intervention. Similarly, the posterior probability of a nonnull effect of the intervention on total cesarean deliveries was only 0.6%. This finding confirms the lack of effect. For a previous probability of 99%, the posterior probability decreased to 83.7%.

**Comment**

In our population with abnormal cardiotocography in labor, intrapartum ST-segment analysis was not associated with a decrease in the rate of operative delivery for NRFS but was associated with a substantially decreased need to test scalp pH during labor. Our results were disappointing in comparison with those from the first 2 trials of the STAN. The Plymouth trial studied 2434 women in high-risk labor in 1 center and observed a 46% (9.2%-5.1%) decrease in operative deliveries for fetal distress in the STAN group (P < .001). There was a trend towards less metabolic acidosis in the STAN arm. ST analyses in that study were performed without the automatic log procedure, which is now included in the STAN 21 that we used. The Swedish trial of 4966 women in high-risk labor in 3 major obstetrics departments found a significant, but nonetheless somewhat small, reduction in the rate of operative delivery for fetal distress in the STAN group compared with the cardiotocography group (9%-8%; P = .047). The decrease in the metabolic acidosis rate was more important (2%-0.7%; P = .02). Our population was at higher risk than either of these populations, because STAN was considered only for abnormal cardiotocography or thick meconium or both. Maternal disorders, epidurals, and induction were not inclusion criteria in our study.

More recently, in a general population, Ojala et al did not find a lower rate of operative delivery for NRFS in the STAN group. Bayesian models of our data show that the probability that cardiotocography + STAN would prevent unnecessary cesarean delivery effectively was small. All the models showed that this probability decreased such that even with a very high previous probability of

### Table 3

Results of neonatal outcomes in each group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiotocography + ST (n = 399)</th>
<th>Cardiotocography (n = 400)</th>
<th>RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal neonatal outcome*</td>
<td>26/372 (7.0 [4.6-10.1])</td>
<td>24/377 (6.4 [4.1-9.3])</td>
<td>1.10 (0.64-1.88)</td>
</tr>
<tr>
<td>pH &lt; 7.05 (n)</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>BD_{ecf}&gt;12 (n)†</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5min Apgar score &lt; 7 (n)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Neonatal intensive care unit admission (n)</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Convolusions (n)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neonatal death (n)</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as n/N (% [95% CI]).
† Cases for which late clamping is unlikely (arterial pCO2 [KPa] – venous pCO2 [KPa] is > 0.5).
an effect (optimistic prior, 99% probability of true effect), the posterior probability is only 88.2, which is not sufficient to warrant its use in everyday practice. These results strongly suggest that STAN is not useful in reducing operative deliveries.

All published trials that used the STAN method permitted scalp pH testing. Analysis of these trials suggests that the rate of scalp pH testing in the cardiotocography group appears to determine whether the rate of operative delivery for fetal distress decreases with ST-segment analysis. A reasonable step in using STAN equipment should thus focus on decreasing metabolic acidosis and encephalopathy that results from hypoxia rather than operative delivery. Another important step is to confirm its performance at centers that do not use scalp pH testing. We substantially decreased the rate of scalp pH in our high-risk population without any negative impact on outcome, but the power of our study was not sufficient to detect a potential unfavorable outcome that is related to metabolic acidosis.

Our study has several limitations that must be considered. First, it was not powerful enough to address the question of metabolic acidosis, and the lack of difference between the groups for unfavorable fetal outcomes must also be interpreted with caution. Second, scalp pH tests were performed liberally in our population (63% in the cardiotocography group). This raises the question of whether these results can be generalized reasonably to centers with different practices.

In conclusion, in a population with abnormal cardiotocography in labor, ST-segment analysis that is used together with cardiotocography did not reduce...
the rate of operative delivery. In a setting where scalp pH was freely used, ST-segment analysis did decrease substantially the need for this procedure.

ACKNOWLEDGMENTS
We thank all midwives and obstetricians of the Departments of Obstetrics and Gynecology of CMCO and Hautepierre in Strasbourg for their cooperation in the study and Sylvie Metzger for supervising the data collection.

REFERENCES
Intrapartum electronic fetal heart rate monitoring and the identification of metabolic acidosis and hypoxic-ischemic encephalopathy

Joel D. Larma, MD; Anadir M. Silva, MD; Cynthia J. Holcroft, MD; Richard E. Thompson, PhD; Pamela K. Donohue, ScD; Ernest M. Graham, MD

OBJECTIVE: The purpose of this study was to determine whether electronic fetal monitoring can identify fetuses with metabolic acidosis and hypoxic-ischemic encephalopathy.

STUDY DESIGN: The cases were 107 nonanomalous chromosomally normal fetuses with an umbilical arterial pH < 7.0 and base excess ≤ 12 mmol/L. Controls were the subsequent delivery that was matched by gestational age and mode of delivery. The last hour of electronic fetal monitoring before delivery was evaluated by 3 obstetricians who were blinded to outcome.

RESULTS: Cases had a significant increase in late and prolonged decelerations/hour and late decelerations/contractions. Those fetuses with hypoxic-ischemic encephalopathy had significant increases in bradycardia, decreased variability, and nonreactivity but no difference in late or variable decelerations/hour. For the identification of hypoxic-ischemic encephalopathy, the sensitivity, specificity, and positive and negative predictive values were 15.4%, 98.9%, 66.7%, and 89.4%, respectively, for bradycardia; 53.8%, 79.8%, 26.9%, and 92.6%, respectively, for decreased variability; 92.3%, 61.7%, 2.7%, and 82.9%, respectively, for nonreactivity; and 7.7%, 98.9%, 50.0%, and 88.6%, respectively, for all 3 abnormalities combined.

CONCLUSION: Fetal metabolic acidosis and hypoxic-ischemic encephalopathy are associated with significant increases in electronic fetal monitoring abnormalities, but their predictive ability to identify these conditions is low.

Key words: electronic fetal monitoring, fetal acidosis, hypoxic-ischemic encephalopathy


Although electronic fetal monitoring (EFM) has been found to have a low predictive ability in the identification of cerebral palsy1 and periventricular leukomalacia,2 most of these neurologic abnormalities are not hypoxic in origin. The causes of neonatal encephalopathy are heterogeneous, with hypoxic-ischemic encephalopathy (HIE) comprising a subset of < 10% of all cases of neonatal encephalopathy.3 Since EFM was introduced as a screening test to identify intrapartum hypoxia-ischemia that was severe enough to lead to neurologic injury or death, EFM might be shown to have a higher predictive value when reserved for brain injury cases that are hypoxic in origin. Many nonspecific markers have been used to attempt to define the presence of intrapartum hypoxia-ischemia, including meconium-stained fluid, abnormal fetal heart rate patterns, and low Apgar scores. An umbilical arterial pH < 7.0 and base excess ≤ 12 mmol/L are more objective measures of intrapartum hypoxia-ischemia, because depletion of oxygen in brain tissue (hypoxia) will not occur without depletion of oxygen in blood (hypoxemia).4 Fetal metabolic acidosis of this severity is rare, occurring in only 3 in 1000 deliveries,5 and has been associated with an increased risk for neonatal hypotonia and seizures.6 When EFM was introduced clinically in the late 1960s, it was assumed that at least one-half of the cases of childhood neurologic injury were related to intrapartum hypoxia;7 however, subsequent research has shown that the prevalence of the target disorder for EFM is much lower,8 which may explain the poor predictive ability of EFM to identify neurologic injury. Our objective in this study was to determine whether EFM can identify the fetus with metabolic acidosis and HIE by focusing on cases with intrapartum hypoxia-ischemia, as objectively measured by an umbilical arterial gas at birth.

MATERIALS AND METHODS
This was an institutional review board–approved case-control study that identified all infants who were born with metabolic acidosis (umbilical arterial pH < 7.0 and base excess ≤ 12 mmol/L), which is an essential criteria for the diagnosis of intrapartum hypoxia-ischemia that is severe enough to lead to neurologic injury, at this single university hospital between April 1991 and February...
Infants with major congenital malformations and chromosomal abnormalities were excluded. There were 107 cases that met these inclusion criteria that were matched to 107 control infants with an umbilical arterial pH of >7.0 at birth, with the subsequent delivery matched by gestational age within 7 days and mode of delivery. The last hour of EFM before delivery was reviewed by 3 obstetricians, who were blinded to outcome and who used the guidelines that were developed by the National Institute of Child Health and Human Development (NICHD) research planning workshop. The 3 reviewers were a chief resident who was pursuing a fellowship in maternal-fetal medicine (J.D.L.), a third-year fellow in maternal-fetal medicine (A.M.S.), and a board-certified maternal-fetal medicine attending (E.M.G.). Each reviewer recorded the baseline fetal heart rate (FHR), the time with FHR of >160 beats/min (tachycardia) or <110 beats/min (bradycardia), the number of accelerations, reactivity, the total number of decelerations, and the number of late, variable, or early decelerations. Reactivity was defined as the presence of at least 2 FHR accelerations that peak (but do not necessarily remain) at least 15 beats/min above the baseline and last 15 seconds from baseline to baseline within a 20-minute period. Variability was classified by 4 grades: grade 1 indicated undetectable variability, grade 2 indicated minimal variability with amplitude range of ≤5 beats/min, grade 3 indicated moderate variability with amplitude range from 6-25 beats/min, and grade 4 indicated marked variability with amplitude range of >25 beats/min. Grades 1 and 2 were considered decreased variability. Severe variable decelerations were those with a drop to <70 beats/min or lasting >60 seconds. The number of prolonged decelerations that lasted 2-10 minutes and the nadir and length of the most severe prolonged deceleration were recorded. The number of contractions in the last hour before delivery was counted, and the ratio of late decelerations/contractions and variable decelerations/contractions was expressed as a percentage. Cases and control infants were then compared with respect to these various EFM parameters.

In addition to determining the predictive value of EFM in identifying the presence of metabolic acidosis, we wished to determine whether EFM could identify those infants with HIE. Sarnat and Sarnat have divided postanoxic encephalopathy into 3 stages, with stage 1 lasting <24 hours and consisting of hyperalertness with normal electroencephalogram (EEG); stage 2 marked by obtundation, hypotonia, strong distal flexion, and multifocal seizures; and stage 3 marked by flaccidity with suppressed brain stem and autonomic functions. This staging system for postanoxic encephalopathy makes no attempt to define hypoxia. Within the case group, all of whom had an umbilical arterial pH of <7.0 and base excess of ≤12 mmol/L, there were 13 neonates with neurologic complications, which included 8 neonates with seizures, 1 neonate with bilateral 3rd intraventricular hemorrhage, and 4 neonates who died. All 13 of the neonates with severe metabolic acidosis and neurologic morbidity or death had clinical features that were consistent with at least Sarnat stage 2. All these neonates were diagnosed with HIE by the neonatologist who managed their case. The EFM tracings of these 13 infants were compared with the other 94 infants with metabolic acidosis who were without neurologic injury.

Intrauterine growth restriction was defined as a birthweight <10th percentile for gestational age. The clinical diagnosis of chorioamnionitis was made when there was maternal fever, with the presence of at least 1 other finding of fetal tachycardia, uterine tenderness, or purulent vaginal discharge. The placentas for these infants were examined by an attending pathologist within our institution, and histologic chorioamnionitis was diagnosed when any polymorphonuclear leukocytes were seen in either the chorion or amnion or in significant amounts in the subchorionic space. Histologic funisitis was diagnosed when polymorphonuclear leukocytes were seen in the umbilical cord. Placental infarcts were defined as focal lesions that were identified macroscopically in which more than one-half the cross-sectional area showed the classic histologic appearance of necrosis of all cellular elements of the villi and collapse of the intervillous space, with consequent aggregation of villi. Respiratory distress syndrome was defined as a requirement for mechanical ventilation for >24 hours and renal dysfunction as urine output of <1 mL/kg/hr after the first 24 hours or serum creatinine level of >1.2 mg/dL. The readings of each EFM tracing by the 3 obstetrician reviewers were averaged. We calculated kappa statistics to assess the interobserver reliability in classifying the categoric EFM parameter reactivity and variability. Kappa values of >0.75 represent excellent reproducibility, 0.4-0.75 represent good reproducibility, and <0.42 represent marginal reproducibility. We calculated the Pearson correlation coefficient to assess the interobserver reliability in the classification of continuous EFM parameters, such as FHR baseline, accelerations, and decelerations. Because each case with metabolic acidosis was matched in a 1:1 fashion by gestational age and mode of delivery to a control infant with a normal cord gas (pH > 7.0), the means of continuous variables were compared with the use of a paired t-test; categoric variables were compared with the use of McNemar’s test, with a probability value of <.05 considered significant. The Wilcoxon matched pair signed rank test was used to compare gravidity and parity. Linear regression was used to examine the relationship among the number of late decelerations, which indicate utero-placental insufficiency, during the hour before delivery and umbilical arterial pH and base excess. Within the 107 cases with metabolic acidosis, the 13 neurologically injured neonates with HIE were compared with the 94 neonates without injury with the use of independent sample t-tests and chi-square test. Multivariable logistic regression models were used to determine the predictive ability of EFM to identify metabolic acidosis and HIE. When infants with metabolic acidosis were compared with those infants without metabolic acidosis, the regression models included adjustments for...
gestational age and mode of delivery. For each model, receiver operator characteristic (ROC) curves were produced. The area under the ROC curve represents the predictive power of the model to identify metabolic acidosis or HIE, for which values close to 0.5 represent little predictive power and values close to 1 represent strong predictive power. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the EFM abnormalities that were statistically significant. Analyses were performed with Stata software (version 7.0; Stata Corp, College Station, TX).

### RESULTS

The gestational age distribution for each group included 68 infants (64%) who were born at ≥37 weeks of gestation, 32 infants (30%) who were born at 29-36 weeks of gestation, and 7 infants (6%) who were born at 24-28 weeks of gestation. For both groups, 76 infants (71%) were delivered by cesarean delivery; 18 infants (16.8%) were delivered by spontaneous vaginal deliveries; 9 infants (8.4%) experienced vacuum-assisted delivery, and 4 infants (3.7%) were delivered with forceps. There were no differences in maternal demographics between groups (Table 1). Although there was no statistically significant difference in birthweight, the cases had significantly more infants with intrauterine growth restriction. Umbilical arterial gases showed that all cases had metabolic acidosis, which put them at significantly increased risk for long-term neurologic morbidity, and were significantly more depressed at birth, as shown by lower 1- and 5-minute Apgar scores. Significantly more of the cases with metabolic acidosis experienced pulmonary and renal dysfunction (Table 1).
The kappa correlation between the 3 blinded reviewers was 0.42 for reactivity and 0.51 for variability at <5 beats/min, which indicated good reproducibility. The Pearson correlation coefficient (r) for the 3 reviewers was 0.92 for FHR baseline, 0.73 for accelerations, 0.79 for total decelerations, and 0.66 for late decelerations. Evaluation of the last hour of FHR tracing before delivery showed an 87% increase in late decelerations, an 83% decrease in early decelerations, and a 121% increase in late decelerations/contractions for the cases that was statistically significant (Table 2). The total number of decelerations was not different between the groups. There were significantly more cases with prolonged decelerations that lasted 2-10 minutes. Linear regression showed that, as the number of late decelerations during the hour before delivery increased, there was a statistically significant decrease in umbilical arterial pH (r = −0.17; P = .02) and base excess (r = −0.20; P = .02).

The area under the ROC curve, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for late decelerations (0.59, 52.3%, 61.7%, 57.7%, 56.4%, respectively), late decelerations/contractions (0.61, 37.6%, 73.3%, 58.5%, 54.0%, respectively), and prolonged decelerations (0.63, 57.9%, 65.4%, 62.6%, 60.9%, respectively). The ability of these EFM parameters to identify metabolic acidosis did not change substantially when all 3 were placed together in the regression model (0.66, 57.4%, 69.3%, 65.2%, 62.0%, respectively). Although low, these values are elevated by not including all neonates with normal cord blood gases from the entire population.

Within the case group with metabolic acidosis, the 13 neurologically injured infants with HIE were compared with the 94 infants without injury, and no difference was found in maternal age, pregnancy history, race, or mode of delivery (Table 3). The neonates had no difference in gestational age, birthweight, umbilical arterial base excess, or neonatal length of stay. There was a trend toward the infants with HIE having a higher incidence of clinical chorioamnionitis and a lower cord pH that did not quite reach the level of statistical significance. The HIE infants had a higher incidence of meconium-stained fluid, lower 5-minute Apgar scores, and more renal dysfunction. Placental histopathologic evaluation was performed for 9 of 13 of the neurologically injured neonates (69.2%) and 51 of 94 of those infants without injury (54.3%). More placental abnormalities were seen in the injured infants that included histologic chorioamnionitis/funisitis and placental infarcts. Neurologically injured infants were more likely to have a positive blood culture during the neonatal period.

When the FHR tracing for the last hour before delivery was compared between the neurologically injured and noninjured infants within the metabolic acidosis group, the infants with HIE were

### Table 2

Evaluation of the EFM tracing during the last hour before delivery by 3 obstetricians blinded to outcome for cases with metabolic acidosis and controls with normal cord gases

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 107)</th>
<th>Control subjects (n = 107)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (beats/min)*</td>
<td>143 ± 14</td>
<td>142 ± 15</td>
<td>.56</td>
</tr>
<tr>
<td>Time baseline &gt; 160 beats/min (min)*</td>
<td>8.2 ± 16</td>
<td>8.2 ± 18</td>
<td>.99</td>
</tr>
<tr>
<td>Time baseline &lt; 110 beats/min (min)*</td>
<td>0.9 ± 3.1</td>
<td>1.0 ± 5.2</td>
<td>.89</td>
</tr>
<tr>
<td>Baseline variability &lt; 5 beats/min (n)†</td>
<td>30 (28.0%)</td>
<td>22 (20.6%)</td>
<td>.24</td>
</tr>
<tr>
<td>Accelerations*</td>
<td>4.1 ± 5.3</td>
<td>5.4 ± 5.3</td>
<td>.10</td>
</tr>
<tr>
<td>Reactive (n)†</td>
<td>37 (34.6%)</td>
<td>50 (46.7%)</td>
<td>.10</td>
</tr>
<tr>
<td>Decelerations*</td>
<td>10.4 ± 7.7</td>
<td>9.7 ± 6.9</td>
<td>.46</td>
</tr>
<tr>
<td>Late decelerations*</td>
<td>2.8 ± 4.0</td>
<td>1.5 ± 2.7</td>
<td>.006†</td>
</tr>
<tr>
<td>Variable decelerations*</td>
<td>7.4 ± 7.1</td>
<td>7.8 ± 6.4</td>
<td>.57</td>
</tr>
<tr>
<td>Severe variable decelerations*</td>
<td>1.3 ± 2.0</td>
<td>1.0 ± 2.1</td>
<td>.19</td>
</tr>
<tr>
<td>Early decelerations*</td>
<td>0.1 ± 0.5</td>
<td>0.6 ± 1.3</td>
<td>.001†</td>
</tr>
<tr>
<td>Prolonged decelerations 2-10 min (n)†</td>
<td>62 (57.9%)</td>
<td>37 (34.6%)</td>
<td>.0006†</td>
</tr>
<tr>
<td>Nadir (beats/min)*</td>
<td>71 ± 20</td>
<td>80 ± 23</td>
<td>.06</td>
</tr>
<tr>
<td>Length (min)*</td>
<td>7.6 ± 14.8</td>
<td>5.0 ± 2.6</td>
<td>.18</td>
</tr>
<tr>
<td>Contractions/hr*</td>
<td>16.7 ± 10.8</td>
<td>17.9 ± 9.2</td>
<td>.37</td>
</tr>
<tr>
<td>Late decelerations/contractions (%)*</td>
<td>21.7 ± 34.7</td>
<td>9.8 ± 22.2</td>
<td>.004‡</td>
</tr>
<tr>
<td>Variables/contractions (%)*</td>
<td>76.9 ± 147</td>
<td>52.4 ± 58.7</td>
<td>.12</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD for continuous variables.
† Categoric variables.
‡ Indicates statistical significance with P < .05.
found to have statistically significant differences in baseline FHR, bradycardia, decreased variability, and reactivity (Table 4). There was no difference between the neurologically injured and noninjured infants in total, late, or prolonged decelerations. Bradycardia, decreased variability, and nonreactivity were repressed to determine the area under the ROC curve, sensitivity, specificity, and positive and negative predictive values in the identification of neurologic injury within this group of severely acidicotic infants (Table 5).

**Comment**

Placental histopathologic studies have shown that disturbed placental circulation underlies the development of periventricular leukomalacia in most cases with prenatal and peripartum brain injury. Childhood neurologic injury has been linked to clinical and histologic chorioamnionitis and culture-positive infection during the neonatal period. Our study likewise found that placental abnormalities, such as histologic chorioamnionitis/funisitis and infarcts, and culture positive neonatal infection are associated with an increased risk of neurologic injury in neonates with metabolic acidosis.

A study of 12 chronically catheterized near-term fetal lambs that were asphyxiated by umbilical cord occlusion until the fetal arterial pH was <6.9 and base

---

**Table 3**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>+HIE (n = 13)</th>
<th>−HIE (n = 94)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>27.8 ± 8.1</td>
<td>26.6 ± 6.9</td>
<td>.55</td>
</tr>
<tr>
<td>Median gravidity (n)†</td>
<td>3</td>
<td>3</td>
<td>.23</td>
</tr>
<tr>
<td>Median parity (n)†</td>
<td>1</td>
<td>1</td>
<td>.14</td>
</tr>
<tr>
<td>Race (n)†</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>White</td>
<td>4 (30.8%)</td>
<td>31 (33%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>9 (69.2%)</td>
<td>59 (62.8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>4 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>Delivery mode (n)†</td>
<td></td>
<td></td>
<td>.82</td>
</tr>
<tr>
<td>Spontaneous vaginal</td>
<td>2 (15.4%)</td>
<td>16 (17.0%)</td>
<td></td>
</tr>
<tr>
<td>Cesarean</td>
<td>11 (84.6%)</td>
<td>65 (69.1%)</td>
<td></td>
</tr>
<tr>
<td>Vacuum</td>
<td>0</td>
<td>9 (9.6%)</td>
<td></td>
</tr>
<tr>
<td>Forceps</td>
<td>0</td>
<td>4 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Meconium (n)†</td>
<td>7 (53.8%)</td>
<td>19 (20.2%)</td>
<td>.01‡</td>
</tr>
<tr>
<td>Clinical chorioamnionitis (n)‡</td>
<td>3 (23.1%)</td>
<td>6 (6.4%)</td>
<td>.08</td>
</tr>
<tr>
<td>Histologic chorioamnionitis + funisitis (n/N)‡</td>
<td>2/9 (22.2%)</td>
<td>0/51</td>
<td>.0006‡</td>
</tr>
<tr>
<td>Placental infarct (n/N)‡</td>
<td>3/9 (33.3%)</td>
<td>3/51 (5.9%)</td>
<td>.01‡</td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>35.4 ± 5.4</td>
<td>37.0 ± 4.2</td>
<td>.21</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>2342 ± 1049</td>
<td>2864 ± 1082</td>
<td>.11</td>
</tr>
<tr>
<td>pH*</td>
<td>6.84 ± 0.12</td>
<td>6.90 ± 0.09</td>
<td>.051</td>
</tr>
<tr>
<td>Base excess (mmol/L)*</td>
<td>-16.3 ± 3.4</td>
<td>-15.9 ± 5.5</td>
<td>.82</td>
</tr>
<tr>
<td>Neonatal length of stay (d)*</td>
<td>18.8 ± 15.9</td>
<td>12.7 ± 19.6</td>
<td>.28</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)†</td>
<td>4 (30.8%)</td>
<td>17 (18.1%)</td>
<td>.29</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (n)‡</td>
<td>0</td>
<td>2 (2.1%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Renal dysfunction (n)‡</td>
<td>5 (38.5%)</td>
<td>9 (9.6%)</td>
<td>.02‡</td>
</tr>
<tr>
<td>Positive blood culture (n)†</td>
<td>4 (30.8%)</td>
<td>5 (5.3%)</td>
<td>.01‡</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD for continuous variables

† Categoric variables.

‡ Indicates statistical significance with P < .05.
excess was ≤20 mEq/L correlated FHR abnormalities with brain histologic features and found that decreased long-term FHR variability and the sinusoidal pattern were indicators of the severity of asphyxial histologic damage in the ovine fetal brain, but the severity of histologic brain damage could not be predicted during or immediately after the asphyxial insult. There was delayed recovery of FHR variability that took up to 40 minutes after the insult in those infants who were destined to have severe brain damage, which was not related to the degree of acidosis. They noted that the recovery of FHR variability is similar to the rapid recovery of electroencephalogram (EEG) activity after a severe asphyxial insult in ovine fetuses with mild histologic brain damage and that the similarity of recovery of EEG and FHR variability suggests that the FHR is, to some extent, a reflection of an intact central and peripheral nervous system.

FHR abnormalities that occur in response to uterine contractions can be a measure of placental function and fetal perfusion. In chronically catheterized monkeys, it was shown that late decelerations were the first FHR consequence of uteroplacental hypoxia. During the course of progressive hypoxia that led to death over 2-13 days, late decelerations

### TABLE 4
Evaluation of the EFM tracing during the last hour before delivery by 3 obstetricians blinded to outcome for neurologically injured (+HIE) and noninjured (−HIE) neonates with metabolic acidosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>+HIE (n = 13)</th>
<th>−HIE (n = 94)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (beats/min)*</td>
<td>134 ± 16</td>
<td>144 ± 14</td>
<td>.02†</td>
</tr>
<tr>
<td>Time baseline &gt;160 beats/min (min)*</td>
<td>2.1 ± 5.8</td>
<td>9.0 ± 17.1</td>
<td>.15</td>
</tr>
<tr>
<td>Time baseline &lt;110 beats/min (min)*</td>
<td>3.3 ± 7.3</td>
<td>0.6 ± 1.9</td>
<td>.003†</td>
</tr>
<tr>
<td>Baseline variability &lt;5 beats/min (n)‡</td>
<td>8 (62%)</td>
<td>22 (23%)</td>
<td>.008†</td>
</tr>
<tr>
<td>Accelerations*</td>
<td>2.0 ± 3.8</td>
<td>4.4 ± 5.4</td>
<td>.11</td>
</tr>
<tr>
<td>Reactive (n)‡</td>
<td>1 (7.7%)</td>
<td>36 (38%)</td>
<td>.032†</td>
</tr>
<tr>
<td>Decelerations*</td>
<td>11.5 ± 10.1</td>
<td>10.3 ± 7.4</td>
<td>.59</td>
</tr>
<tr>
<td>Late decelerations*</td>
<td>3.7 ± 4.4</td>
<td>2.7 ± 4.0</td>
<td>.42</td>
</tr>
<tr>
<td>Variable decelerations*</td>
<td>7.6 ± 9.7</td>
<td>7.3 ± 6.8</td>
<td>.92</td>
</tr>
<tr>
<td>Severe variable decelerations*</td>
<td>0.9 ± 1.7</td>
<td>1.4 ± 2.0</td>
<td>.44</td>
</tr>
<tr>
<td>Early decelerations*</td>
<td>0.2 ± 0.6</td>
<td>0.1 ± 0.5</td>
<td>.66</td>
</tr>
<tr>
<td>Prolonged decelerations 2-10 min (n)‡</td>
<td>9 (69.2%)</td>
<td>53 (56.4%)</td>
<td>.37</td>
</tr>
<tr>
<td>Nadir (beats/min)*</td>
<td>71 ± 13</td>
<td>71 ± 21</td>
<td>1.0</td>
</tr>
<tr>
<td>Length (min)*</td>
<td>5.9 ± 2.2</td>
<td>7.9 ± 15.9</td>
<td>.40</td>
</tr>
<tr>
<td>Contractions/hr*</td>
<td>11.5 ± 10.8</td>
<td>17.4 ± 10.6</td>
<td>.06</td>
</tr>
<tr>
<td>Late decelerations/contractions (%)*</td>
<td>41.0 ± 54.4</td>
<td>19.4 ± 31.2</td>
<td>.05</td>
</tr>
<tr>
<td>Variables/contractions (%)*</td>
<td>136 ± 308</td>
<td>69.7 ± 114</td>
<td>.16</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD for continuous variables.
† Indicates statistical significance with P < .05.
‡ Categoric variables.
developed before acidemia occurred, and variability disappeared as acidemia developed. In the present study, although late decelerations were significantly more common in the presence of metabolic acidosis, they were unable to identify the presence of HIE.

Some studies have found that EFM correlates well with metabolic acidosis and neurologic injury. A study of 488 term fetuses for whom the last 2 hours of FHR tracing before delivery was analyzed according to the NICHD guidelines found that the best intrapartum FHR parameter to predict the development of significant acidemia was minimal/absent variability for at least 1 hour as a solitary abnormal finding or in conjunction with late decelerations in the absence of accelerations.24 A case-control study that reviewed 4 hours of FHR tracing before delivery for 71 asphyxiated infants with an umbilical arterial base excess of $>$16 mmol/L and 71 control infants with normal cord gases found that a narrow 1-hour window of FHR patterns before delivery that contain minimal baseline variability and late or prolonged decelerations will predict fetal asphyxial exposure before decompen- sation and newborn morbidity.25 The sensitivity of these EFM abnormalities in the identification of asphyxia was 93%, but the positive predictive value was only 3%-18%. They concluded that careful interpretation of FHR patterns could be a useful screening test for fetal asphyxia; however, they noted that supplemental tests are required to confirm the diagnosis and to identify the large number of false-positive patterns to avoid unnecessary intervention. Some investigators have claimed that, in their experience of allegations of malpractice, injury could be timed 85%-90% of the time and defined a new cardiocotograph pattern, the conversion pattern, which they claimed was a specific marker of ischemic injury.26 They believed that, irrespective of the amplitude, duration, or configuration of decelerations during labor, if decelerations are not accompanied by a change in baseline rate and variability, they cannot represent developing tissue hypoxia or significant ischemia.27 In our study, in neither the fetuses with metabolic acidosis nor acidosis with neurologic injury were such patterns present during the hour before delivery, but our data agree with their finding that decelerations were unable to identify the presence of HIE.

Other studies have not found EFM to be clinically helpful in the identification of fetal acidosis or neurologic injury. A recent case-control study (in which the last 2 hours of FHR tracing before delivery were reviewed according to the NICHD workshop guidelines) for 25 term neonates who experienced seizures within 48 hours of birth and had an umbilical artery pH of $<$7.0 compared with 25 control infants matched by gestational age and cord gas but who did not experience seizures) did not find any difference in specific FHR abnormalities but did find a significantly longer duration of abnormal FHR patterns.28 A prospective study of 2200 consecutive deliveries found that 80% of cases with adverse outcome exhibited good FHR variability and that low FHR variability occurred in only 11.5% of newborn infants with adverse outcome.29 They found that decreased FHR variability had a low sensitivity (20.3%) and low positive predictive value (11.6%) in the identification of adverse fetal outcome. A prospective observational study of women whose last FHR tracing was performed within 4 hours of delivery found that computerized quantification of accelerations and variability were unable to predict umbilical arterial pH.30 A case-control study of the FHR tracings of 91 cases of neonatal encephalopathy at term compared with 89 control infants found that 89% of patients with neonatal encephalopathy had abnormal intrapartum FHR patterns, especially decreased variability, but that 52% of the control group also had abnormal FHR patterns.31 They concluded that, given the low incidence of neonatal encephalopa- thy, the predictive value of an abnormal FHR tracing is clinically unhelpful.

The present study shows that, although fetuses with metabolic acidosis have a significant increase in late and prolonged decelerations and late decel- erations/contractions during the last hour before delivery (even in this matched case-control sample that enhances the predictive power by not including all normal patients), the predictive power is not high. For fetuses with metabolic acidosis and neurologic injury because of HIE, which is the group that EFM was designed specifically to prevent, there were significant increases in bradycardia, decreased variability, and nonreactivity; however, these EFM abnormalities were poorly predictive of injury. We conclude that EFM is not a precise tool in the identification of metabolic acidosis or HIE, which emphasizes the importance of the development of other forms of intrapartum monitoring that may identify the hypoxic fetus more accurately.

REFERENCES
10. National Institute of Child Health and Human Development Research Planning Workshop. Electronic fetal heart rate monitoring: re-
Predictors of umbilical artery acidosis in preterm delivery

Marianna Andreani, MD; Anna Locatelli, MD; Francesca Assi, MD; Sara Consonni, MD; Silvia Malguzzi, MD; Giuseppe Paterlini, MD; Alessandro Ghidini, MD

OBJECTIVE: The purpose of this study was to investigate the significance of preterm acidosis and its risk factors.

STUDY DESIGN: From a cohort of 786 consecutive singleton neonates who were born after spontaneous or iatrogenic preterm delivery at 24.0–33.6 weeks of gestation from January 1993 to December 2005 with an evaluation of umbilical artery pH at delivery, we extracted demographic, obstetric, neonatal, and placental histologic variables and related them to umbilical artery evidence of fetal acidemia, which was defined as pH < 7.10. Excluded were stillbirths and neonates with major congenital anomalies. Fetal distress was defined as nonreassuring fetal heart rate tracing or biophysical profile or appearance of thick meconium at delivery. Statistical analysis included 1-way analysis of variance and logistic regression with a probability value of <.05 considered significant.

RESULTS: Neonates with umbilical cord evidence of acidosis (n = 34) were born more frequently after abruption (P < .001), fetal distress (P < .001), and by cesarean delivery (P < .04) and were born less frequently after a complete course of corticosteroids (P = .03) and labor (P = .05) than nonacidotic babies (n = 752). Acute inflammatory lesions at placental histologic evaluation were less frequent (P = .049), and placental vascular lesions were more common in acidic than in nonacidotic preterm neonates (P = .039). Logistic regression analysis demonstrated that cord acidosis was associated independently with the occurrence of abruptio placentae (odds ratio, 7.3; 95% CI, 2.9, 18.8), fetal distress (odds ratio, 12.0; 95% CI, 4.9, 18.3), and vascular placental lesions (odds ratio, 2.8; 95% CI, 1.2, 6.8)

CONCLUSION: In preterm infants, umbilical artery acidosis is significantly more common in the presence of placental abruption, fetal distress, and histologic evidence of placental vascular disease.

Key words: fetal distress, placenta, placental abruption, preterm delivery, umbilical artery acidosis


A n association between umbilical cord pH and subsequent adverse outcome has been reported for preterm infants, with worsening acidosis being associated with greater risk of respiratory distress syndrome, intraventricular hemorrhage, and periventricular leukomalacia. However, the predictors of umbilical cord acidosis in preterm neonates have not been investigated. Two recent studies on neonates who were born at ≤34 weeks of gestation that aimed at exploring the relationship between intrauterine infection (clinical or histologic) and acidosis found a protective effect of infection on neonatal acidosis, with higher umbilical artery pH2 and lower umbilical artery base excess values3 in the presence of infection. One of the 2 studies noted that placental histopathologic findings of infarcts or thrombosis and hypermature villi were associated with a significant decrease in umbilical arterial pH.2 These results confirmed those of a previous study that was based on placental histologic findings in neonates who were born at <32 weeks of gestation, which demonstrated an inverse relationship between neonatal umbilical cord (venous and arterial) pH and the presence of placental vascular disease, in particular abruptio placentae, villous infarct, villous hypovascularity, and avascular villi.4 If fetal acidosis is related to placental vascular lesions, it would be relevant clinically to know whether such lesions can be predicted before delivery. For example, Low et al5 demonstrated that nonreassuring findings at biophysical profile and electronic fetal heart rate monitoring are predictors of asphyxia in preterm infants.

The goal of our study was to evaluate the clinical, biophysical, and histologic predictors of cord acidosis in a cohort of infants who are delivered at <34 weeks of gestation.

MATERIALS AND METHODS We have accessed a prospectively collected database of consecutive singleton neonates who were born after preterm membrane rupture, spontaneous preterm labor, or iatrogenic preterm delivery at 24.0–33.6 weeks of gestation from January 1993 to December 2005 at St. Gerardo Hospital, Monza, Italy.

Maternal medical history, pregnancy complications, results of monitoring of fetal well-being, delivery characteristics, and perinatal outcome were recorded. Inclusion criteria for this study were singleton, liveborn neonates with no major anomalies and available results of umbilical artery pH at delivery.

Fetal heart rate was monitored continuously in the presence of labor, and the tracing was interpreted according to
the Boylan classification.6 Non reassuring tracing was considered tachycardia >180 beats/min, reduced or absent variability, bradycardia <100 beats/min lasting longer than 10 minutes, repetitive late decelerations, or severe variable decelerations. Fetal distress was defined as non reassuring fetal heart rate tracing, biophysical profile <6/8, or appearance of thick meconium at delivery.

Umbilical artery pH and base excess values were determined routinely at the time of delivery. The samples were collected and placed in ice by nursing personnel immediately after delivery for all infants who were deemed to be viable. Gas analysis was completed usually within 15 minutes of delivery (Blood Gas Analyzer OMNI 3 e OMNI S; Roche Laboratories, Basel, Switzerland).

Neonatal outcome was evaluated for occurrence of respiratory distress syndrome, which was defined as the presence of respiratory distress shortly after delivery (tachypnea, retractions, and/or nasal flaring), with a persistent need for respiratory support (oxygen or positive pressure) for >24 hours and typical chest x-ray findings (reticular granular appearance of pulmonary parenchyma) without clinical, laboratory, or radiologic signs of infection; of early neonatal sepsis, which was defined as positive blood cultures within 72 hours of birth; or of intraventricular hemorrhage, which was defined as the presence of hyperechogenicity in the lateral ventricles and classified in 4 grades according to Volpe et al7: of periventricular leukomalacia, which was defined as periventricular echodense lesions that persisted for >15 days that did or did not evolve into cystic lesions or cystic lesions in the absence of a previous echodense lesion and classified in 3 grades according to De Vries8: white matter damage, which was defined as intraventricular hemorrhage with white matter involvement (grade 3 plus), periventricular leukomalacia of any grade, or ventriculomegaly; necrotizing enterocolitis, which was suspected or proved by radiography or surgery; and disseminated intravascular coagulation, which was suspected clinically and supported by laboratory findings of platelets <150 × 10⁹/dL and fibrinogen <150 mg/dL.9 It was reported by laboratory findings of platelets <150 × 10⁹/dL and fibrinogen <150 mg/dL.9

Histopathologic examination of the placenta was performed by observers who were blinded to the neonatal outcome with the exception of gestational age and was classified according to standard published protocols.12 Acute inflammatory lesions in the amnion, umbilical, and chorionic vessels and choriodicidae were classified as present or absent independently from the severity or extent of the lesions. Uteroplacental vascular disease, which included absent or incomplete conversion of the basal spiral arteries, abruption, villous infarcts and fibrosis, fibrinoid necrosis, atherosis, increased syncytial knotting, and X-cell proliferation, was classified as present or absent independently from the severity or extent of the lesions.

Statistical analysis
The main outcome variable was presence of fetal acidaemia, which was defined as an umbilical artery pH <7.10, which corresponds to the second SD below the mean for our population. Clinical and histologic variables were related to the occurrence of umbilical artery acidaemia with 1-way analysis of variance, Fisher’s exact test, chi-square test, and backward logistic regression, with a probability value of <.05 or odds ratio with 95% CI not inclusive of the unity considered significant. The study was approved by the Institutional Review Board.

RESULTS
From the cohort of 889 consecutive singleton neonates who were born after spontaneous or iatrogenic preterm delivery at 24.0-33.6 weeks of gestation,
786 neonates (88%) had evaluation of umbilical artery pH at birth and constituted the study population. Mean umbilical artery pH was 7.30 ± 0.10. Using a cut-off of umbilical artery pH <2 SD to define acidosis (ie, <7.10), we compared the frequency of antenatal and delivery variables between cases with vs without acidosis (Table 1). Neonates who were born with umbilical artery pH <7.10 had significantly higher rates of clinical abruption, fetal distress on admission or in labor and cesarean delivery, and lower rates of steroids administration. Figure 1 displays the distribution of umbilical artery pH among the 4 most common clinical causes of preterm delivery; the median values of pH were essentially identical for preeclampsia (7.32), abruption (7.32), clinical chorioamnionitis (7.33), and spontaneous prematurity (7.33; Figure 1).

Histopathologic examination of the placenta was performed in 697 of 786 neonates (89%). Acute inflammatory lesions were less frequent with umbilical artery acidosis (3/29 [10.3%] vs 193/668 [28.9%]; \( P = .049 \); odds ratio, 0.28; 95% CI, 0.09, 0.89), whereas placental vascular lesions were more represented in cases with umbilical artery acidosis (19/29 [65.5%] vs 296/668 [44.3%]; \( P = .039 \); odds ratio, 2.39; 95% CI, 1.11, 5.12). Figure 2 shows the mean umbilical artery pH in relation to placental histologic findings. Umbilical artery pH was significantly lower with placental vascular lesions than with inflammatory lesions.

Follow-up was available for an average of 49 ± 36 months (range, 12-96 months). Neonatal cord acidosis predicted a higher risk of neonatal morbidity, which included intraventricular hemorrhage, respiratory distress, and disseminated intravascular coagulation, but not white matter damage (Table 2).

To identify the independent predictors of fetal acidosis, variables with a probability value of <.10 at univariate analysis were included in a multivariate model. Only fetal distress, clinical diagnosis of abruptio placentae, and histologic evidence of placental vascular lesions were associated independently with umbilical artery acidosis (Table 3).

**COMMENT**

We have found that, in infants who were born at <34 weeks of gestation, umbilical cord acidosis can be predicted by clinical evidence of placental abruption or nonreassuring findings at fetal heart rate monitoring or biophysical profile. These results are in line with those of a previous small series of 30 cases with neonatal asphyxia, which observed a correlation between abnormalities at fetal heart rate tracing and severity of umbilical cord acidosis. Other prenatal clinical variables (such as labor, corticosteroid administration, and mode of delivery) were associated with cord acidosis at univariate analysis in our series but lost significance at multivariate anal-

![Figure 1](image1.png)

**Figure 1**

Box plot shows the distribution of umbilical artery pH among the most common causes of prematurity

The vertical axis represents the umbilical artery pH. The boxes indicate the interquartiles; the vertical lines indicate the range. PTL, preterm labor; PROM, premature rupture of membranes.

![Figure 2](image2.png)

**Figure 2**

Box plot shows a comparison of distribution of umbilical artery pH based on histologic placental findings

Umbilical artery pH (vertical axis) was significantly different between cases with placental vascular lesions (7.30 ± 0.10) vs those with inflammatory lesions (7.32 ± 0.07; \( P = .003 \)), but not between the former vs those without either type of lesions (7.31 ± 0.09; \( P = .07 \)) or between inflammatory lesions and absence of either type of lesions (\( P = .2 \)). With the use of Bonferroni correction for multiple comparisons, only a probability value of <.017 was considered significant.
ysis. Placental vascular disease was also associated significantly with increased risk of neonatal acidosis, which confirms the results of a histopathologic series of placentas that are delivered at <32 weeks of gestation, which observed a similar contribution of metabolic and respiratory acidosis at birth in preterm infants, and a similar predictive relationship for adverse outcome of arterial and venous pH, which suggests the primary role of the placenta in the determination of fetal compromise.

We have used the second SD below the mean to define umbilical artery acidosis in our population. Such a cut-off (7.10) is identical to that reported by Victory et al13 in a population of very preterm neonates,1 which suggested that it is a reproducible threshold value for a definition of acidosis in very preterm neonates. However, caution should be exercised to assume that values within a normal range are reassuring, because rates of neonatal complications increase progressively with worsening neonatal acidosis. Of interest, the distribution of umbilical artery pH in very preterm neonates is similar to that reported in a population at term (pH = 7.24 ± 0.07).

Neither clinical nor histologic chorioamnionitis was associated with umbilical artery acidosis in our series, which confirms previous findings in populations at similar gestational ages.2 At variance with Richardson et al3 our results suggest that acute placental inflammation is not protective against acidosis per se, but rather it signals the absence of the concomitant presence of vascular lesions, which are the true correlates of cord acidosis. As expected, we did not find an association between acidosis and neonatal periventricular leukomalacia or white matter damage, which are 2 causes of neurologic damage of preterm infants that usually are related to an acute inflammatory or infectious cause.

Because it has already been reported for the term infant, also in the very preterm neonate umbilical artery acidosis is an important prognosticator of adverse neonatal and long-term outcome. In our series, cord pH <7.10 was associated with nearly a 4-fold increased risk of intraventricular hemorrhage and respiratory distress syndrome and a 6-fold increased risk of disseminated intravascular coagulation. Similarly, Low et al15 reported that fetal asphyxia in preterm gestations carries a greater risk of subsequent long-term cardiovascular, respiratory, and neurologic morbidity and death, which reflected the distinctive response of the immature fetus to asphyxia. Animal studies have demonstrated that, although preterm fetal sheep are neurologically less susceptible to 10-20 minutes of cord occlusion,14-16 they manifest sudden

### TABLE 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH &lt;7.10 (n = 35)</th>
<th>pH ≥7.10 (n = 758)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at birth (wk)*</td>
<td>29.7 ± 2.6</td>
<td>30.3 ± 2.6</td>
<td>—</td>
<td>.17</td>
</tr>
<tr>
<td>Male gender (n)</td>
<td>18 (52.9%)</td>
<td>380 (50.5%)</td>
<td>1.05 (0.51, 2.18)</td>
<td>.92</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>1218 ± 498</td>
<td>1360 ± 512</td>
<td>—</td>
<td>.11</td>
</tr>
<tr>
<td>Birthweight &lt;10th percentile (n)</td>
<td>6 (17.1%)</td>
<td>143 (19%)</td>
<td>0.89 (0.32, 2.30)</td>
<td>.97</td>
</tr>
<tr>
<td>5-Min Apgar score &lt;7</td>
<td>16 (48.1%)</td>
<td>58 (7.7%)</td>
<td>10.6 (4.86, 23.27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intraventricular hemorrhage (n)</td>
<td>9 (25.7%)</td>
<td>61 (8%)</td>
<td>3.95 (1.64, 9.33)</td>
<td>.001</td>
</tr>
<tr>
<td>Periventricular leukomalacia (n)</td>
<td>0</td>
<td>20 (3%)</td>
<td>0.00 (0.96, 5.50)</td>
<td>.67</td>
</tr>
<tr>
<td>White matter damage (n)</td>
<td>3 (8.8%)</td>
<td>37 (4.9%)</td>
<td>1.87 (0.43, 6.80)</td>
<td>.53</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td>22 (62.8%)</td>
<td>241 (31.8%)</td>
<td>3.63 (1.71, 7.76)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (n)</td>
<td>7 (20.0%)</td>
<td>73 (9.6%)</td>
<td>2.35 (0.90, 5.88)</td>
<td>.08</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (n)</td>
<td>3 (8.6%)</td>
<td>18 (2.3%)</td>
<td>3.85 (0.85, 14.87)</td>
<td>.09</td>
</tr>
<tr>
<td>Sepsis (n)</td>
<td>3 (8.6%)</td>
<td>58 (7.6%)</td>
<td>1.13 (0.27, 4.02)</td>
<td>1</td>
</tr>
<tr>
<td>Retinopathy of prematurity (n)</td>
<td>6 (17.1%)</td>
<td>94 (12.4%)</td>
<td>1.46 (0.53, 3.82)</td>
<td>.57</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation (n)</td>
<td>4 (11.4%)</td>
<td>15 (2%)</td>
<td>6.39 (1.68-22.27)</td>
<td>.003</td>
</tr>
<tr>
<td>Neonatal death (n)</td>
<td>5 (14%)</td>
<td>52 (7%)</td>
<td>2.26 (0.74, 6.46)</td>
<td>.50</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.

### TABLE 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular placental lesions</td>
<td>.019</td>
<td>2.84</td>
<td>1.19, 6.78</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>.000</td>
<td>7.33</td>
<td>2.86, 18.80</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>.000</td>
<td>12.00</td>
<td>4.91, 29.33</td>
</tr>
</tbody>
</table>
decompensation with profound hypoten-
sion and cerebral hypoperfusion to
longer periods of hypoxia or ischemia. We
hypothesize that abnormal fetal testing sig-
als the presence of an underlying intra-
uterine process of such severity or duration
as to compromise fetal metabolic status.
Further research is needed to clarify the
value of the monitoring of fetal well-being
in very preterm infants for the prediction
of adverse neurologic outcome.

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Cesarean delivery outcomes after a prolonged second stage of labor

Joyce F. Sung, MD; Kay I. Daniels, MD; Laura Brodzinsky, MD; Yasser Y. El-Sayed, MD; Aaron B. Caughey, MD, PhD; Deirdre J. Lyell, MD

OBJECTIVE: We hypothesized that prolonged second stage of labor increases the incidence of unintentional hysterotomy extensions at cesarean delivery.

STUDY DESIGN: A retrospective cohort of term pregnant women who underwent primary cesarean delivery after failed second stage of labor at Stanford University was assessed for hysterotomy extensions and other maternal and neonatal morbidities. Groups included second stage length of 1-3 hours and >4 hours. Data were analyzed with the use of chi-square and Fisher’s exact tests.

RESULTS: Of the 239 women who were studied, the second stage of labor lasted 1-3 hours in 82 patients and >4 hours in 157 patients. Prolonged second stage of labor was associated with unintentional hysterotomy extensions (40% vs 26%; P = .03), particularly to the cervix (29% vs 5%; P = .005), and with surgery that lasted >90 minutes (9% vs 1%; P = .01). The incidence of hysterotomy extensions was associated positively with the length of the second stage. Other maternal and neonatal morbidities were similar between groups.

CONCLUSION: Prolonged second stage of labor is associated with an increase in unintentional hysterotomy extensions at cesarean delivery and prolonged operative time. The future risk of hysterotomy extensions merits further investigation.

Key words: cesarean delivery, hysterotomy extension, maternal morbidity, prolonged second stage of labor


The second stage of labor historically was limited to <2 hours, based on the observations that most nulliparous patients delivered within this time period1 and that neonatal mortality rates increased when the second stage was prolonged.2 With the advent of noninvasive fetal monitoring, obstetricians allowed the second stage to last >2 hours.3 As regional anesthesia became increasingly common, the limit of second stage was extended further; most nulliparous patients with regional anesthesia were found to deliver within 3 hours of second stage vs 2 hours in those patients without regional anesthesia.4 Indeed, allowing additional time in second stage >2 hours results in an increased overall vaginal delivery rate.5 A second stage lasting up to 3 hours is considered appropriate,6,6 and second stages that last >4 hours are not uncommon.7-10 Although a prolonged second stage of labor does not appear to increase neonatal morbidities,6,7,11 maternal morbidities are increased and include operative vaginal delivery, anal sphincter tear, cesarean delivery, and postpartum hemorrhage.6,7,11

When compared with cesarean deliveries in the first stage of labor, cesarean deliveries in the second stage have been associated with longer surgery time,12 increased postoperative fevers,12 maternal intraoperative trauma,13 and composite maternal morbidity.14 Although 1 small study showed increased neonatal morbidity,12 larger studies have shown no differences in neonatal morbidity.13,14

We hypothesized that prolonged second stage of labor that results in cesarean delivery is associated with more frequent unintentional hysterotomy extensions, when compared with a shorter second stage. Further, we sought to identify whether cesarean delivery after prolonged second stage of labor is associated with increased composite maternal and neonatal morbidity and to identify any factors that may help predict increased morbidity.

MATERIALS AND METHODS

We analyzed a retrospective cohort of patients who underwent primary cesarean delivery during the second stage of labor at Lucile Packard Children’s Hospital at Stanford University between 2001 and 2004. This is a tertiary care academic center that includes both a private service and a faculty practice with resident involvement. All resident deliveries were supervised by a faculty physician. At our institution, the routine practice for cesarean delivery after a prolonged second stage of labor does not differ substantially from cesarean deliveries for other indications. After prolonged second stage of labor, surgeons often elevate the infant’s head vaginally before cesarean delivery. In all cesarean deliveries, patients receive antibiotic prophylaxis after cord clamp and com-
Cases were patients with a second stage from complete dilation until delivery. \textit{Second stage of labor} was defined as the time from complete dilation until delivery. Cases were patients with a second stage of >4 hours (>240 minutes), which we refer to as “prolonged second stage”; and control subjects were patients with a second stage of labor that lasted 1-3 hours (60-180 minutes). We chose a second stage that lasted 1-3 hours for the control group to further dichotomize any potential differences in outcomes that were based on duration of the second stage. Exclusion criteria were multiple gestation, previous myomectomy, type I diabetes mellitus, steroid-dependent disease, anticoagulation, and body mass index \geq 40 kg/m². All cases and control subjects were identified with the use of the Lucile Packard Children’s Hospital Perinatal Database.

The primary outcome was unintentional hysterotomy extension at caesarean delivery. This was assessed by review of the surgeon’s dictated operative notes that identified any description of an unintentional hysterotomy extension. The location and number of extensions were noted. We assumed a 22.5% incidence of hysterotomy extension when the second stage lasted 1-3 hours and a 45% incidence with a >4-hour second stage of labor. To have 80% power to detect a 50% difference in hysterotomy exten-

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cesarean delivery after second stage that lasted 1-3 hours (n = 82)</th>
<th>Cesarean delivery after second stage that lasted &gt;4 hours (n = 157)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)*</td>
<td>32.3 (\pm) 5.2</td>
<td>30.9 (\pm) 5.7</td>
<td>.07</td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
<td></td>
<td>.34</td>
</tr>
<tr>
<td>White (45%)</td>
<td>32 (40%)</td>
<td>76 (48%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic (23%)</td>
<td>21 (26%)</td>
<td>34 (22%)</td>
<td></td>
</tr>
<tr>
<td>Asian (24%)</td>
<td>20 (25%)</td>
<td>37 (23%)</td>
<td></td>
</tr>
<tr>
<td>African American (2.5%)</td>
<td>4 (5%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
<tr>
<td>Pacific Islander (1.3%)</td>
<td>0</td>
<td>3 (2%)</td>
<td></td>
</tr>
<tr>
<td>Other (4.2%)</td>
<td>4 (5%)</td>
<td>6 (4%)</td>
<td></td>
</tr>
<tr>
<td>Public insurance: 25% (n)</td>
<td>20 (24%)</td>
<td>40 (25%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nulliparity (n)</td>
<td>61 (75%)</td>
<td>141 (89%)</td>
<td>.005</td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>39.8 (\pm) 1.1</td>
<td>39.6 (\pm) 1.2</td>
<td>.21</td>
</tr>
<tr>
<td>&lt;40 (n)</td>
<td>32 (39%)</td>
<td>70 (44%)</td>
<td></td>
</tr>
<tr>
<td>(\geq) 40 (n)</td>
<td>50 (61%)</td>
<td>88 (56%)</td>
<td></td>
</tr>
<tr>
<td>Induction of labor (n)</td>
<td>38 (46%)</td>
<td>69 (44%)</td>
<td>.78</td>
</tr>
<tr>
<td>Indication for induction of labor (n)</td>
<td></td>
<td></td>
<td>.07</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>9 (13%)</td>
<td></td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>2 (5%)</td>
<td>5 (7%)</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>3 (8%)</td>
<td>5 (7%)</td>
<td></td>
</tr>
<tr>
<td>Postdue date</td>
<td>20 (51%)</td>
<td>29 (41%)</td>
<td></td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
<td>5 (13%)</td>
<td>15 (21%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (5%)</td>
<td>17 (11%)</td>
<td>.15</td>
</tr>
<tr>
<td>General anesthesia during cesarean delivery</td>
<td>1 (1%)</td>
<td>6 (4%)</td>
<td>.43</td>
</tr>
<tr>
<td>Epidural during labor</td>
<td>79 (96%)</td>
<td>154 (97%)</td>
<td>.69</td>
</tr>
<tr>
<td>Operative vaginal delivery attempt</td>
<td>17 (21%)</td>
<td>33 (21%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>3601 (\pm) 446</td>
<td>3676 (\pm) 446</td>
<td>.22</td>
</tr>
<tr>
<td>&gt;4000 (n)</td>
<td>15 (18%)</td>
<td>45 (28%)</td>
<td>.12</td>
</tr>
</tbody>
</table>

| * Data are presented as mean \(\pm\) SD. |
sions, with an alpha of .05 and a beta of .2, 74 patients per arm were needed.

Secondary outcomes were composite and individual maternal morbidities, included chorioamnionitis, disseminated intravascular coagulation, uterine atony, length of stay of >4 days, postoperative fever (temperature, >38°C), intensive care unit admission, repeat surgery, blood transfusion, blood loss, postoperative complications (including wound seroma, ileus, anemia, disseminated intravascular coagulation, deep venous thrombosis, pulmonary embolism), and length of surgery. Neonatal outcomes included 5-minute Apgar score of <7, presence of meconium, arterial cord pH <7.2, neonatal intensive care unit (NICU) admission, and NICU length of stay. All patients underwent continuous electronic fetal monitoring throughout labor.

Data were entered into a STATA database (Statacorp, College Station, TX). Univariate statistical tests with the chi-square test of proportions and the Fisher’s exact test were considered statistically significant if the probability value was <.05. Multivariable logistic regression analysis was used to control for potential confounding variables. We received approval for this study from the Committee on Human Research at Stanford University Medical Center.

### RESULTS

Two hundred forty patients met eligibility criteria; 1 patient was not included because her chart was unavailable for review. Of the 239 patients whose cases were assessed, 82 patients had a second stage of labor that lasted 1-3 hours, and 157 patients had a second stage of labor that lasted >4 hours. Maternal and neonatal demographics were similar between groups (Table 1). All hysterotomies were low transverse.

Prolonged second stage of labor was associated with unintentional hysterotomy extensions (Table 2). The incidence of hysterotomy extensions increased with the length of the second stage (25% at 1-3 hours, 32% at 4-5 hours, and 47% at >5 hours; *P* = .01). Prolonged second stage of labor was associated with an increase in the incidence of hysterotomy extensions to the cervix (29% vs 5%; *P* = .005; Table 3). By location, hysterotomy extensions occurred most frequently in the lower uterine segment, followed by the cervix, other locations, and the broad ligament (Table 3). The occurrence of >1 hysterotomy extension was not more frequent with a prolonged second stage of labor.

Total operative time was longer after a prolonged second stage of labor. Cesarean deliveries more frequently lasted >90 minutes among women with a prolonged second stage of labor (Table 2).

There were no differences in the other studied maternal morbidities between groups, nor was there a difference in composite morbidity (Table 2).
A multivariable logistic regression analysis was performed that controlled for potential confounding variables (Table 4). Unintentional hysterotomy extensions were >2 times more frequent with a prolonged second stage of labor and 2 times more frequent when oxytocin was used during labor, independent of the length of the second stage. The other variables that were examined were not associated independently with hysterotomy extensions.

Neonatal outcomes were not significantly different between groups (Table 5).

**Comment**

In our study, cesarean delivery after a second stage of labor that lasted >4 hours was associated with unintentional hysterotomy extensions, particularly to the cervix. The risk appears to increase with the duration of the second stage, because we found a positive association between the length of a prolonged second stage of labor and the incidence of extensions. Use of oxytocin during labor also conferred an independent risk of unintentional hysterotomy extensions. Both findings were independent of attempted operative vaginal delivery, obesity, and birthweight. Prolonged labor may increase attenuation of the lower uterine segment and impaction of the fetal head, giving rise to a thin, easily lacerated lower uterine segment and cervix. Oxytocin use may be a proxy for an abnormal labor or may lead to greater attenuation of the lower uterine segment. Our findings are in contrast to those of Allen et al,13 who found an association between intraoperative trauma during cesarean delivery in second stage, compared with first stage, but not when prolonged second stage of labor of >4 hours was compared with second stage of ≤4 hours.

Operative times were increased among patients with a prolonged second stage of labor, likely because of the increased time that was needed to repair hysterotomy extensions. We did not identify an increase in any of the other maternal morbidities that we examined, which included blood loss and bladder injury, but we were not powered to identify small differences.

Several recent studies have shown that prolonged second stage of labor does not affect neonatal morbidity, regardless of mode of delivery.6,7,11 We assessed a limited number of neonatal outcomes and

---

**TABLE 3**

Location of hysterotomy extensions

<table>
<thead>
<tr>
<th>Location</th>
<th>Hysterotomy extensions with cesarean delivery after second stage that lasted 1-3 hours (n = 21)</th>
<th>Hysterotomy extensions with cesarean delivery after second stage that lasted &gt;4 hours (n = 63)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower uterine segment (n)</td>
<td>19 (90%)</td>
<td>38 (64%)</td>
<td>.87</td>
</tr>
<tr>
<td>Cervix (n)</td>
<td>1 (5%)</td>
<td>17 (29%)</td>
<td>.005</td>
</tr>
<tr>
<td>Broad ligament (n)</td>
<td>0</td>
<td>2 (3%)</td>
<td>.87</td>
</tr>
<tr>
<td>Other (n)</td>
<td>1 (5%)</td>
<td>2 (3%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**TABLE 4**

Hysterotomy extensions and prolonged second stage of labor, multivariable outcomes*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4-Hour second stage of labor</td>
<td>2.18 (1.13-4.22)</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>1.67 (0.89-3.13)</td>
</tr>
<tr>
<td>Age ≥35 years</td>
<td>0.49 (0.24-1.00)</td>
</tr>
<tr>
<td>Parity</td>
<td>0.93 (0.53-1.64)</td>
</tr>
<tr>
<td>Operative vaginal delivery attempt</td>
<td>0.71 (0.33-1.53)</td>
</tr>
<tr>
<td>Body mass index ≥30 kg/m²</td>
<td>0.78 (0.42-1.44)</td>
</tr>
<tr>
<td>Birthweight ≥4000 g</td>
<td>0.76 (0.38-1.53)</td>
</tr>
<tr>
<td>Public insurance</td>
<td>0.68 (0.27-1.73)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>1.61 (0.33-7.74)</td>
</tr>
<tr>
<td>Oxytocin augmentation</td>
<td>2.01 (1.08-3.75)</td>
</tr>
</tbody>
</table>

* Variables that were controlled for: chorioamnionitis, age ≥35 years, parity, operative vaginal delivery attempt, body mass index ≥30 kg/m², birthweight ≥4000 g, public insurance, preeclampsia, and oxytocin augmentation.

**TABLE 5**

Neonatal outcomes*

<table>
<thead>
<tr>
<th>Outcome</th>
<th>1-3 Hours</th>
<th>&gt;4 Hours</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Minute Apgar score &lt;7†</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td>.61</td>
</tr>
<tr>
<td>Meconium‡</td>
<td>29 (42)</td>
<td>44 (43)</td>
<td>1.00</td>
</tr>
<tr>
<td>Arterial cord pH &lt;7.2ª</td>
<td>20 (50)</td>
<td>26 (35)</td>
<td>.12</td>
</tr>
<tr>
<td>Neonatal intensive care unit admission¹</td>
<td>27 (34)</td>
<td>58 (37)</td>
<td>.67</td>
</tr>
<tr>
<td>Negative</td>
<td>2.9 ± 1.4</td>
<td>3.3 ± 2.1</td>
<td>.63</td>
</tr>
</tbody>
</table>

* Data were not available for all patients.
† N = 183.
‡ N = 172.
ª N = 115.
¹ N = 237.
* N = 82.
* Data are presented as mean ± SD.
also did not identify an increase in neonatal morbidity with prolonged second stage of labor. Of note, a large number of newborn infants in our study were admitted to the NICU, likely because it is routine practice at our institution to admit all newborn infants with the diagnosis of chorioamnionitis to the NICU for evaluation.

Our study was limited by its retrospective nature. The presence and details of unintentional hysterotomy extensions relied on the surgeon’s accurate dictation. Further, because this was not a randomized study, the findings may be biased because of confounding. We attempted to control for potential confounding with multivariable logistic regression; however, residual confounding may exist because of confounders that we did not consider or abstract from the medical records.

Our data may be helpful in encouraging obstetricians to modify labor management and cesarean technique and with counseling patients who are considering intentional hysterotomy extensions at the time of cesarean delivery increase the risk of future cervical insufficiency is unknown, but plausible. The increase in hysterotomy extensions may have as yet unknown effects on future pregnancies, including increased uterine rupture risk or risk for cervical insufficiency. Both of these questions deserve further investigation.

REFERENCES
Predictors of failed operative vaginal delivery: a single-center experience

Avi Ben-Haroush, MD; Nir Melamed, MD; Boris Kaplan, MD; Yariv Yogev, MD

OBJECTIVE: The purpose of this study was to identify factors that predict operative vaginal delivery.

STUDY DESIGN: A retrospective cohort study was conducted that included all women who underwent a trial of operative vaginal delivery between 1993 and 2006 at a major tertiary center.

RESULTS: Operative vaginal delivery was attempted in 5120 of 83,351 deliveries (6.1%): 4299 vacuum extractions (84.0%) and 821 forceps deliveries (16.0%). Failures occurred in 8.6% of trials, more often with vacuum extraction (10.0% vs 1.3%; P < .001). Most vacuum extraction failures (72.6%) were followed by a trial of forceps delivery, which failed in 3.5% of cases. On multivariate logistic regression analysis, the use of forceps (vs vacuum; odds ratio [OR], 0.4; 95% CI, 0.2-0.7) and administration of analgesia (epidural: OR, 0.4 [95% CI, 0.2-0.7]; intravenous opiates: OR, 0.2 [95% CI, 0.1-0.6]) were associated with a lower risk of failure, persistent occiput posterior position (OR, 2.2; 95% CI, 1.4-3.5) and birthweight >4000 g (OR, 2.8; 95% CI, 1.6-4.9), with a higher risk.

CONCLUSION: Fetal weight and head position should be evaluated carefully before operative vaginal delivery, and the use of analgesia should be encouraged.

Key words: forceps, operative vaginal delivery, vacuum extraction

O
erative vaginal delivery (OVD), either with forceps or vacuum-assisted, is used to facilitate childbirth and to avoid cesarean section delivery (CS) and its associated morbidities. Nevertheless, operative techniques are associated with a greater tendency for birth injury than spontaneous delivery.1 Furthermore, failed OVD followed by CS is associated with significantly higher rates of subdural or cerebral hemorrhage, convulsions, and mechanical ventilation than is spontaneous delivery or successful vacuum extraction (VE).2 Prompted by these findings, we sought to identify maternal and fetal factors that are associated with failed OVD to prevent excessive morbidity.

MATERIALS AND METHODS

A retrospective cohort study was conducted that included all women who underwent a trial of OVD between 1993 and 2006 at our university-affiliated tertiary medical center. The study protocol was approved by the local institutional review board.

The indications for OVD at our center are prolonged second stage, as stipulated in the guidelines of the American College of Obstetricians and Gynecologists for nulliparous and multiparous women,3 and nonassuring fetal heart rate. We performed only low or outlet instrumental deliveries as defined by the American College of Obstetricians and Gynecologists.3 Mid and rotational deliveries were prohibited. The choice of VE or forceps delivery for the initial attempt was left to the discretion of the attending physician.

In most cases, metal-cup vacuum extractors (5-6 cm in diameter) were used. In the absence of epidural analgesia, local infiltration usually was added. Failed VE is defined as 2 cup detachments or no progression of the fetal head, despite appropriate traction. In cases of failed VE, either a CS or a trial of forceps delivery was performed.

Data for the study were drawn from the computerized birth certificate records and their linked maternal/child hospital discharge records. All cases in which a singleton infant was born by CS and had a code for OVD (VE or forceps) on the birth certificate were entered into the study group. Cases in which OVD was performed successfully constituted the control group.

Outcome was compared between failed and successful vacuum delivery, failed and successful forceps delivery, and failed and successful OVD (whole sample). Statistical analyses included the Student’s t test, chi-square test, and multivariate logistic regression. Differences were considered significant when the probability value was <.05. All data were managed and analyzed with the SPSS software (version 15.0 for Windows; SPSS Inc, Chicago, IL).
RESULTS

OVD was attempted in 5120 of the total 83,351 deliveries (6.1%) that were performed at our center during the study period (Figure). VE was used more often than forceps as the initial procedure (84.0% vs 16.0%; \( P < .001 \)).

Primary failure of OVD occurred in 8.6% of cases (Figure) and was significantly more common with VE than with forceps delivery (10.0% vs 1.3%; \( P < .001 \)). CS was performed in all cases of failure of primary forceps delivery. When VE failed, a trial of forceps delivery was undertaken in 72.6% of the cases; the secondary failure rate was 3.5% (Figure). CS was performed in 27.4% of patients with primary VE failure and in all patients in whom the post-VE trial with forceps delivery failed as well. Comparison of the failed and successful OVD groups (whole sample and by specific technique) yielded no differences in baseline characteristics (Table 1).

On univariate analysis, the failed OVD group (whole sample) was characterized by higher rates of birthweight >3500 g and >4000 g, absence of systemic or regional analgesia during labor (epidural or intravenous opiates), persistent occipit posterior position, and less frequent use of episiotomy (Table 2). Similar findings were noted on separate analysis of the cases in which VE was the initial procedure. In the primary forceps delivery group, failure was associated only

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**TABLE 1**

Characteristics of the women with failed and successful OVDs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>VE</th>
<th>Forceps</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Success (n = 4170)</td>
<td>Failure (n = 129)</td>
<td>Success (n = 4980)</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>28.4 ± 4.7</td>
<td>27.9 ± 4.6</td>
<td>28.4 ± 4.7</td>
</tr>
<tr>
<td>Parity (n)*</td>
<td>1.2 ± 0.6</td>
<td>1.3 ± 0.8</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Nulliparity (n)</td>
<td>3370 (81%)</td>
<td>105 (81%)</td>
<td>4009 (80%)</td>
</tr>
<tr>
<td>Previous cesarean delivery (n)</td>
<td>284 (7%)</td>
<td>11 (9%)</td>
<td>327 (7%)</td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>39.5 ± 1.6</td>
<td>39.6 ± 1.3</td>
<td>39.4 ± 1.6</td>
</tr>
<tr>
<td>Preterm delivery (n)</td>
<td>146 (4%)</td>
<td>2 (2%)</td>
<td>186 (4%)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD.
with a higher rate of birthweight >3500 g and less frequent use of episiotomy (Table 2).

On multivariate logistic regression analysis, the use of forceps (rather than VE) and the administration of analgesia (epidural or intravenous opiates) were associated with a lower risk of OVD failure, whereas persistent occiput posterior position and birthweight >4000 g were associated with an increased risk of OVD failure (Table 3).

**Comment**

The present study of 5120 attempts at OVD, which represents 6.1% of all deliveries at our tertiary center, yielded several key findings: (1) the rate of failed OVD was 8.6% and was significantly higher for VE than for forceps delivery. (2) Most of VE failures (72.6%) were followed by a trial of forceps delivery. The failure rate in these cases was 5.5%. (3) On multivariate logistic regression analysis, the factors that were associated significantly and independently with failed OVD were the use of VE instead of forceps, absence of systemic or regional analgesia, persistent occiput posterior head position, and birthweight >4000 g.

The medical literature contains several retrospective studies of OVD failure. In a large study of 1750 failed OVDs and 3500 control subjects that was derived from the birth certificate records of Washington State, Gopalan et al. found that failure was associated with increased maternal age, African American race, higher maternal body mass index, maternal diabetes mellitus, polyhydramnios, induction of labor, dysfunctional labor, prolonged labor, and birthweight >4000 g. However, this study was subject to potential confounders because of its inclusion of data from several levels from various medical centers with different management protocols. By contrast, our data were based on the experience of a single major medical facility.

Al-Kadri et al. compared 155 failed instrumental deliveries, accounting for 5.9% of all attempts, with 204 successful OVDs. In accordance with our findings, the failure rate was significantly higher for VE than for forceps delivery and for fetal positions other than occiput anterior. Additional factors that were identified were fetal station <0, nulliparity, and history of CS. Conversely, in a pro-
spective and randomized study of 637 OVDs, Boffil et al found that the efficacy of forceps and VE was similar. Accordingly, in the study of Sheiner et al of 113 VE failures of 2111 trials (5.35%), significant risk factors for failure were large gestational-age infant, birthweight >4000 g, and lack of prenatal care.

In a report from Thailand, the incidence of failed OVD was only 0.5% (n = 70 cases), and failure occurred more often in primigravid women. Lack of clinician appreciation of the true level of the fetal head and too early intervention accounted for 50% of the failures.

The higher VE failure rate in the delivery of infants in the occiput posterior position, as in our study, and the occiput anterior position may be explained by the general use of forceps, mainly in cases of low or outlet position, and their lack of force limitation. Forceps deliveries usually are performed by more experienced and older physicians, which emphasizes the need for a better teaching of forceps to clinicians in training. Unfortunately, we do not have information regarding the head station at the time of OVD. Another limitation of the database is that it did not include body mass index data, which could be an important variable that affects procedure success or failure.

The absence of systemic or regional analgesia during delivery may have led to a lower success rate because it is associated with pain and lack of patient cooperation, which can affect the decision of the physician to continue with OVD or to use forceps on VE failure. Epidural-associated dystocia reflects mainly reduced power and therefore is associated less often with cephalopelvic disproportion, which is a risk factor for failure.

Recent studies reported that digital examination during instrumental delivery fails to identify the correct fetal head position in 25% to 65% of cases. Therefore, the accuracy of intrapartum transvaginal ultrasound evaluation in providing objective information on the fetal head station, direction, and progression of labor has been investigated for purposes of the prediction of successful OVD. Because birthweight and fetal head position are consistent risk factors of failed OVD, some authors recommended the routine performance of abdominal and transvaginal ultrasound scanning in the labor room.

In conclusion, failure of OVD occurs more often with VE than with forceps and is more common in cases of fetal macrosomia, fetal head in the occiput posterior position, and the absence of systemic or regional analgesia. The importance of continued training in the use of obstetric forceps must be emphasized. Fetal weight and head position should be evaluated carefully before OVD, and the use of analgesia should be encouraged.

### TABLE 3
Factors that predict failure of VE, forceps delivery, or OVD in general

<table>
<thead>
<tr>
<th>Factor</th>
<th>VE</th>
<th>Forceps</th>
<th>Any*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Forceps delivery</td>
<td>—</td>
<td>—</td>
<td>0.4</td>
</tr>
<tr>
<td>Birthweight &gt;4000 g</td>
<td>3.0 1.7-5.2</td>
<td>—</td>
<td>2.8 1.6-4.9</td>
</tr>
<tr>
<td>Epidural†</td>
<td>0.3 0.2-0.6</td>
<td>—</td>
<td>0.4 0.2-0.7</td>
</tr>
<tr>
<td>Intravenous opiates†</td>
<td>0.2 0.1-0.6</td>
<td>—</td>
<td>0.2 0.1-0.6</td>
</tr>
<tr>
<td>Persistent occiput posterior</td>
<td>2.1 1.3-3.4</td>
<td>—</td>
<td>2.2 1.4-3.5</td>
</tr>
<tr>
<td>Birthweight &gt;3500 g</td>
<td>5.2 1.2-22.5</td>
<td>5.2 1.2-22.5</td>
<td></td>
</tr>
<tr>
<td>Maternal age &gt;35 y</td>
<td>6.5 1.5-29.1</td>
<td>6.5 1.5-29.1</td>
<td></td>
</tr>
</tbody>
</table>

Values reflect the results of multivariate logistic regression analysis that controlled for the variables that are detailed in Tables 2 and 3.

* VE or forceps delivery.
† Compared with no analgesia.

### REFERENCES


Risk factors for sonographic internal anal sphincter gaps 6-12 months after delivery complicated by anal sphincter tear

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OBJECTIVE: The objective of the study was to identify risk factors for internal anal sphincter (IAS) gaps on postpartum endoanal ultrasound in women with obstetric anal sphincter tear.

STUDY DESIGN: This prospective study included 106 women from the Childbirth and Pelvic Symptoms Imaging Supplementary Study who had third- or fourth-degree perineal laceration at delivery and endoanal ultrasound 6-12 months postpartum. Data were analyzed using Fisher’s exact and t tests and logistic regression.

RESULTS: Mean (± SD) age was 27.7 (± 6.2) years. Seventy-nine women (76%) were white and 22 (21%) black. Thirty-seven (35%) had third-degree tears and episiotomy are associated with more frequent sonographic IAS gaps. Risk factors for gaps included fourth- vs third-degree perineal laceration (odds ratio [OR] 15.4, 95% confidence interval [CI] 4.8, 50) and episiotomy (OR 3.3, 95% CI 1.2, 9.1). Black race (OR 0.23, 95% CI 0.05, 0.96) was protective.

CONCLUSION: In women with obstetric anal sphincter repairs, fourth-degree tears and episiotomy are associated with more frequent sonographic IAS gaps.

Key words: anal sphincter laceration, endoanal ultrasound, postpartum fecal incontinence

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Materials and Methods

Two hundred fifty-six CAPS-IS subjects were recruited from 921 women enrolled in the Childbirth and Pelvic Symptoms (CAPS) study performed by the Pelvic Floor Disorders Network, a cooperative
agreement network sponsored by the National Institute of Child Health and Human Development. Six clinical sites (with 1 site recruiting from 2 hospitals) and the data coordinating center received institutional review board approval, and women provided written informed consent for both CAPS and CAPS-IS studies. CAPS was a prospective cohort study that focused on postpartum fecal and urinary incontinence.7 CAPS-IS, a supplementary study performed in a subset of CAPS participants, also included postpartum imaging studies of the anal sphincter.8

Methods of the CAPS and CAPS-IS studies have been reported in detail and are briefly summarized here.5,7 CAPS subjects included 3 groups of primiparous women identified while hospitalized after a singleton term delivery: (1) 407 women delivered vaginally with a clinically evident anal sphincter tear, (2) 390 women delivered vaginally without clinical evidence of an anal sphincter tear, and (3) 124 women delivered by cesarean section before labor. CAPS-IS subjects were recruited from each of these 3 groups. Only CAPS-IS participants from the first group with third-degree obstetric perineal tears (into or through the anal sphincter) and fourth-degree tears (through the anal sphincter and rectal mucosa) were included in this analysis because the aim was to identify risk factors for internal anal sphincter defects after a recognized and repaired sphincter laceration. Women were invited to participate in CAPS-IS at or after their 6 month postpartum CAPS interviews. Demographic data and a medical history were obtained by interview while the participant was in the hospital after the delivery. Data on the delivery were abstracted from the medical record.

Ultrasounds were performed and interpreted by physician investigators blinded to CAPS-IS cohort group and symptoms. At the time of imaging, all subjects were between 6 and 12 months postpartum and had negative urine pregnancy testing. To improve consistency in performing and interpreting the ultrasound, investigators underwent centralized training. The ultrasound was performed in the left lateral decubitus position using a 10 MHz probe. Details of the ultrasound training process, the technique, and quality control measures used have been previously reported.5 Ultrasound images were studied at contiguous 5 mm intervals throughout the anal sphincter. The internal anal sphincter was identified as a concentric hypoechoic band surrounding the anal mucosa. The external anal sphincter was identified lateral to the internal sphincter as a concentric band of mixed echogenicity. Structural gaps were defined as echogenic or echolucent disruptions in the sphincter(s) seen on at least 1 image.

Associations between internal anal sphincter gaps on ultrasound and demographic and obstetrical variables were examined using the Fisher’s exact and Student’s t tests for categorical and continuous variables, respectively. Multivariate analysis was performed using logistic regression. Variables associated with the outcome, internal anal sphincter gap on ultrasound, at a P ≤ .1 were included in the models. Models were fit in an exploratory manner to identify the primary factors that related to the outcome. For this reason the findings are regarded as preliminary and will require confirmation in future studies. We chose to use a relatively liberal 5% level of significance for defining statistical significance (rather than a more conservative alpha meant to adjust for multiple comparisons) because we wanted to minimize the risk of a type II (false-negative) error. Based on our sample size of 106, we had 80% power to identify risk factors that have a correlation of 0.3 or greater with internal anal sphincter gaps on ultrasound when testing at a 5% level of significance.

RESULTS

One hundred six CAPS-IS subjects with sphincter laceration at the time of vaginal delivery underwent endoanal ultrasound and were included in this analysis. Most subjects (92%) had their ultrasounds performed 6-9 months after delivery; the remainder was performed 10-12 months postpartum. The mean (± SD) age of the women was 27.7 ± 6.2 years. The average prepregnancy body mass index (BMI) was 25.8 ± 6.2 kg/m². Seventy-nine of the women (74.5%) were of white race, 22 (20.8%) were black, 3 (2.8%) were of other racial backgrounds, and 2 (1.9%) were unknown.

Thirty-seven women (35%) had internal anal sphincter gaps on ultrasound. The majority (78%) of women with internal anal sphincter gaps had concomitant external sphincter gaps. Univariate associations between subject characteristics and obstetric variables and the presence of an internal anal sphincter gap on ultrasound are presented in Table 1. A greater extent of perineal laceration (fourth vs third degree), episiotomy, and length of second stage were associated with the presence of an internal sphincter gap on ultrasound. Women who were married or living as married were also more likely to have an internal sphincter gap on ultrasound. Every woman in the study had an epidural during labor, so this could not be studied as a potential risk factor for persistent sphincter gaps. Over 90% of the episiotomies were midline, so all episiotomy types were grouped as a single potential risk factor. Sphincter repair technique (method of approximating the external sphincter, suture type, etc) could also not be included in the analyses because in most cases these data were not provided in the medical record.

In the multivariate analysis (Table 2), only the extent of the perineal laceration, episiotomy, and race were associated with internal sphincter gaps on ultrasound. Fourth-degree perineal lacerations were strongly associated with internal sphincter gaps on ultrasound when compared with third-degree lacerations. Weaker associations were found between internal sphincter gaps and episiotomy and race. Among all women who had a sphincter laceration at delivery, those who had episiotomy performed were more likely to have a persistent internal gap on ultrasound. Women who reported their race as black were less likely to have a persistent internal sphincter gap on ultrasound when compared with those who reported white or “other” race.
TABLE 1
Univariate associations between potential risk factors for an internal anal sphincter gap on ultrasound 6-12 months after vaginal delivery complicated by a sphincter laceration*

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>IAS gap (n = 37)</th>
<th>No IAS gap (n = 69)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.3 ± 6.1</td>
<td>27.3 ± 6.3</td>
<td>.41</td>
</tr>
<tr>
<td>BMI (prepregnancy) (kg/m²)</td>
<td>25.9 ± 6.2</td>
<td>25.7 ± 6.3</td>
<td>.90</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>32 (88.9%)</td>
<td>47 (69.1%)</td>
<td>.09</td>
</tr>
<tr>
<td>Black</td>
<td>4 (11.1%)</td>
<td>18 (26.5%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0%)</td>
<td>3 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>Married/living as married</td>
<td>29 (78.4%)</td>
<td>38 (55.1%)</td>
<td>.02</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>7 (18.9%)</td>
<td>19 (27.5%)</td>
<td>.36</td>
</tr>
<tr>
<td>More than high school</td>
<td>30 (81.1%)</td>
<td>50 (72.5%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>8 (21.6%)</td>
<td>10 (14.5%)</td>
<td>.42</td>
</tr>
<tr>
<td>Baby weight (g)</td>
<td>3668 ± 400</td>
<td>3554 ± 460</td>
<td>.20</td>
</tr>
<tr>
<td>Baby head circumference (cm)</td>
<td>35.9 ± 8.4</td>
<td>34.5 ± 1.7</td>
<td>.32</td>
</tr>
<tr>
<td>Length of second stage (min)</td>
<td>154.1 ± 105.6</td>
<td>106.3 ± 75.4</td>
<td>.03</td>
</tr>
<tr>
<td>Delivery type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forceps</td>
<td>11 (31.4%)</td>
<td>20 (29.0%)</td>
<td>.96</td>
</tr>
<tr>
<td>Vacuum</td>
<td>9 (25.7%)</td>
<td>18 (26.1%)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>15 (42.9%)</td>
<td>31 (44.9%)</td>
<td></td>
</tr>
<tr>
<td>Episiotomy</td>
<td>25 (67.6%)</td>
<td>26 (37.7%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Perineal laceration type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third degree</td>
<td>16 (43.2%)</td>
<td>63 (91.3%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fourth degree</td>
<td>21 (56.8%)</td>
<td>6 (8.7%)</td>
<td></td>
</tr>
</tbody>
</table>

IAS, internal anal sphincter.
* Data presented as n (percent) or mean ± SD.
† Two-tailed P values calculated using Fisher’s exact or Student’s t tests.

COMMENT
In this study our multivariate analyses revealed increased degree of laceration and episiotomy as likely factors predicting postrepair internal anal sphincter gaps, whereas black race was protective. Although risk factors for anal sphincter laceration itself are well described, including primiparity, birthweight, forceps delivery, and episiotomy, predictors of persistent sphincter gaps identified by ultrasound after delivery have not been well studied. The pathophysiology responsible for sonographic sphincter gaps and their associated fecal incontinence symptoms is even less clear. It is possible that more severe perineal injury, or a particular type of injury to the sphincter muscles, nerves, or other connective tissue may predispose to postrepair sphincter gaps.

Risk factors identified in this study, including extent of laceration and episiotomy, appear to fall into this category. On the other hand, gaps may result because of poor repair technique or impaired healing. In this study, variables that might be related to healing (age, education, smoking, BMI) were not associated with the finding of postrepair sphincter gaps. We were unable to study associations between repair technique and postpartum sphincter gaps because these data were not reported consistently in the medical record.

Similar to our findings, a recent prospective study from a single institution found a higher rate of postpartum sphincter gaps on ultrasound in women with fourth-degree lacerations, compared with third-degree lacerations. At 6 weeks after delivery, more women with fourth-degree tears vs third-degree tears had combined internal and external anal sphincter gaps on ultrasound (48% vs 8%, P = .002). Bowel symptoms (fecal urgency and/or anal incontinence) were also significantly higher in women with a more severe sphincter tear (59% vs 28% for fourth-degree vs third-degree laceration, P = .03). This high prevalence of postrepair defects and fecal incontinence symptoms, especially with fourth-degree lacerations, confirm the detrimental effects of obstetrical sphincter trauma in general and suggest the importance of...
the internal anal sphincter for maintaining continence.

Among obstetrical variables, we found episiotomy to be associated with a near doubling of postrepair gaps in the internal anal sphincter. Episiotomy may simply be a marker of increased birth trauma, with more difficult deliveries requiring episiotomy. However, based on this hypothesis, we would anticipate other obstetrical factors such as forceps delivery and birthweight to be associated with postrepair gaps, and they were not in this study.

Obstetrical factors, including episiotomy, have been linked with levator ani injury after childbirth, another type of postdelivery radiological finding associated with pelvic floor conditions. Kearney et al found that episiotomy, forceps use, anal sphincter rupture, and length of second stage were associated with levator ani defects on magnetic resonance images. In our study, the length of the second stage was longer in the women with internal sphincter gaps (154 vs 106 minutes), but this variable was not independently associated with sphincter gaps in the multivariable analysis.

Race was also marginally associated with postpartum internal sphincter gaps, with black race being protective. Black women have decreased rates of pelvic organ prolapse and urinary incontinence, compared with white women, and a recent study suggested that pelvimetry might explain these differences. The anthropoid pelvis is more prevalent in black women and is least likely to be associated with pelvic floor disorders, possibly because this pelvic type is less frequently associated with injury of the pelvic floor during pregnancy or delivery. Perhaps lower rates of postrepair gaps in black women may be due to less severe birth trauma and pelvic floor disruption when compared with white women.

Limitations of this theory are similar to those previously discussed for episiotomy, namely the lack of other significant obstetrical factors in our results. Race may also be associated with other variables that were not included in this analysis. For example, repairs might heal better in black women because of unmeasured factors such as genetics (collagen composition, muscle bulk, etc.), diet, postpartum bowel consistency, or even differences in breast-feeding patterns.

Strengths of this study include its multicenter design, which provided regional and racial diversity to the study group. Also, the ultrasonographers were blinded to delivery events to decrease bias in the identification of anal sphincter gaps. We acknowledge several limitations as well. Our findings were based on the analysis of 37 women with internal sphincter gaps (of 106 women studied), limiting our power to study a greater number of risk factors. Additionally, because CAPS participants were recruited after delivery, delivery events (including the diagnosis and repair of the anal sphincter tears) were not standardized among study centers and often poorly documented. Therefore, we could not study associations between these factors (for example, repair technique or experience level of the clinician performing the repair) on the presence of postpartum anal sphincter gaps.

In summary, sonographic internal anal sphincter gaps detected 6-12 months after vaginal delivery with anal sphincter repair are associated with increased extent of perineal laceration and episiotomy and inversely associated with black race. If confirmed in other studies, our findings may help clinicians to identify a group of women at particular risk for the development of fecal incontinence after obstetrical sphincter laceration.

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The authors thank Dr. Robert Park, Chair of the Pelvic Floor Disorders Network Steering Committee, for his contributions to the network. The authors gratefully acknowledge the other investigators who performed the imaging studies at all of the clinical sites including Mark Lockhart, MD, Gregg Shore, MD, and Franklin Tessler, MD, University of Alabama at Birmingham; Bruce Brown, MD, and Alan Stolpen, PhD, University of Iowa; Susan Gearhart, MD, and Harpreet Pannu, MD, Johns Hopkins Medical Institutes; Caryl Solomon, MD, Loyola University; and Julia Fielding, MD, University of North Carolina.

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Stepwise sequential screening for fetal aneuploidy

Peter A. Benn, DSc; Winston A. Campbell, MD; Carolyn M. Zelop, MD; Charles Ingardia, MD; James F. X. Egan, MD

OBJECTIVE: The purpose of this study was to evaluate stepwise sequential screening for fetal aneuploidy.

STUDY DESIGN: Women who received first-trimester screening were also offered second-trimester tests with second-trimester risks that were based on both sets of markers. Screen-positive rates, use of second-trimester testing and invasive testing, sensitivity, and changes in risks were evaluated.

RESULTS: Of 1528 women who received first-trimester screening, 133 women (8.7%) had an indication for invasive testing that was based on first-trimester results alone; 1173 women (76.8%) received second-trimester tests, which reduced the net number of women with an indication for invasive testing to 105 (6.9%). In unaffected pregnancies, the addition of the second-trimester testing reduced the median Down syndrome risk from 1:2368 to 1:10,301. Six of 10 chromosome abnormalities (60%) were identified by first-trimester screening, and 9 of 10 chromosome abnormalities (90%) were identified by sequential screening.

CONCLUSION: Sequential screening can be introduced successfully into clinical practice, is effective, and can reduce the number of invasive tests that are performed.

Key words: amniocentesis, chorionic villus sampling, Down syndrome, prenatal screening, trisomy 18

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First-trimester screening that involves the measurement of nuchal translucency thickness (NT), maternal serum human chorionic gonadotropin (hCG), and pregnancy-associated plasma protein-A (PAPP-A) has been shown to be effective in screening for fetal Down syndrome.1,4 Second-trimester quadruple screening by the determination of maternal serum concentrations of alpha-fetoprotein, hCG, unconjugated estriol, and inhibin-A has comparable efficacy.3,4 Approaches that incorporate both sets of tests are expected to be more efficacious.3,4

There are a number of practical approaches that can be used to incorporate both first- and second-trimester screening tests into routine obstetric practice.7 In the first trimester, NT, hCG, and PAPP-A can be measured, and the risk can be presented to patients. For those patients who do not seek definitive diagnosis through chorionic villus sampling, the second-trimester quadruple test can be provided. The second risk Figure incorporates both first- and second-trimester tests. This approach is called “stepwise sequential screening.” When there is nondisclosure of the first-trimester risk Figure with all pregnant women who receive a combination of both first- and second-trimester tests, the approach is referred to as “integrated screening.”6,8 Another variation involves “contingency screening,” which limits the second-trimester tests to those patients with intermediate first-trimester risks.9,10 Separate provision of first- and second-trimester tests without the incorporation of the first-trimester markers into the final risk estimate, “independent screening,” is not recommended because of the invalidity of the second risk figures and because it leads to an unacceptable false-positive rate.11-13

The potential benefits of stepwise sequential screening are recognized in current guidelines that have been issued by the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine.14 However, it has been suggested that sequential screening protocols with disclosure of risks may not achieve their theoretic expectations for reduced invasive testing because the protocol itself may create anxiety and therefore increase the number of women who seek invasive testing.15 No data have yet been published that indicate that stepwise sequential screening can be introduced successfully into routine obstetric care or that, in fact, it will be beneficial. We present our experience with the introduction of stepwise sequential screening into clinical practice.

MATERIALS AND METHODS

Women who were referred to our maternal-fetal medicine departments for first-trimester screening received information from a genetic counselor or obstetrician about the risks and benefits of screening and invasive testing before any risk evaluation. This information included a recommendation that a second-trimester risk assessment also be carried out for all women who do not choose to receive invasive testing on the basis of their first-trimester results alone. After the completion of the first-trimester screening, risks were presented to the women by their genetic counselor or obstetrician, and their follow-up options were reviewed again. Second-trimester risk assessments were based on both the first- and second-trimester tests considered together.
We searched our laboratory database for women with singleton pregnancies who received first- and second-trimester Down syndrome and trisomy 18 screening between September 2004 and October 2006. Two groups of women were included in this study. Group 1 consisted of women who received both their first-trimester screening and any follow-up second-trimester screening tests exclusively through our program (1528 cases). These women had their first-trimester risk assessments based on maternal age, NT, PAPP-A, and total beta-hCG. Their second-risk assessment was based on maternal age, second-trimester alpha-fetoprotein (AFP) level, total beta-hCG, unconjugated estriol, and inhibin-A together with the previously measured NT and PAPP-A. The results for these patients were used to evaluate the extent to which second-step screening and invasive testing were used. Group 2 consisted of women who had received their first-trimester testing through other laboratories and had been referred to us for their second-step risk assessment (1283 cases). These women had their first-trimester risk based on maternal age, NT, PAPP-A, and free beta-hCG. Their second-trimester risks were based on these markers plus AFP, total beta-hCG, unconjugated estriol, and inhibin-A. Data from groups 1 and 2 were combined to compare risk estimates at the 2 stages of screening based on the expectation that the use of alternative forms of hCG or both forms at different gestational ages has only a minimal effect on screening performance.10,16

NT measurements were performed by accredited sonographers and maternal fetal medicine physicians. All Down syndrome risks were recalculated using the Serum, Urine and Ultrasound Screening Study statistical parameters.3 Trisomy 18 risks were computed with the use of statistical parameters from several sources,17–19 with NT assumed to be uncorrelated with serum markers in affected pregnancies. Second-trimester risks of >1:270 for Down syndrome and >1:100 for trisomy 18 were used as cutoffs at both stages of the screening. A priori risks for Down syndrome and trisomy 18 were based on maternal age at estimated date of delivery for all women, except when there was a history of a potentially viable trisomic pregnancy and a maternal age <38.8 years. For these latter women, the a priori risk was increased to that associated with a maternal age of 38.8 years.20

Information on invasive test use, chromosome abnormalities that are detected prenatally, and any abnormalities detected at birth was collected from our cytotogenetics laboratory database and from information provided to us from referring physician offices. On the basis of previous studies, our ascertainment of chromosome abnormalities should be substantially complete.21 This study was based on existing patient record data and was therefore deemed exempt by the University of Connecticut Health Center Institutional Review Board.

**RESULTS**

A total of 1528 women received their first-trimester screening through our program (group 1). After adjustment for maternal age and a history of trisomy 21 or trisomy 18 pregnancies, the effective median age of these women was 36.2 years at estimated date of delivery. The race/ethnicity classifications for these women were 77% white, 9% black, 7% Hispanic, and 7% other (primarily Asian).

The Figure summarizes the screening results and additional testing that was received by these women. In total, 1173 of the 1528 women (76.7%) received a second-risk assessment. Of the 133 women who were screen-positive in the first trimester, 88 women (66.1%) chose to receive a second risk evaluation; of these 88 women, 58 women (65.9%) received a screen-negative second result. Of the 1395 women who were initially screen-negative, 1085 women (77.8%) proceeded to second-trimester screening, and 30 of these women (2.8%) received a second-step screen-positive result.

The net number of women with an indication for invasive testing that was based on the screening was 105 (6.9%). This included 45 women who received only first-trimester screening, 30 women...
who were screen-positive at both steps, and 30 women who became screen-positive at the second step. This 6.9% rate for invasive testing compared favorably with the 8.7% with an indication for invasive testing that was based on first-trimester screening alone (McNemar test, \( P < .001 \)).

The 45 women who were screen-positive in the first trimester and did not receive any further screening had relatively high first-trimester high risks (median, 1:91), compared with the 88 women who were also first-trimester screen-positive (median, 1:156), but chose to obtain a second risk estimate. Many of these first-trimester high-risk women who did not pursue additional screening, instead, proceeded directly to invasive testing (30 of the 45 women; 66.7%). Conversely, the 310 screen-negative women who received only first-trimester screening tended to have slightly lower first-trimester risks, compared with the 1085 women who chose a second-step risk assessment (median first-trimester risks, 1:3803 vs 1:3273).

For the 60 women who had a positive second-step screening result, 18 women (30%) received amniocentesis. Of the 58 women who were initially screen-positive and subsequently screen-negative, only 2 women (3.4%) received amniocentesis. The rate of amniocentesis in women who were screen-negative at both stages of screening was 5 in 1055 (0.5%). Overall, only 55 women (3.6%) in group 1 received invasive testing.

There were 5 pregnancies with chromosome abnormalities in the group 1 patients. Three abnormalities (2 trisomy 21, 1 trisomy 18) were identified on the basis of positive first-trimester testing alone, and 2 abnormalities (1 trisomy 21 and 1 trisomy 18) were among women who had positive first- and second-trimester risk assessments.

Because this number of chromosome abnormalities was very small, we also chose to consider results for additional patients who had received their first-trimester testing through other laboratories and their second-trimester tests through us (group 2). These 1283 women with unaffected pregnancies had a median risk of 1:10,301 for Down syndrome and <1:100,000 for trisomy 18. After the completion of the second screening step, the median risks were reduced to 1:10,301 for Down syndrome and <1:100,000 for trisomy 18. Very similar reductions in risk were observed for women who received first-trimester total beta-hCG (group 1), compared with those who received first-trimester free beta-hCG (group 2). For women with unaffected pregnancies, 74% of women had their fetal Down syndrome risk reduced by the second-trimester tests. Correspondingly, 94% of women with unaffected pregnancies had their fetal trisomy 18 risk reduced by the second-trimester tests.

**TABLE**

<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotype</th>
<th>Screening test</th>
<th>First screening</th>
<th>Sequential screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+21</td>
<td>Down syndrome</td>
<td>+</td>
<td>1:152</td>
</tr>
<tr>
<td>2</td>
<td>+21</td>
<td>Down syndrome</td>
<td>+</td>
<td>1:91</td>
</tr>
<tr>
<td>3</td>
<td>+21</td>
<td>Down syndrome</td>
<td>+</td>
<td>1:110</td>
</tr>
<tr>
<td>4</td>
<td>+21</td>
<td>Down syndrome</td>
<td>+</td>
<td>1:50</td>
</tr>
<tr>
<td>5</td>
<td>+21</td>
<td>Down syndrome</td>
<td>+</td>
<td>1:81</td>
</tr>
<tr>
<td>6</td>
<td>+21</td>
<td>Down syndrome</td>
<td>-</td>
<td>1:447</td>
</tr>
<tr>
<td>7</td>
<td>Del (11q)</td>
<td>Down syndrome</td>
<td>-</td>
<td>1:754</td>
</tr>
<tr>
<td>8</td>
<td>+18</td>
<td>Trisomy 18</td>
<td>-</td>
<td>1:225</td>
</tr>
<tr>
<td>9</td>
<td>+18</td>
<td>Trisomy 18</td>
<td>+</td>
<td>1:7</td>
</tr>
<tr>
<td>10</td>
<td>Triploidy</td>
<td>Trisomy 18</td>
<td>-</td>
<td>1:219</td>
</tr>
</tbody>
</table>

**COMMENT**

We have evaluated the acceptance of stepwise sequential screening by determining the uptake of the second step component among women who were referred early in pregnancy for aneuploidy...
screening. We found that a high proportion of women (77%) received both first- and second-trimester risk assessments. Both first-trimester screen-positive and screen-negative women proceeded to the second step of screening. Those women who did not continue with second-trimester screening often had risks that more strongly indicated a need for invasive testing or may have had more reassuring risk assessments that reduced the patient’s desire for further risk refinement.

The application of the second step of screening reduced the number of women for whom invasive testing was indicated. Based on first-trimester screening alone, 8.7% of women had an indication for chorionic villus sampling or amniocentesis. After including the second step in the protocol, this was reduced to 6.9%. A comparable reduction in the number of women with an indication for amniocentesis probably could have been achieved by providing the second-trimester tests to fewer women because those women with extremely high or low first-trimester risks are unlikely to be reclassified. From the perspective of the detection of the maximum number of Down syndrome–affected pregnancies while the amount of testing was minimized, maximum efficiency would be achieved with a contingency screening protocol in which the second-trimester tests are restricted to those with intermediate first-trimester risks. Using computer modeling for the United States pregnancy population, Benn et al10 showed that almost as many cases of Down syndrome would be detected when the second-trimester tests are limited to the 19% of women with risks between 1:30 and 1:300 after first-trimester screening. However, the provision of the second-trimester tests to additional women is most consistent with maximizing a woman’s choices. The provision of screening tests at both stages provides an opportunity to identify other specific abnormalities that are identifiable only by first- or second-trimester protocols alone or which will more likely be detected as a result of the combination.22-24 Additionally, some women do require second-trimester serum screening to provide the AFP test. Current guidelines recommend neural tube defect screening through AFP testing or by targeted second-trimester ultrasonography.14 Because the AFP test helps identify a broader range of fetal defects and pregnancy complications,25 also offering the AFP test to women who are to receive a second-trimester ultrasound evaluation would seem to be justifiable. We have not yet performed a cost/benefit analysis to evaluate the economics of the additional second-trimester testing that would be involved.

We chose to implement our 2-step sequential screening protocol by presenting the results as screen-positive or screen-negative, on the basis of cut-offs that had been a widely accepted standard of care for second-trimester screening alone (1:270 for Down syndrome and 1:100 for trisomy 18). All results were presented as second-trimester risks, even when the testing was performed in the first trimester. This was done because most invasive testing is carried out in the second trimester, and changes in the cut-off as gestation progresses can be confusing for patients. All women must be informed of their individual risk,14 by raising the question whether it is necessary to classify pregnancies as “screen-positive” or “screen-negative” on the basis of specific cut-offs. Although the somewhat arbitrary definitions of screen-positive may be anxiety provoking, we believe that it is useful to identify specifically subgroups at the highest risk and that it is logical to have consistent definitions that are independent of the type of screening that is provided.

A multistep screening protocol generates additional subsets of patients who have positive results; potentially, all such women could request invasive testing.26 In fact, we found little evidence that 2-step screening resulted in additional invasive testing. Among the 58 women who were screen-positive in the first trimester and screen-negative in the second trimester, only 2 women received amniocentesis. This suggests that women did find the revised risk to be reassuring.

Much larger datasets are required to verify theoretic expected detection rates for complex screening protocols, such as stepwise sequential screening, and to compare them with other approaches. However, our observations for 10 abnormal cases indicated superior detection with the 2-step approach, with 6 cases screen-positive with first-trimester screening alone and 9 screen-positive after the second step. In most cases, risks were markedly higher after the completion of all tests (Table). This aspect is important because acceptance of invasive testing is dependent on the risk 

Although our data show that stepwise sequential screening is advantageous, we conclude with a note of caution. Case 2 (Table) illustrates that there will be occasional cases in which the second step of screening is falsely reassuring. It must be remembered that uncertainty is intrinsic to all types of prenatal screening.

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Cervical length ≤25 mm in low-risk women: a case control study of cerclage with rest vs rest alone

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OBJECTIVE: The purpose of this study was to evaluate the clinical utility of cerclage in low-risk women with cervical length (CL) ≤25 mm at transvaginal ultrasound (TVU).

STUDY DESIGN: This was a retrospective cohort study of women with CL ≤25 mm identified incidentally at TVU examinations between 16⁶/₇ to 24⁶/₇ weeks, with no history of previous preterm birth or midtrimester losses. The primary study outcome was rate of preterm delivery <35 weeks' gestation.

RESULTS: Women undergoing cerclage placement (n = 31) had shorter CL (P < .001) and lower gestational age at presentation (P < .001) than those managed with rest alone (n = 36). Gestational age at delivery was 37.6 ± 3.6 vs 38.5 ± 2.1 weeks (P = .17), and delivery at <35 weeks occurred in 5/31 versus 2/36 cases, respectively (P = .23). The lack of a significant association between cerclage and rate of delivery <35 weeks persisted after controlling for gestational age at TVU and initial CL (P = .81).

CONCLUSION: Cerclage placement does not improve pregnancy outcome in low-risk women with incidental detection of CL ≤25 mm in the early second trimester.

Key words: cervical cerclage, cervical incompetence, preterm birth, short cervix

P reterm birth is the single most important and persistent problem in obstetrics. Preterm deliveries are responsible for 75% of nonanomalous neonatal deaths, and nearly one half of all cases of congenital neurologic disability, including cerebral palsy. The events that culminate in spontaneous preterm birth are not completely understood. Cervical incompetence is 1 of the factors that have been thought to play a role in the causation of spontaneous prematurity. Since adoption of transvaginal ultrasonography (TVU), a short cervical length (CL) has been consistently found to be 1 of the best predictors of preterm birth, and when it is observed in the absence of uterine contractions, it is often considered to be indicative of cervical incompetence. From a practical clinical standpoint, the sonographic diagnosis of cervical incompetence is meaningful only if some intervention can be shown to reduce the risk of preterm delivery. Whereas preliminary evidence suggests that in women with short cervix and a history of prior preterm delivery cerclage may reduce the risk of preterm delivery, the efficacy of the procedure in women without such history is currently unknown. A recent metaanalysis concluded that there was potential benefit of cerclage in the presence of CL < 25 mm in a mixed population of women at high- and low-risk for preterm delivery (RR 0.75; 95% CI 0.58-0.97).

A large multicenter trial has demonstrated no benefit of cerclage placement for CL ≤15 mm in low-risk women.

We have evaluated the clinical utility of cerclage in exclusively low risk women with findings of cervical shortening ≤ 25 mm at TVU.

MATERIALS AND METHODS

We conducted a retrospective cohort study of women with short cervix identified by TVU examinations at the Perinatal Diagnostic Center of Inova Alexandria Hospital from January 2001-December 2005. Inclusion criteria were CL ≤25 mm incidentally found at TVU at the time of midtrimester fetal anatomy survey, gestational age between 16⁶/₇ to 24⁶/₇ weeks at the time of TVU scan, and no history of a prior preterm birth or midtrimester losses. We excluded women with multiple gestations, prophylactic cervical cerclage placed during the index pregnancy, history of cervical incompetence, dilation of the external os or membranes bulging in the vagina, presence of regular uterine contractions at tocometry, uterine malformations, and major fetal anomalies. The diagnosis of cervical shortening was made in women who had been referred for suspected cervical shortening either at routine prenatal visits or at sonograms done elsewhere for fetal anatomy survey.

The counseling of women with the above characteristics changed over the years, adapting to emerging scientific evidence. During the first half of the study period, low-risk women with CL ≤25
mm were counseled about potential benefits of cerclage placement and most agreed to the procedure. Cerclage was always performed using the surgical technique of McDonald, and patients were counseled to reduce physical activity for the remainder of the pregnancy. The cerclage was removed between 36 and 37 weeks’ gestation, at onset of active labor unresponsive or with contraindications to tocolytic therapy, or in the presence of confirmed ruptured membranes. In 2003, several metaanalyses or reviews were published that suggested a probable lack of benefit of cerclage in low risk women with cervical shortening; therefore, women with CL ≤25 mm were informed of the new evidence, they were discouraged from undergoing cerclage placement, and they were instructed only to reduce physical activity.

Relevant medical history and obstetric complications, such as preeclampsia, gestational diabetes, gestational cholestasis, or placenta previa, were abstracted from the maternal obstetric records. Delivery information recorded included gestational age at delivery, mode of delivery, and occurrence of any complication in the peripartum period. From the neonatal records we recorded sex, birthweight, Apgar scores, and admission to the neonatal intensive care unit.

The study was approved by the institutional review board committee.

### Statistical analysis

The primary outcome variable was rate of spontaneous preterm delivery at <35 weeks’ gestation. Univariate analysis was performed using Fisher’s exact test or χ² for categorical variables, and Wilcoxon rank-sum test for continuous variables (SPSS v 13.0; SPSS, Inc, Chicago, IL). Logistic regression analysis was used to control for confounders. Differences in intervals from TVU to delivery were tested using Cox proportional hazard survival curves, with nonspontaneous deliveries considered censored observations. We considered significant a 2-tailed P < .05 or an odds ratio (OR) with 95% confidence interval (CI) not inclusive of unity.

### Results

A total of 67 women underwent TVU between 16⁶/₇ to 24⁶/₇ weeks from January 2001-December 2005 and fulfilled the study criteria. Cervical cerclage was performed in 31 of them (46%) whereas the remaining 36 were managed with rest alone. In the 2001-2003 period, 21 low-risk women with short cervix requested cerclage placement and 11 preferred rest alone; in the 2004-2005 period, 10 such women requested cerclage placement and 25 preferred rest alone.

Table 1 shows the population characteristics in relation to cerclage. Women who underwent cerclage placement had significantly lower gestational age at TVU and shorter cervical length than those who did not undergo the procedure. No difference was present between the 2 groups in rates of cervical surgery (ie, LEEP procedure).

There was no difference in gestational age at delivery between the 2 groups (Table 2). Five women (16%) delivered before 35 weeks in the cerclage group and 2 (5.5%) in the control group (P = .23; Table 2). The lack of a significant association between cerclage and rate of delivery <35 weeks persisted after controlling for gestational age at TVU and initial cervical length (P = .81; OR = 1.3; 95% CI, 0.12-1.43). Our study had adequate statistical power (alpha = 0.05; beta = 0.20) to detect a difference of 14% in rate of preterm delivery before 35 weeks between the 2 groups. A sample size of 179 women in each group would be required for the observed difference to achieve statistical significance.

There was a significant correlation between CL at TVU and gestational age at delivery (R = 0.28; P = .02). Cox proportional hazards showed a longer interval between TVU and delivery in the cerclage group (P = .08; Figure 1). However, after controlling for gestational age at TVU (OR = -0.46; 95% CI, 0.42-0.26) and cervical length at TVU

**Table 1** Population characteristics in relation to cerclage

<table>
<thead>
<tr>
<th></th>
<th>Cerclage (n = 31)</th>
<th>No cerclage (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>32.7 ± 4.7</td>
<td>31.2 ± 4.4</td>
<td>.21</td>
</tr>
<tr>
<td>Nulliparae</td>
<td>14 (45.1%)</td>
<td>22 (61.1%)</td>
<td>.29</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>12 (38.7%)</td>
<td>14 (38.8%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Medical history</td>
<td>5 (16.1%)</td>
<td>3 (8.3%)</td>
<td>.45</td>
</tr>
<tr>
<td>Obstetric complications</td>
<td>9 (29.0%)</td>
<td>7 (19.4%)</td>
<td>.52</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.1 ± 5.7</td>
<td>26.6 ± 5.4</td>
<td>.77</td>
</tr>
<tr>
<td>History of cervical surgery</td>
<td>17 (54.8%)</td>
<td>22 (61.1%)</td>
<td>.79</td>
</tr>
<tr>
<td>Gestational age at TVU (wks)</td>
<td>20.1 ± 2.0</td>
<td>21.6 ± 2.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cervical length at TVU (mm)</td>
<td>14 ± 5</td>
<td>21 ± 3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gestational age at cerclage (wks)</td>
<td>21.0 ± 2.2</td>
<td>NA</td>
<td>/</td>
</tr>
</tbody>
</table>

TVU, transvaginal ultrasound. Number (%) or mean ± standard deviation.
weeks or prolongation of gestation. This finding adds to the observations of previous studies in women with short CL, which evaluated mixed populations at high and low risk for preterm delivery.9

We excluded both women with history-indicated cerclage and those with physical exam-indicated cerclage, as these populations have already been shown to benefit from the procedure.9,14

The evolving paradigm of the management of cervical shortening during the study period provided an ideal setting to perform our case-control study testing the efficacy of cerclage. At the beginning of the study period, TVU detection of short cervix in the lack of uterine activity was thought to identify a homogeneous clinical entity with the characteristics of the traditional cervical incompetence. Evidence from randomized clinical trials in mixed populations for obstetric risk for preterm delivery and with cervical shortening suggested a possible benefit of cerclage.8 The evidence from such studies was extrapolated also to low-risk women with cervical shortening. As additional cohort and randomized trials were published, and their findings underwent metaanalyses, it emerged that cervical shortening at TVU is similarly predictive of preterm delivery in women with a history of mid trimester losses or preterm delivery, as in those without such history.15

However, obstetric history appeared to play a critical role in the benefit of cerclage placement: cerclage was shown to be beneficial in populations at high risk for preterm delivery, whereas in low-risk women the benefit of the intervention was unclear. In 2004, To et al demonstrated that cerclage placement did not reduce rate of preterm delivery in low-risk women with cervical shortening <1.5 cm.10 Our data suggest that even in milder forms of cervical shortening (ie, CL <2.5 cm) cerclage does not improve outcome. That our population consisted

(OR = 2.37; 95% CI, 0.23-2.64), there was no significant difference in interval to delivery between the 2 groups (OR = -0.01, 95% CI, -2.01-2.00; P = .92, Figure 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Outcome in relation to cerclage</th>
<th>Cerclage (n = 31)</th>
<th>No cerclage (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm labor or pPROM</td>
<td>5 (16.1%)</td>
<td>4 (11.1%)</td>
<td>.81</td>
</tr>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>37.6 ± 3.6</td>
<td>38.5 ± 2.1</td>
<td>.28</td>
</tr>
<tr>
<td>Gestational age at delivery &lt; 35 weeks</td>
<td>5 (16.1%)</td>
<td>2 (5.5%)</td>
<td>.23</td>
</tr>
<tr>
<td>Interval TVU delivery (d)</td>
<td>122.6 ± 30.3</td>
<td>117.0 ± 19.3</td>
<td>.008</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>10 (32.7%)</td>
<td>11 (30.5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Male sex</td>
<td>16 (51.6%)</td>
<td>19 (52.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2979 ± 772</td>
<td>3202 ± 575</td>
<td>.70</td>
</tr>
<tr>
<td>5-minute Apgar score &lt; 7</td>
<td>0 (0%)</td>
<td>2 (5.5%)</td>
<td>.54</td>
</tr>
<tr>
<td>NICU admission</td>
<td>7 (22.5%)</td>
<td>3 (8.3%)</td>
<td>.20</td>
</tr>
</tbody>
</table>

TVU, transvaginal ultrasound; pPROM, preterm premature rupture of membranes.

Number (%) or mean ± standard deviation
of low-risk women is confirmed by the rate of preterm birth <35 weeks in our control group without a cerclage (5.5%), which compares favorably with the rate reported in a previous study on a similar population (4%).

Strengths of our cohort study include a population homogeneous for absence of risk factors for preterm delivery, and the consistent management protocol during the study period. However, the group of women who underwent cerclage happened to have shorter CL and lower gestational age at TVU than those managed with rest alone. Therefore, we utilized multivariate analyses to control for these important biases of entry, finding no benefit of cerclage. This finding was surprising as women in the 2 groups had similar gestational ages at delivery, although those who underwent cerclage were at an important clinical disadvantage compared with those managed expectantly, ie, they had shorter cervixes at earlier gestational ages.

Sonographic evidence of cervical shortening identifies women at increased risk for preterm delivery, but the processes underlying the cervical changes are probably heterogeneous. Obstetric history of preterm delivery increases the likelihood that cervical shortening may be the manifestation of cervical incompetence, and thus such history identifies candidates who may benefit from cerclage. In the absence of such history, the obstetrician faces an obstetrical dilemma, with sonographic evidence of increased risk for preterm delivery, yet no interventions available which have been documented to improve the outcome. Further studies are needed to explore whether interventions other than cerclage (eg, progesterone administration or vaginal pessary) may reduce the hazard of preterm delivery in low-risk women with short cervix at transvaginal sonogram.

REFERENCES

Transabdominal cerclage after comprehensive evaluation of women with previous unsuccessful transvaginal cerclage

Robert H. Debbs, DO; Guillermo A. DeLa Vega, MD; Stephanie Pearson, MD; Harish Sehdev, MD; Dominic Marchiano, MD; Jack Ludmir, MD

OBJECTIVE: The purpose of this study was to assess the outcome after transabdominal-cerclage placement during pregnancy in women with previous unsuccessful transvaginal cerclage.

STUDY DESIGN: We conducted a retrospective case series that described pregnancy outcome in women who were treated with transabdominal cerclage between 1994 and 2006.

RESULTS: Seventy-five women with negative evaluation for recurrent pregnancy loss and 1 previous unsuccessful transvaginal cerclage procedures were treated with transabdominal cerclage. The median gestational age at the time of cerclage placement was 13 weeks, and the median gestational age at delivery was 36 weeks. Seventy-two women delivered after 24 weeks of gestation, and 3 women delivered ≤24 weeks of gestation. The fetal-salvage after transabdominal cerclage was 96%.

CONCLUSION: Our findings suggest that, in women with a history of 1 failed transvaginal cerclage, transabdominal cerclage is an effective procedure.

Key words: cervical insufficiency, pregnancy, transabdominal cerclage

Cervical insufficiency is estimated to complicate as many as 1 in 217 pregnancies.1 The diagnosis has been established traditionally by an obstetric history of recurrent painless dilation of the cervix in the second trimester, which results in early pregnancy loss or preterm birth. The efficacy of transvaginal cerclage (TVC) is uncertain. Women in whom the TVC is unsuccessful are often advised to undergo transabdominal cerclage (TAC). Case series of TAC report varied success rates.2-20 This may be the result of incomplete preoperative evaluation to exclude causes other than cervical insufficiency or of treatment of women who do not have a history of early preterm birth. We report pregnancy outcome after TAC in carefully selected women with ≥1 unsuccessful TVC procedures.

MATERIALS AND METHODS

One hundred fourteen patients were referred to a single Maternal Fetal Medicine specialist (R.H.D.) between 1994 and 2006. Strict criteria were used to identify appropriate candidates for TAC placement. Preconception work-up included a hysterosalpingogram or sonohysterogram for uterine cavity evaluation (75 patients). Thrombophilia work-up was performed that included anticardiolipin antibody (ACA) immunoglobulin G/M, lupus anticoagulant (LAC), protein S and C levels, antigen (Ag), antithrombin III level and antigen, and activated protein C resistance before 1997 (15 patients). After 1997, factor V Leiden and prothrombin gene mutation DNA analysis were added (60 patients). Parental blood chromosome analysis was performed (75 patients). Cervicovaginal Neisseria gonorrhoea, Chlamydia, group B streptococcus, Ureaplasm, and Mycoplasma cultures were performed in 45 patients (up to 2001) after which Ureaplasm and Mycoplasma cultures were abandoned (30 patients). Placental pathologic condition, autopsy, and records from previous losses were also requested and reviewed (70 patients). Thirty-nine women were excluded, which included 13 women with a history of cervical surgery, 10 of who had previously delivered at term and 3 of whom had no previous pregnancies. Fifteen patients who were thought to have recurrent infection from a previous cerclage or chorioamnionitis were excluded when the infection was confirmed historically. Two patients with a uterine septum, 2 patients with intracavitary myomas, 1 patient with a large endometrial polyp, and 1 patient with uterine didelphys were excluded. Five patients were excluded with previously undiagnosed thrombophilia. Two patients had antiphospholipid syndrome with persistently elevated immunoglobulin G anticardiolipin antibody; 1 patient had compound heterozygous factor V Leiden and prothrombin gene mutations; 1 patient had antithrombin III deficiency, and 1 patient had activated protein C deficiency. Seventy-five patients met the study criteria.

Outcome data that were collected included complications of surgery, gesta-
tional age at delivery, and fetal outcomes. The same perinatologist (R.H.D.) performed all procedures between 12 and 19 weeks of gestation. All women were counseled before the procedure regarding the risks and benefits of TAC. All procedures were performed with spinal anesthesia. Cefazolin (1 g) was given intravenously before incision. A Foley catheter was placed. A Pfannenstiel incision was used in 73 patients, and a vertical incision was used in 2 patients because of advanced gestational age. The bowel was packed; the vesicouterine peritoneum was incised and was developed to allow exposure to the isthmic region. The uterus was exteriorized whenever possible. Adhesiolysis was performed when necessary. The surgeon grasped the uterine vessels at the cervicoisthmic junction with his hand and fingers, and a window was made with a right-angle clamp entering from anterior to posterior, 1 cm medial and 1 cm superior to the uterosacral ligaments. An assistant then passed a 5-mm Mersilene suture on a long Kelly clamp to the open right-angle clamp. The suture was brought from posterior to anterior. A sponge was then placed both posteriorly and anteriorly to tamponade the puncture sites, while the contralateral side was addressed. The same procedure was performed on the opposite side. The suture was then pulled taut and tied anteriorly. The posterior puncture sites were sutured with a figure 8 of 2-0 Vicryl for hemostasis. The anterior puncture sites were then sutured in a similar fashion. After hemostasis was confirmed, the uterus was placed back into the abdominal/pelvic cavity. If cramping was encountered, 50 mg of oral indomethacin was administered every 6 hours.

Institutional Review Board approval for the collection of data was obtained from The University of Pennsylvania Health System office of Regulatory Affairs.

Statistical analysis was performed with SPSS software (version 10.0; SPSS Inc, Chicago, IL). Categoric data were compared with the use of the Pearson $\chi^2$ test and Fisher exact test, as appropriate.

Continuous variables were compared with the use of the Student $t$ test.

RESULTS

The results after TAC are shown in Table 1. Seventy-five women underwent 75 TAC procedures in singleton pregnancies. Seventy-two patients had no intraoperative or postoperative complications of TAC placement. The most serious complication of TAC placement was a bowel perforation that occurred while the right angle clamp passed through the broad ligament. This was oversewn with no subsequent sequelae. One woman had an incisional hernia, and another woman had a wound infection. No patients required a blood transfusion after the TAC procedure.

Of the 75 pregnancies, there was 1 woman who had ruptured membranes within 24 hours of the procedure; dilation and evacuation was accomplished through the cerclage at 13 weeks of gestation. One patient delivered at 19 weeks of gestation after preterm premature rupture of membranes, and another patient delivered at 22 weeks of gestation because of severe preeclampsia. Both women were delivered by hysterotomy. Seventy-two women delivered live born infants, for a fetal salvage rate of 96%. Eighteen patients (25%) delivered before 37 weeks of gestation, the earliest at 31 weeks. Nine women (12.5%) delivered early because of preterm labor.

All patients were delivered by cesarean section with the stitch left intact. There were no intraoperative complications because of the stitch at the time of cesarean section delivery, and none of the complications required a blood transfusion or hysterectomy. There were no identified acute or chronic bladder injuries. Four patients (6%) were diagnosed with postpartum endometritis, all of whom recovered uneventfully. Six patients (8%) had wound infections or seroma formation. Two of these wounds were reopened for seroma drainage.

Table 2 shows the outcome of previously published retrospective cohorts with fetal survival between 60%-100%.

### TABLE 1

Demographics and outcomes with transabdominal cerclage

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>n = 75</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32 (23-42)</td>
<td></td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>17</td>
<td>22.7</td>
</tr>
<tr>
<td>Nonblack</td>
<td>58</td>
<td>77.3</td>
</tr>
<tr>
<td>Previous pregnancies (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>49</td>
<td>65.3</td>
</tr>
<tr>
<td>6-12</td>
<td>26</td>
<td>34.7</td>
</tr>
<tr>
<td>Previous losses (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>68</td>
<td>91</td>
</tr>
<tr>
<td>6-12</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>Gestational age at abdominal cerclage (wk)*</td>
<td>13 (12-19)</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (wk)$^+$</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Preterm labor at 24-36 wk (n)</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Live births (n)</td>
<td>72</td>
<td>96</td>
</tr>
<tr>
<td>Perinatal deaths or deliveries at &lt;24 wk (n)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Preterm premature rupture of membranes (n)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Complications (n)</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

* Data are given as median (range).
$^+$ Data are given as median.
Cervical insufficiency often is diagnosed on the basis of an obstetric history of repeated painless mid-trimester losses or preterm deliveries. Although TVC has been an accepted management for cervical insufficiency for decades, its efficacy remains in question. TAC has been used in women with congenitally short or extensively amputated cervices, markedly scarred or lacerated cervices, cervico-vaginal fistulas, subacute cervicitis, or failed TVC. In this retrospective series, we present a large series of patients who underwent TAC after previously failed TVC procedures. Fetal survival was high, with an acceptably low rate of complications. All patients had a history of unsuccessful TVC, and all patients had a negative work up for other causes of second-trimester loss. In addition, the cases of most patients had an extensive examination of records, autopsy results, and placental pathologic results from previous pregnancies.

Potential advantages of TAC include placement of the stitch at the level of the internal os, decreased slippage of the suture, lack of a foreign body within the vagina that could act as a source of infection, and the ability to leave the suture in place for future pregnancies. One known disadvantage of TAC is the need for at least 2 laparotomies during pregnancy. However, few major complications and minimal maternal morbidity were encountered in our patients. Placement of the stitch in pregnancy may have multiple benefits that include easier visualization of anatomy and the tension applied to the Mersilene band judged more accurately. Some researchers have recommended that TAC should be performed as an interval procedure in the nonpregnant state to avoid 2 surgeries during pregnancy and to reduce operative blood loss, but heavy bleeding has been reported in both interval and cerclage placement during pregnancy. With experienced operators, blood loss during TAC is minimal.

Late prenatal complications including intrauterine fetal death, intrauterine growth restriction, suture migration, uterine rupture, preterm premature rupture of membranes, rectovaginal fistula, and persistent maternal discomfort have been reported with TAC. These complications were uncommon in this series because other causes for recurrent loss were excluded.

The cerclage suture was left in place in all patients, and no long-term complications were reported (such as cervical erosion). Several authors have described successful future pregnancies with the same TAC. We prefer placement of the stitch after 10 weeks of gestation because the risk of miscarriage remains substantially high until the completion of the first trimester. In addition, ultrasound evaluation can confirm viability and detect major fetal anomalies after week 12, and first trimester screening and chorionic villus sampling can be performed before patients are subjected to the procedure. Finally, laparoscopic placement of abdominal cerclage has been described multiple times in the literature. This technique limits the number of abdominal laparotomies that the patient would require. Some authors believe that adhesion formation can be reduced by laparoscopy. Although this procedure may gain popularity in the future, the technical difficulty of this approach to TAC limits its use to only those surgeons with experience.
We recognize the ongoing controversy about the indications for TVC. Our data indicate that TAC is a successful option for carefully selected women with previous unsuccessful TVC.

REFERENCES

Histologic evidence of inflammation and risk of placental abruption

Carl A. Nath, MD; Cande V. Ananth, PhD, MPH; John C. Smulian, MD, MPH; Susan Shen-Schwarz, MD; Lillian Kaminsky, MD; for the New Jersey–Placental Abruption Study Investigators

OBJECTIVE: The objective of the study was to determine whether placental abruption is associated with an increased incidence of histologic chorioamnionitis among singleton gestations and whether this association is dependent on its severity.

STUDY DESIGN: Data were derived from the New Jersey–Placental Abruption Study, an ongoing, multicenter, case-control study conducted in New Jersey since August 2002. Subjects were women with a clinical diagnosis of abruption, and controls were matched to cases based on parity and maternal race/ethnicity. Two perinatal pathologists, blinded to the case-control status, performed all histologic examination based on standardized protocol. The association between chorioamnionitis and abruption was quantified based on odds ratio (OR) with 95% confidence interval (CI), after adjustment for potential confounders, and all analyses were stratified based on preterm birth (less than 37 weeks) status.

RESULTS: At preterm gestations (n = 141), chorioamnionitis was present in 30.8% and 12.5% of abruption cases and controls, respectively (OR 3.6, 95% CI 1.7 to 10.5). At term gestations (n = 205), the corresponding rates were 34.6% and 20.4%, respectively (OR 2.8, 95% CI 1.3 to 6.1). Severe chorioamnionitis was 7.2 (95% CI 1.6 to 20.1) and 18.3 (95% CI 2.2 to 150.4) times more common in abruption patients at preterm and term gestations, respectively.

CONCLUSION: Histologic chorioamnionitis is associated with placental abruption. The association was strongest in the presence of severe chorioamnionitis at term and, to a lesser extent, at preterm gestations. These observations suggest that the histologic findings in abruption are accompanied by severe inflammation, in both preterm and term gestations.

Key words: chorioamnionitis, histology, inflammation, placental abruption, preterm birth

Histologic chorioamnionitis is associated with placental abruption. The pathways by which these characteristics contribute to placental abruption can be categorized as either chronic processes or acute inflammation–associated processes.

We have previously proposed that acute and chronic inflammatory processes that activate cytokines, such as interleukin (IL)-1β and tumor necrosis factor-α, may lead to placental abruption. These cytokines have been shown to up-regulate the production and activity of matrix metalloproteinases in the trophoblast. The result is destruction of the extracellular matrices and cell-cell interactions, which may lead to disruption of the normal placental attachment and lead to the premature separation of the placenta. Nevertheless, evidence that supports the mechanisms by which inflammatory lesions lead to placental abruption remains sparse.

To better define how inflammation may be linked to abruption, we tested the hypothesis that acute and severe inflammatory processes defined by histologic

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examination of the placenta are associated with placental abruption. Specifically, we evaluated the severity of infiltration of neutrophils into the subchorion, the chorion, and the amnion.

**Materials and Methods**

The New Jersey–Placental Abruption Study (NJ-PAS) is an ongoing, matched case-control study conducted at 2 hospitals in New Brunswick, New Jersey, since August 2002. Both hospitals, Robert Wood Johnson University Hospital and Saint Peter’s University Hospital, are tertiary, level III, regional perinatal centers. The original study was undertaken to evaluate the association of thrombophilia status and placental abruption. Each study participant had a structured in-person interview questionnaire to collect details regarding maternal and paternal demographic characteristics, lifestyle, and behavioral and general health conditions. In addition, each participant gave consent to abstract the medical and prenatal care records from the index and all previous pregnancies and outcomes.

Placental abruption cases were identified, with gestational age greater than or equal to 20 weeks’ gestation, based on a clinical diagnosis. Women with placental abruption were identified using 1 of 2 criteria. The criteria for diagnosis of abruption included the classical signs and symptoms of painful vaginal bleeding or hemorrhage, uterine pain or tenderness, uterine hypertonicity, retroplacental clot, or hematoma on the placental surface or on the basis of prenatal sono- graphic diagnosis. Controls were enrolled on an ongoing basis following the recruitment of a subject. Further details of the NJ-PAS is described elsewhere. One (S.S.-S.) of 2 perinatal pathologists, who were blinded to abruption status, performed the histologic evaluation of the placentas, and each placenta was reviewed for gross findings and histologic lesions. The histologic classification of lesions was standardized through a protocol and tested in a pilot study for reproducibility prior to initiation of the study (unpublished data). Chorioamnionitis was defined by the presence of inflammatory infiltrates of neutrophils at 2 or more sites on the chorionic plate and extraplacental membranes. The degree of chorioamnionitis was then subclassified into the following categories: none, mild, moderate, and severe. Mild chorioamnionitis was defined by the presence of a few scattered (5–10 per high-powered field) neutrophils in the subchorionic space and adjacent chorion; moderate chorioamnionitis by many (11–30 per high powered field) neutrophils in the lower half of the chorionic plate; and severe chorioamnionitis by dense infiltrates of neutrophils (more than 30 per high powered field) throughout the chorionic plate into the amnion.

**Statistical analysis**

Distributions of maternal sociodemographic and behavioral characteristics were examined according to preterm and term birth status for both cases and controls. The association between chorioamnionitis and case-control status was based on odds ratio with 95% confidence interval derived from logistic regression models before and after adjustment for potential confounding factors. The confounders considered for adjustment included maternal age (grouped as younger than 20, 20–24, 25–34, and 35 years old or older), maternal education (coded as below or above high school), marital status (single or married), maternal smoking during pregnancy (non-smoker or smoker), and prepregnancy body mass index. Body mass index was derived as the ratio between prepregnancy weight (in kilograms) to squared height (in inches). Confounders were retained in the regression models for adjustment if their presence changed the odds ratio (between chorioamnionitis and placental abruption) by 10% or more. In addition, we further adjusted all analyses for parity (parity 0, parity 1, and parity 2 or greater) and maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other race/ethnicity).

We further examined the association between chorioamnionitis and abruption based on severity of chorioamnionitis (grouped as mild, moderate, and severe grades as well as mild/moderate and severe). All analyses were further stratified based on preterm birth status (20–36 completed weeks). All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Ethics approval was received by the institutional review boards of both hospitals as well as by the Institutional Review Board of University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, New Brunswick, NJ.

**Results**

There were 170 placental abruption cases during the study period, of which 68.8% were preterm and 31.2% were term. Preterm and term subjects and controls were examined for differences in clinical and maternal sociodemographic and behavioral characteristics (Table 1). Premature rupture of membranes approached statistical significance for preterm births, with a higher incidence in subjects (24.1%) than controls (8.3%). There were no differences between subjects and controls based on maternal age, parity, marital status. For term births, several differences were observed, including maternal race, education, and smoking.

The overall frequency of chorioamnionitis in the study group was 32%, and 69% of abruption cases occurred at less than 37 weeks’ gestation. Figure 1 shows the overall frequency of severity of chorioamnionitis for abruption subjects and
controls. The frequency of chorioamnionitis was greater for subjects in comparison with controls for the overall chorioamnionitis-abruption relationship and was most striking for severe chorioamnionitis (P < .05).

The comparison of histologic findings of chorioamnionitis between abruption cases and controls is shown in Table 2. The associations between chorioamnionitis and placental abruption at preterm (odds ratio [OR] 3.6, 95% confidence interval [CI] 1.7 to 10.5) and term (OR 2.8, 95% CI 1.3 to 6.1) gestations were significant. Severe chorioamnionitis was equally strongly associated with placental abruption at both preterm (OR 7.2, 95% CI 1.6 to 20.1) and term (OR 18.3, 95% CI 2.2 to 150.4). However, mild and moderate chorioamnionitis was not statistically significant.

Because premature rupture of membranes has been associated with increased risk of histologic chorioamnionitis, we performed a separate analysis of abruption and premature rupture of membranes (PROM) (Table 3). PROM was more frequent in abruption cases as the severity of chorioamnionitis increased, with PROM present in 35.3% of abruption cases with severe chorioamnionitis. Among abruption cases, there was an apparent trend for earlier delivery in PROM abruption cases vs non-PROM cases. This pattern of earlier delivery was most striking for severe chorioamnionitis, with delivery occurring on average 4.5 weeks earlier in PROM cases (P <

TABLE 1
Demographic characteristics of placental abruption cases and controls delivered at preterm and term gestations

<table>
<thead>
<tr>
<th></th>
<th>Preterm birth (20-36 wks)</th>
<th></th>
<th>Term birth (37 wks or longer)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (%) (n = 117)</td>
<td></td>
<td>Controls (%) (n = 24)</td>
<td></td>
</tr>
<tr>
<td><strong>Maternal age (y)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Younger than 20</td>
<td>3.4</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>18.8</td>
<td></td>
<td>25.0</td>
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<tr>
<td>25-34</td>
<td>52.1</td>
<td></td>
<td>45.8</td>
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<tr>
<td>35 or older</td>
<td>25.6</td>
<td></td>
<td>29.2</td>
<td></td>
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<td><strong>Maternal race/ethnicity</strong></td>
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<tr>
<td>Caucasian</td>
<td>22.2</td>
<td></td>
<td>20.8</td>
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</tr>
<tr>
<td>African American</td>
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<td>Hispanic</td>
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<td>37.5</td>
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<tr>
<td>Other</td>
<td>12.8</td>
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<td>12.5</td>
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<tr>
<td><strong>Parity</strong></td>
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<tr>
<td>Parity 0</td>
<td>38.5</td>
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<td>Parity 1</td>
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<td>Parity 2 or greater</td>
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<td><strong>Education below high school</strong></td>
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<td>Single marital status</td>
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<td>Maternal smoking</td>
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<tr>
<td><strong>Prepregnancy body mass index</strong></td>
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<tr>
<td>PROM</td>
<td>25.4 ± 5.7</td>
<td></td>
<td>25.0 ± 3.4</td>
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</tr>
<tr>
<td>Acute deciduitis</td>
<td>8.6</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wks)*</td>
<td>30.8 ± 3.8</td>
<td></td>
<td>32.7 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

* Data represented as mean ± SD.

FIGURE 1
Frequency of severity of chorioamnionitis among placental abruption cases and controls

COMMENT

Although many risk factors have been identified in association with placental abruption, causal pathways remain largely speculative. We report on the first prospectively collected series of placental abruption cases, with a complete histological evaluation of the placenta using standardized methodology with well-defined pathologic criteria for inflammatory lesions. Our findings corroborate previous findings that placental abruption is associated with inflammatory lesions of the placenta, especially at preterm gestations. Moreover, a novel finding was the strong association between more severe chorioamnionitis and abruption at term. This finding opens new insight into the importance of inflammatory processes in abruption by suggesting that perhaps inflammatory processes, when severe, may be involved in the pathway that leads to abruption irrespective of gestational age.

Recent evidence has also linked neutrophil infiltration into the decidua with preterm PROM and placental abruption. It has been demonstrated that the risk of abruption is 3.6-fold higher among women with preterm PROM, compared with women with intact membranes. When preterm PROM is accompanied by intrauterine infection, the risk of abruption is 9.0-fold higher, compared with women with intact membranes and no infection. It is known that abruptions are associated with a thrombin-enhanced expression of IL-8, a potent neutrophil chemoattractant, which leads to a marked infiltration of decidual neutrophils. This influx of neutrophils into the decidua is a rich source of proteases that can degrade extracellular matrix, leading to premature rupture of the fetal membranes. Therefore, it is difficult to judge definitively whether neutrophil infiltration into the decidua is secondary to vascular disruption or whether it is the primary cause of abruption through inflammation. In our study, the frequency of acute deciduitis was higher in subjects than controls at both preterm (8.6% vs 0%, respectively) and term (11.5% vs 4.6%) gestations, supporting the role of chronic inflammatory environment associated with increased risk of placental abruption.

We have previously demonstrated that neutrophils are increased in the chorion of placentas in women with placental abruption in comparison with controls. Furthermore, the histologic finding of neutrophil infiltration into the decidua was 1.6-fold more common with vaginal birth than with abdominal birth. Our current study further demonstrates that severe acute inflammatory processes are indeed important in term abruption as well.

We speculate that the placental lesions that manifest as severe chorioamnionitis are indicative of an intense inflammatory process at the interface of the decidua and chorion, which stimulate inflammatory cytokines and chemokines. The result of this cascade of events is destabilization of the uteroplacental interface, culminating in placental abruption, premature rupture of membranes, and preterm labor. Because the development

| TABLE 2 |
| Association between histologic chorioamnionitis and placental abruption at preterm and term gestations |
| Chorioamnionitis | Preterm birth (20-36 wks) | Term birth (37 wks or longer) |
| | Cases (%) | Controls (%) | Odds ratio* (95% CI) | Cases (%) | Controls (%) | Odds ratio* (95% CI) |
| None | 69.2 (n = 117) | 87.5 (n = 24) | 1.0 (reference) | 65.4 (n = 53) | 79.6 (n = 152) | 1.0 (reference) |
| Any | 30.8 | 12.5 | 3.6 (1.7 to 10.5) | 34.6 | 20.4 | 2.8 (1.3 to 6.1) |
| Mild | 12.0 | 8.3 | 1.6 (0.3 to 8.4) | 23.1 | 13.8 | 2.3 (1.0 to 5.8) |
| Moderate | 7.7 | 4.2 | 1.5 (0.2 to 14.7) | 3.8 | 5.3 | 1.5 (0.3 to 9.4) |
| Mild/moderate | 19.7 | 12.5 | 1.6 (0.4 to 6.3) | 26.9 | 19.1 | 2.1 (0.9 to 4.9) |
| Severe | 11.1 | 0.0 | 7.2 (1.6 to 20.1) | 7.7 | 1.3 | 18.3 (2.2 to 150.4) |

* Odds ratios are adjusted for the confounding effects of maternal age, parity, maternal race/ethnicity, maternal education, marital status, and maternal smoking during pregnancy through multivariable logistic regression.

| TABLE 3 |
| Incidence of PROM and mean gestational age at delivery by severity of histologic chorioamnionitis among placental abruption cases |
| Chorioamnionitis | PROM n (%) | Gestational age (wks)* | Non-PROM | PROM* | P value |
| | | | | | |
| None | 17 (15.6) | 34.5 ± 4.0 | 31.7 ± 3.4 | .008 |
| Mild | 2 (7.7) | 33.2 ± 6.6 | 28.0 ± 4.2 | .301 |
| Moderate | 3 (27.3) | 32.1 ± 5.2 | 30.0 ± 5.3 | .586 |
| Severe | 6 (35.3) | 31.0 ± 6.0 | 26.5 ± 2.8 | .020 |

* Table entries denote mean (± SD).
of severe histologic chorioamnionitis takes time, this further provides evidence for abruption as a more chronic process.

One potential explanation for our findings is that abruptions elicit an intense production of thrombin from the decidua that in turn leads to a massive recruitment of neutrophils. Therefore, the presence of neutrophils in the chorion may be a manifestation of a pathway that begins with abruption-related hemorrhage, leading to decidual cell production of tissue factor and eventual conversion of prothrombin to thrombin.

Others have described apoptotic cell death in the placenta of patients with histologic evidence of chorioamnionitis. Apoptotic nuclei are twice as common in the chorion of subjects with histologic chorioamnionitis, compared with those without chorioamnionitis. Chorioamnionitis may induce hypoxia with production of cytokines and other bioactive mediators, such as nitric oxide, superoxide, and peroxynitrite. These mediators may lead to cell death and subsequent placental abruption. In the study by Nakatsuka et al., placentas of patients with chorioamnionitis and abruption demonstrated a similar up-regulation of inducible nitric oxide synthase and other molecules involved in the apoptotic cascade.

Our study has several strengths: First, the data came from a well-designed prospective, matched case-control study. Furthermore, the collection of data was of very high quality (carried out by patient interview and critical evaluation of medical records), and detailed pathologic examination of the placentas was available for all recruited patients.

A few limitations of this study remain despite the prospective study design. Patients were matched according to parity and maternal race/ethnicity; however, there were several factors such as smoking and cocaine status, which may have been related in the causal pathway that leads to abruption. Specifically, it was tempting to control for preterm PROM and evaluating the latency period to delivery. However, earlier studies have suggested preterm PROM to be implicated in the causal pathway of the exposure-disease relationship. Therefore, PROM cannot be used as a variable to adjust the analysis. In addition, the specific demographic characteristics of our population may limit our ability to generalize the results of our study; however, our population was diverse. In addition, the association between chorioamnionitis and placental abruption has previously been described in other populations, making it more likely the results will be generalizable.

In conclusion, our findings suggest that more severe inflammatory lesions of the placenta, characterized by neutrophil infiltration of the chorion and amnion, are associated with placental abruption in both preterm and term gestations. However, a cause-and-effect relationship could not be established. These findings should stimulate further research, including animal models, into the histological evaluation of the placenta to elucidate the inflammatory changes that occur at the level of the uteroplacental interface, culminating in placental abruption.

REFERENCES

APPENDIX

Investigators currently participating or who have participated in the New Jersey–Placental Abruption Study include: Cande V. Ananth, PhD, MPH (principal investigator), Darios Getahun, MD, MPH, Neela Srinivas, MD, MPH, Celeste DeMarco, RN, BSN, Denise Elsasser, MPH, Yu-Ling Lai, RN, and Shelby Pitts, RN (Division of Epidemiology and Biostatistics); John C. Smulian, MD, MPH, Wendy L. Kinzler, MD, Morgan R. Peltier, PhD, and Marian Lake, RN, MPH (Division of Maternal-Fetal Medicine), all in the Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School; Claire Philipp, MD (Department of Medicine), University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School; and George G. Rhoads, MD, MPH (Department of Epidemiology), and Dirk F. Moore, PhD (Department of Biostatistics) at the University of Medicine and Dentistry of New Jersey–School of Public Health.

Other investigators that were involved with the study included: Rima Rozen, PhD, and Jacques Genest, MD (McGill University, Montreal, Canada); Susan Shen-Schwarz, MD (Department of Pathology, Saint Peter’s University Hospital, New Brunswick, NJ), and Vinay Prasad, MD (Department of Pediatric Pathology, Arkansas Children’s Hospital, University of Arkansas Medical Sciences, Little Rock, AR).
Discussion: ‘Complications of labor induction among multiparous women’ by Battista et al

William A. Grobman, MD, MBA, George A. Macones, MD, MSCE, Associate Editor

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:


DISCUSSION QUESTIONS

- What is the primary aim of this study?
- Is this an important study question?
- What was the study design?
- What are the advantages of this study design?
- What were the inclusion and exclusion criteria of the study?
- Can you describe external and internal validity?
- How would you judge the external and internal validity of this study?
- What is confounding bias, and is it present in this study?
- Is there any downside to using exclusion criteria?
- Why was random effect logistic regression used?
- What are the conclusions of this study?
- Why would induction with oxytocin alone be associated with a significantly greater risk of postpartum hemorrhage?
- Do multiparous women who are electively induced have an increased risk for cesarean delivery?
- Would this study change your clinical practice?

In 1620, philosopher Francis Bacon observed, “Nature cannot be ordered about, except by obeying her.” That was long before labor induction was commonplace. In 1990, 9.5% of all births in the United States were induced; that figure climbed to 19.4% by 1998. At one community hospital, the induction rate climbed from 32% to 43% between 1990 and 1997, a finding that was not necessarily exceptional. The procedure can be accompanied by complications in nulliparous women, but the data are inconsistent among multiparous women. In investigating this important area, Battista and colleagues made a series of good design decisions, which led to very useful information.

This interesting observational study provides a good opportunity to review the concept of validity, which reflects whether study results correctly represent the associations of interest. Both external and internal validity have to be assessed before readers can determine if research findings apply to their own patients.

SHORT-SITED?

External validity, a measure of generalizability, gauges the applicability of study results to people other than study participants. It can be maximized by including subjects from as wide a cross section of the population as is possible. Conversely, a more limited study population yields less generalizable results.

For example, if a study included only women of upper socioeconomic status, its results would not necessarily be applicable to women who were not in that so-
cial stratum. In some cases, including the study by Battista et al, there can be good reason to limit external validity. Because the researchers excluded nulliparous women from their analysis, their results cannot be extended to a nulliparous population. But that is more than compensated for by the greater focus on labor induction in multiparous women, a previously understudied group.

As noted in this month’s Journal Club discussion, external validity can be compromised when all patients are from a narrow geographic region. These participants may share attributes that are tied to their locale; characteristics that may not be easily quantified. For example, certain local practice patterns may produce associations that would not exist when other approaches to medical management are applied. Study subjects were delivered at 4 different hospitals, but all institutions belonged to a single health system. Journal Club members pointed out that some care plans could be particular to those locales and not generalizable to other institutions or regions. This is not to say that a study from a single area unavoidably lacks external validity—rather, it should be remembered that the possible effect of a study population’s geographic location should be considered when external validity is assessed.

**Beware of Bias**

Internal validity measures how well the study’s results apply to those who were actually studied. It can be marred by bias, which is nonrandom systematic error. Bias can be introduced in several ways, including in the selection of study subjects (ie, selection bias) or in the collection of data (ie, ascertainment bias). The latter, also called measurement bias or observation bias, can be introduced in multiple ways.

Indeed, this type of bias can be difficult to remove even with the most finely honed study protocol, as it can be introduced during patient care administered outside a protocol. The caregiver who has already read or heard about an association between an exposure and an outcome might be more likely to diagnose the outcome after a given exposure has occurred. Obviously, subjective outcomes are more prone to this form of ascertainment bias than objective outcomes.

Postpartum hemorrhage, for example, is more likely to be documented in a biased fashion than would be hysterectomy or a patient death, but even firmly objective outcomes are not immune from ascertainment bias. Consider a study data collector who might, albeit unknowingly, be more likely to scour the chart or perform a more thorough patient interview in an effort to uncover certain outcomes if a certain exposure is known to have occurred. In the work by Battista et al, a data abstractor who was aware that induction of labor can be complicated by postpartum hemorrhage might be more likely to interpret documentation of bleeding after delivery as postpartum hemorrhage, whether or not the event strictly meets the definition; in the same way, the treating physician could document bleeding as postpartum hemorrhage.

One method for limiting ascertainment bias is to enlist different abstractors for the collection of exposure data and outcome data. Each would then be shielded from the data of the other. Blinding the data collectors helps prevent the introduction of bias through other information they have gleaned. Nevertheless, even this does not remove all possibility of ascertainment bias. Thus, both data sources and methods of data collection should be carefully considered in determining whether ascertainment bias exists, and if it does, the degree to which it might impair internal validity should be weighed.

**Grouped Mentality**

The analysis of an observational study can be complex, since investigators need to consider adjusting for factors that may confound the association between an exposure and an outcome. As has been discussed in prior sessions of the Journal Club, this is usually handled with a multivariable analysis, as when logistic regression is applied to binary outcomes. In this study, the analysis is more complex than usual, because the data are clustered.

Clustered refers to an aggregation of subjects and/or outcomes that is greater than would be expected by chance. The outcomes of subjects within a cluster are not completely independent of each other, and yet, independence is a critical assumption of a standard multivariable analysis. An easy example of clustering in obstetric research is multiple gestations, where the outcome of each neonate is correlated with the outcome of another.

In this manuscript, each individual physician practice can be seen as a cluster, since the management of subjects within a practice and their outcomes are not necessarily independent of each other. For example, some physicians may be more inclined to induce labor than others. Random effects regression modeling is preferable in a study like this, because it takes correlation or clustering of subjects into account. Although a standard model could still be constructed when data are clustered, the chance of a falsely positive result is increased. That is, random effects models tend to be more conservative—they are less likely to find a significant association—than are models that do not incorporate clustering. We do not see a lot of random-effects modeling in obstetric and gynecologic research; probably because most data sets do not include clustered information.

Multiparous women who underwent labor induction had an increased risk of cesarean section, particularly when cervical ripening agents were necessary. Compared with those who went into labor spontaneously, those who were induced also had a greater risk of blood loss exceeding 500 mL and a longer stay in labor and delivery. However, Journal Club participants noted that even with an increased risk of cesarean section, the vast majority would deliver vaginally. Members agreed that the information gleaned from this study would help fine-tune patient counseling.

**REFERENCES**

Overburdened and undernourished

Angelika Bord, MD; Simcha Yagel, MD; Dan V. Valsky, MD

CASE NOTES

A woman, gravida 2, para 1, was admitted at 30 weeks of pregnancy for suspected intrauterine growth restriction. Indeed, fetal ultrasound and echocardiography revealed that the fetus had an estimated weight of 700-800 g and a head-abdomen ratio of 1.26. Fetal biometry was consistent with symmetric intrauterine growth restriction (IUGR). Fetal anatomy, amniotic fluid volume, and placentation and cord insertion were all normal.

Doppler evaluation confirmed normal flow without notching in both uterine arteries. Flow parameters were also normal in a free loop of umbilical artery (peak systole/peak diastole or S/D= 4.25; resistance index or RI=0.76), but diastolic flow was reversed or absent in other areas of the cord. Normal resistance was noted in the umbilical arteries near the placental cord insertion (S/D=4.0; RI=0.75). Elevated diastolic flow (S/D 2.86; RI=0.65) in the middle cerebral artery reflected a brain-sparing effect. Flow in the ductus venosus was normal.

CONCLUSIONS

Signs of severe, early, symmetric IUGR, combined with normal parameters of placental resistance and areas of high umbilical artery resistance, suggested cord obstruction. Close examination of the umbilical cord with 3-dimensional power Doppler ultrasound revealed 4 nuchal cord loops (Figure 1). Color Doppler evaluation of these loops disclosed absent or reversed end-diastolic flow in associated portions of the umbilical arteries (Figure 2).

Betamethasone was administered to promote fetal lung maturation. The fetus was closely followed with fetal monitoring 3 times a day and a daily sonographic biophysical score assessment. In the 31st week of pregnancy, repeated, severe, variable decelerations occurred, and an emergency cesarean section was performed. A male with extreme growth-restriction, weighing only 840 g, had 4 umbilical cord loops tightly encircling his neck (Figure 3). One loop had to be released before the neonate could be extracted. His Apgar scores and pH were normal.

Encirclement of the fetal neck by a nuchal cord occurs in 5.8–29% of all pregnancies and is generally considered benign. Development of fetal growth restriction in some of these cases is well established in the literature, and its severity is positively correlated to the number of encircllements. Generally, the more serious the compression and the...
The longer it persists, the more severe the sequelae that can develop.

The present case represents an interesting example of severe IUGR in which the etiological factor—multiple, tight nuchal cord loops—was suspected when discrepancies in Doppler blood flow parameters were found. Normal transplacental flow parameters were observed in conjunction with increased flow resistance in the umbilical arteries in a free loop of cord and absent-to-reversed flow in the nuchal loops.

Optimal management and timing of delivery for growth-restricted fetuses with cord abnormalities remain unresolved. In cases of IUGR resulting from chronic cord compression, it might not be feasible to stall delivery until fetal Doppler evaluations suggest that parameters and biophysical profile are worsening, because fetal deterioration or demise can be unpredictable. Early delivery is preferred when adequate neonatal intensive care is available.

REFERENCES
Comment on single-dose methotrexate regimen in the treatment of low-risk gestational trophoblastic neoplasia

TO THE EDITORS: We commend the work of Chan et al1 and agree that use of single-dose methotrexate regimen is an effective option for women with low-risk gestational trophoblastic neoplasia without metastases and a low pretreatment human chorionic gonadotropin level. According to Miller and Lurain,2 the term gestational trophoblastic neoplasia is no longer used because invasive moles are not true neoplasms. We suggest the use of gestational trophoblastic tumor instead of neoplasia.

We hope further discussion and suggestion will contribute to advancement and popularity of the findings of the authors among practicing gynecologists.

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REFERENCES

REPLY

We thank Dr Mahajan et al for their interest in our work.1 The terminology for gestational trophoblastic diseases had indeed been under a lot of debate, and different terms had been used in the past. A consensus was reached after much discussion from experts from the International Society for the Study of Trophoblastic Disease and the International Gynecologic Cancer Society in 2000. The revised classification was accepted by the International Federation of Gynecology and Obstetrics (FIGO) Oncology Committee in 2002.2 We understand that no single definition would be completely applicable in every situation; however, it is important to use an agreed-upon terminology so that data can be compared throughout the world. We have therefore adopted the FIGO 2000 classification system2 in our report.

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REFERENCES

Randomized, double-blind, placebo-controlled trial of transdermal nitroglycerin for preterm labor

TO THE EDITORS: We read with great interest the recent paper by Smith et al,1 who studied the effect of transdermal nitroglycerin on preterm labor. However, we have some comments pertaining to the design of the trial.

The authors mentioned that the available tocolytics have poor efficacy and do not improve neonatal outcomes.2 In fact, most trials that came to the same conclusion have included a large proportion of women who were advanced in gestation in which further prolongation of pregnancy might have a marginal effect. This might not hold true for women at the lower extreme of gestational age (24 weeks) included in their trial.

Furthermore, most authorities and medical bodies, including the Royal College of Obstetricians and Gynaecologists, agree that some form of tocolysis should be considered in women with preterm labor, given the evidence that some tocolytics are associated with a significant reduction in the odds of delivering within 48 hours and even 7 days.2,3 This time gained is invaluable to administer corticosteroids and to transfer to a center with better neonatal care facilities.

At the time of the initiation of their trial, at least 1 systematic review documented the efficacy of nifedipine in delaying delivery for more than 48 hours and a lower risk of respiratory...
distress syndrome and admission to a special care unit, compared with beta-agonists.4 Therefore, it is not easy to understand from an ethical point of view how the authors denied many women in the placebo group these potential benefits.

In addition, the authors mentioned in the abstract conclusion that the reduction in the primary outcome is the result of decreased risk of delivery before 28 weeks’ gestation. This is negated in their results section in which this reduction was reported as statistically nonsignificant (relative risk caused by 0.50, 95% confidence interval, 0.23-1.09). Although they theorized that this reduction might be caused by a potential non-tocolytic effect of nitroglycerin, this remains to be quantified in future research.

The authors went further to exclude women who delivered at term “because they likely represent women who were not in true preterm labor.” Although this exclusion might be justifiable on the premise that the outcome in such pregnancies would be optimal irrespective of whether tocolysis was given, those patients most likely were in “true” labor according to the strict inclusion criteria of the study.

We thank Drs Ustar and Nassar for their interest in our trial. Given the increasing incidence of preterm birth, the vast array of tocolytics available, and the ongoing research into such drugs, it would seem apparent that there is ongoing skepticism regarding their efficacy. In contrast to what they report in their letter, the governing bodies do not state that some form of tocolytic should be used. The Royal College of Obstetricians and Gynaecologists, which they quote, states that “it is reasonable and ethical on the premise that the outcome in such pregnancies would be optimal irrespective of whether tocolysis was given, those patients most likely were in “true” labor according to the strict inclusion criteria of the study.

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**REPLY**

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We would disagree with Drs Ustar and Nassar that the women in the trial who delivered at term were in “true” preterm labor because of our inclusion criteria. Given that an approximately 50% of those women who received placebo delivered at term, we can certainly state that these women were not in true preterm labor despite our inclusion criteria. Given what we know about the mechanisms of labor, it is physiologically improbable that any tocolytic agent would prolong pregnancy by more than a few days or weeks at most. Even in a trial setting with strict inclusion/exclusion criteria, we know that clinical assessment of preterm labor is relatively poor in its positive predictive value.

As stated in the article and reiterated by Drs Ustar and Nassar, the mechanism of effect of glyceryl trinitrate (nitroglycerin) at improving neonatal outcome is unknown, and more work should be done, given the real potential neonatal benefit of using glyceryl trinitrate (nitroglycerin) for women in preterm labor.

**REFERENCES**


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Faulty interpretation of observed racial disparity in recurrent preterm birth

TO THE EDITORS: Kistka et al\textsuperscript{1} reported that black women in Missouri had a higher risk of preterm delivery and recurrent preterm delivery than white women and that these differences persisted after adjustment for some variables available from birth certificates. Aside from the statistical error of not accounting for clustering by the mother in the variance estimation, the analysis is remarkable. What is problematic is the authors’ interpretation. The adjusted odds ratio for black race represents the contrast conditioned on measured predictors and demonstrates a persistent excess risk for black women. This excess risk is caused by factors that were not measured, but the authors vigorously asserted that these unmeasured factors are most likely genetic.

This interpretation is fallacious because adjusting for some small set of crudely measured social or environmental variables does not imply that other variables from this general class are balanced across the racial groups.\textsuperscript{2} The argument is especially suspicious here because the modeled covariates were only those available from birth certificates. For example, social status was assessed only by dichotomized education and public assistance program participation. Arguing that conditioning on these few factors makes black and white women indistinguishable with respect to other important social exposures is not credible.

The authors based their assertions about the importance of genetic mechanisms primarily on results for recurrence. These results suggest only that the etiologically important unmeasured factors are stable over time, however, not that they are genetic. The authors’ argument is reminiscent of earlier epimologic myths about ethnic differences, such as the assertion a century ago that tuberculosis was a genetic disease linked to the trait of red hair on the basis of its higher incidence among the Irish. The authors also ignored a substantial literature that contraindicates their proposed explanation. For example, multiple studies have found that the risk of adverse pregnancy outcome for black immigrants to the United States is comparable with that of native-born whites, not to native-born blacks.\textsuperscript{3,4}

Imagine how preposterous it would be if, after having ruled out a few murder suspects named Bob, Fred, and Karl, a police detective were to declare triumphantly that the murderer must therefore have been a woman. Science would be better served if we made well-reasoned inferences about the effects of things we actually measured, rather than unfounded speculations about things that we did not measure.

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REPLY

We appreciate the comments and are sensitive to the issues raised by Kaufman et al\textsuperscript{1} concerning our manuscript. Our results provide supportive evidence that genetic factors are likely to contribute to preterm birth. We acknowledge important social and environmental influences on the racial disparity in preterm birth and maintain that identifying the entire spectrum of risk factors imparting this disparity is essential.

We described 3 observations that suggest a role for genetic factors in prematurity: first, an increased rate of recurrence of preterm birth to black mothers; second, earlier preterm births in blacks; and third, concordance in timing between preterm births to a given mother, regardless of race. This concordance in timing of recurrent preterm birth, with median age 2 weeks earlier in blacks, is especially compelling for genetic contributions.

We welcome alternative testable hypotheses to account for this pattern. Because a primary outcome was to assess recurrence in a multiple birth cohort, providing adjustment for clustering may mask biological differences inherent to the data. Nonetheless, in-
TO THE EDITORS: Kistka et al\(^1\) concluded that racial disparities in preterm birth observed in their study must be caused by an underlying genetic factor because of the observed regularity in timing of repeat preterm births and their control for maternal socioeconomic and medical characteristics. Because of flaws in their analysis, this conclusion is insufficiently supported.

First, the authors overstated the adequacy of their adjustment for socioeconomic status because the only socioeconomic indicator included was receipt of public assistance (Medicaid, food stamps, and WIC). Other aspects of socioeconomic status not included in their analysis are also important independent determinants of preterm birth (eg, education level)\(^2\); thus, the authors could not rule out the contribution of socioeconomic status to observed disparities.

Second, simplistic conclusions of genetic determinism failed to acknowledge ancestral heterogeneity within the American blacks community as well as research findings indicating time lived in the United States to be an important determinant of the elevated preterm birth rate among American blacks relative to American white women. A study by Fang et al\(^3\) found that after adjustment for socioeconomic factors, immigrant black women had preterm birth rates less than native black women and comparable with white American women. Similarly, Howard et al\(^4\) found nonnative black women to have lower rates of preterm birth than native black women, although relative rates varied according to region of origin. Such studies suggest that social exposures specific to residence in the United States are important determinants of observed racial disparities in adverse birth outcomes.

It is dismaying that the authors assumed that regularity in the timing of preterm births could be explained only by genetic variation in preterm birth, like other complex diseases, is likely to contribute to racial health disparities. Indeed, gene variants that increase risk in blacks\(^5\) or are associated with a lower risk of preterm birth in whites\(^6\) as contributing to the racial disparity have recently been reported.

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Racial disparities in preterm birth: the role of social determinants

TO THE EDITORS: Kistka et al\(^1\) concluded that racial disparities in preterm birth observed in their study must be caused by an underlying genetic factor because of the observed regularity in timing of repeat preterm births and their control for maternal socioeconomic and medical characteristics. Because of flaws in their analysis, this conclusion is insufficiently supported.

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Psychosocial contributors to preterm birth must be considered

TO THE EDITORS: The attribution by Kistka et al1 that genetics is the cause of racial disparities is lackng in credibility, given the substantial amount of research suggesting that social factors contribute to the racial/ethnic disparities in birth outcomes. Race is a social construct, so to attribute a health outcome to race without assessing the social implications of the construct gives more credence to a biological definition of race. Furthermore, attributing racial disparities to genetics suggests that all blacks, regardless of nationality, should have similar birth outcomes. This is not the case. In 2005, Acevedo-Garcia et al2 found that there is a differential effect of foreign-born status on low birthweight among blacks. Foreign-born black women were 25% less likely to deliver a low-birthweight infant, compared with US-born black women (odds ratio, 0.75; 95% confidence interval, 0.72-0.78).2 There is a greater protective effect of foreign-born status for black women with less than a high school education. If genetics were the culprits, we would expect there to be no differential between US- and foreign-born black women, with lower education attenuating the effect of foreign-born status because it is a risk factor among American women.

Attribution of preterm birth to genetic etiology also derails focus from the social factors that contribute to the increased incidence of preterm birth among black women, such as racial discrimination.3 The authors suggest that there are “physiological characteristics of the mother that influence the timing of birth,” because preterm births to black mothers occur at roughly the same gestational age in subsequent births. To attribute the physiologic characteristic to genetics ignores the literature that shows that maternal stress and the activity of the hypothalamic–pituitary axis are associated with preterm birth.4 Exposure to chronic stress (ie, racial discrimination) leads to dysregulation of the stress-response system. In response to a stressful event, the hypothalamic–pituitary axis releases the stress hormones corticotropin-releasing hormone (CRH) and cortisol. Exposure to chronic stress allows for repeated release of the hormones, with little time for regulation of the hormone levels. After a time, the stress-response system becomes dysregulated, with the body incessantly functioning at this heightened level of stress response. CRH also plays a role in the stimulation of labor, so it is not inconceivable that chronic stress, in the form of racial discrimination, could lead to preterm labor among black women through this pathway.

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REFERENCES

REPLY

The letters by Moultrie and Spriggs state that our manuscript1 concluded that “genetics is the cause of racial disparities” in preterm birth and “racial disparities in preterm birth must be due to an underlying genetic factor.” Implicit in this misrepresentation of our results is the notion that we assert that there is no role for social and environmental factors. We present information that many social and environmental factors indeed contribute to risk for preterm birth, and these factors may not be equally distributed across races. Nonetheless, after adjusting for many of these factors, including maternal education as indicated in our regression analysis, a large increase in risk for preterm births in blacks remains. This result, taken together with the increase in risk of recurrence, concordant gestational age timing, and earlier preterm births along with recent genetic studies from other groups,2,3 suggests genetic factors contribute to the racial disparity. We explicitly state in our paper that we have not proven a genetic cause, but accumulating information suggests a component of the increase in risk is likely to be heritable.

Data from immigrant studies highlight the complexity of factors that have an impact on risk for preterm birth. However, those studies that have analyzed preterm birth in foreign vs US-born black cohorts consistently demonstrate an increase in risk for blacks above whites, including foreign-born blacks.4-6 Curiously, Fang et al7 adjusted for preterm birth in their regression analysis of nativity on low-birthweight, confounding the interpretation of their results, given the consistent increase in preterm birth risk among blacks.

To say that race is purely a social construct ignores a wealth of information on ancestry-dependent genomic variation and accuracy of self-reported race as reflecting ancestry, which should be brought to bear on the problem of preterm birth.8,9 Multidisciplinary approaches to preterm birth (analyzing social factors as
can be accomplished through immigrant studies or stress pathways, for example) will continue to produce valuable information. We encourage the broad research community interested in preterm birth to remain open minded and collect some DNA along the way.

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Commentary on microarray analysis for gynecologists and obstetricians

TO THE EDITORS: I would like to comment on several issues suggested by a series of articles on microarray analysis that appeared in a recent issue of the Journal.1-4

First, non-PhD clinicians may have difficulty comprehending the technology of microarray analysis and its demanding techniques. Proper training is essential for several reasons. For example, the characteristics of individual specimens—for our specialty, these include blood, amniotic fluid, and placental and other tissues—affect the ability to extract, purify, and isolate samples for use in microarray analysis. In addition, before clinicians attempt to interpret the results, they must understand how greatly sensitivity, specificity, and positive and negative predictive values can vary in this expensive technology, whose use must justify its high cost by providing significant results.

Second, confounding factors such as low-abundance potential biomarkers could be interfered by several groups of high-abundance proteins in human plasma in which a dynamic concentration range of protein component exists.5 Furthermore, how the extent of early detection and the amount of these proteomes could be accurately measured, and the limitation of instruments will be another issue. Therefore, those factors must be addressed before this high-throughput technology is applied to the cells with confidence.

Third, the accurate identification of candidate genes requires close attention to subtleties of the statistical method used and possible effects on results. In addition, clinicians must recognize the need to cross-validate candidate genes and confirm their choice with quantitative real-time polymerase chain reaction.

The greatest challenge faced by clinicians who lack advanced technical training is to interpret the results. In the interest of developing nonbiased study designs and protecting quality assurance, physicians should be alert for such potential roadblocks as sources of human error, technical difficulties, the reproducibility of laboratory procedures, data interpretation, and the interaction between the environment and the detected genes.

Finally, clinicians should prepare themselves to use microarray analysis by taking a biophysical chemistry approach and reconsidering the pathophysiologic mechanism and biologic pathway in light of the biochemical and chemodynamic state of the human body. The implication of those would be a cross-disciplinary integrated collaborative research team. Physicians-in-training should be inculcated into molecular and cellular biology coursework and series of laboratory technique training. Similarly, the basic researcher or scientists should communicate with physicians in terms of probing at clinical-oriented and specifically to problem-solving processes. The evolving of paradigm change of system biology and systems medicine era will replace the traditional medicine in early predictive biomarkers of cancer disease, in promoting preventive health care, and tailored through personalized medicine.6

Besides reading the Journal’s excellent introductory articles on genomic medicine, clinicians who intend to attempt microarray analysis would benefit from reflecting on the nature and scope of knowledge.7

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REFERENCES


REPLY

We thank Dr. Liu for his interest in the series of articles on microarray technology in Obstetrics and Gynecology and our Editorial, on published recently in the Journal. Dr. Liu highlights the complexity of some of the challenges. We agree with many of his points, which are valid to the application of virtually any experimental methodology in high-dimensional biology to any problem within or outside reproductive sciences.

The availability of high throughput methodology and computational biology is likely to make clinical application of the “omics sciences” inevitable. However, we wish to alert readers that the conceptual framework, experimental platforms and analytical methods are still in the process of developing and far from mature. It is necessary to maintain a healthy dose of skepticism as to whether these approaches will deliver on their promises.

Given the “completion” of the human genome as well as the simplicity and robustness of nucleic acid hybridization, the most mature of the “omics sciences” is transcriptomics. This is a reason why we have made this a research priority. Nevertheless, challenges remain as RNA is an intermediate product, which by itself does not determine phenotype. The expectation that proteomics, metabolomics and other “omics” will enhance our understanding of the mechanisms of disease, as well as diagnostic, prognostic and therapeutic approaches remains appealing, but we are among the first to alert readers that the complexity and magnitude of the challenge in reproductive sciences are not trivial. The feasibility of this approach has, however, been demonstrated in selective cases using model systems. Human disease is the next frontier.1-4

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REFERENCES


The corrected Table appears below:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Mean procedure time (minutes)*</th>
<th>Number of procedures</th>
<th>Difference (minutes)</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>With appendectomy</td>
<td>77</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without appendectomy</td>
<td>68</td>
<td>44</td>
<td>8</td>
<td>-3 to 20</td>
<td>.16</td>
</tr>
<tr>
<td>With lysis of adhesions</td>
<td>93</td>
<td>26</td>
<td>28</td>
<td>14 to 42</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Without lysis of adhesions</td>
<td>65</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval.
* Means were stratified by type of concomitant procedure and weighted by sample size of each stratum.


Four of 8 women (50%) with avidity 40 or LESS transmitted herpes simplex virus to the neonate, compared with only 12 of 97 (12%) of women with avidity greater than 40 ($P = .02$)

Bag A was always given 15-60 minutes pre-operatively and B after cord-clamping. The contents were randomized and blinded to everyone except the investigational pharmacy. This is stated correctly in the online version and outlined in Figure 1 of that text.


The “%” symbols were omitted from 64 and 68. The correct sentence should read: “In a survey of 921 primiparous women, only 64% of the women were taught pelvic floor exercises during antepartum or postpartum period, and only 68% of those women reported actually performing the exercises.”
Discussion: ‘Complications of labor induction among multiparous women’ by Battista et al

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:


DISCUSSION QUESTIONS

- What is the primary aim of this study?
- Is this an important study question?
- What was the study design?
- What are the advantages of this study design?
- What were the inclusion and exclusion criteria of the study?
- Can you describe external and internal validity?
- How would you judge the external and internal validity of this study?
- What is confounding bias, and is it present in this study?

- Is there any downside to using exclusion criteria?
- Why was random effect logistic regression used?
- What are the conclusions of this study?
- Why would induction with oxytocin alone be associated with a significantly greater risk of postpartum hemorrhage?
- Do multiparous women who are electively induced have an increased risk for cesarean delivery?
- Would this study change your clinical practice?

INTRODUCTION

In 1620, philosopher Francis Bacon observed, “Nature cannot be ordered about, except by obeying her.” That was long before labor induction was commonplace. In 1990, 9.5% of all births in the United States were induced; that figure climbed to 19.4% by 1998.1 At one community hospital, the induction rate climbed from 32% to 43% between 1990 and 1997, a finding that was not necessarily exceptional.2 The procedure can be accompanied by complications in nulliparous women, but the data are inconsistent among multiparous women. In investigating this important area, Battista et al made a series of good design decisions, which led to very useful information.

William A. Grobman, MD
George A. Macones, MD

BACKGROUND

Grobman: Induction of labor has become an increasingly common obstetrical procedure. Correspondingly, multiple studies have tried to assess the consequences of induction of labor for women and their neonates. There is a reasonable amount of evidence that nulliparous women who undergo induction of labor have a greater risk of cesarean section and other obstetric complications when compared to their spontaneously laboring counterparts. Evidence that labor induction and spontaneous labor have different outcomes has not been demonstrated as consistently in multiparous populations, although the lower frequency of complications in multiparous women may appear to have fewer associations due to Type II error.

STUDY OBJECTIVE AND DESIGN

Grobman: What is the primary aim of this study?
The primary aim of this study is to determine whether the frequency of complications among multiparous women who undergo induction of labor is different from that of multiparous women who labor spontaneously.

**Grobman:** Is this an important study question?

**Dugan:** I do think that this is an important study question. The experience at our own institution is similar to that reported throughout the country; namely, the frequency of induction has increased substantially. Thus, I think it is important to determine what the complications associated with that frequent event might be. This information will allow me to make better medical decisions, and help me to inform my patients, who seem to increasingly inquire about induction of labor and its risks and benefits.

**Grobman:** I couldn’t agree more. It is important to understand outcomes related to labor induction so that we can optimally counsel our patients. What type of study design have the authors used?

**Facco:** They used a retrospective cohort design. After women delivered, the authors abstracted data from the patients’ charts and separated women into 2 groups according to type of exposure—that is, whether they had induced or spontaneous labor. Multiple outcomes were then compared between these 2 exposure groups.

**Grobman:** Can you explain the advantages of using this type of study design for this particular question, as opposed to a case control study?

**Julien:** In a case-control study, the groups would have been defined not by their exposure, but by their outcomes. For example, women would have been classified according to whether they had a cesarean section or vaginal delivery, and then compared with respect to the different exposures that they had experienced, such as labor induction. This study design would have been better if the authors wished, for example, to determine the many different risk factors for a single outcome, such as cesarean delivery. However, as the authors wished to examine multiple obstetrical outcomes, the decision to use a cohort design was a wise one, because it allowed assessment of different outcomes related to a single type of exposure.

**Su:** Additionally, because a cohort design was used, we can understand the incidence of different outcomes in relation to induction of labor. For example, if a case-control design had been used, even if inductions were found to be associated with increased odds of a cesarean delivery, it would not be possible to directly know the probability of having a cesarean section after labor induction from the study results.

**Grobman:** Right. You both bring up really good points. Because labor induction is sufficiently frequent and because the authors wished to examine multiple outcomes, the cohort design was a very good choice.

**Grobman:** What were the inclusion and exclusion criteria of the study?

**Linder:** The authors chose to include women who underwent labor at 37 to 42 weeks’ gestation with singleton pregnancies. Also, these women had no history of a prior cesarean section or a contraindication to a trial of labor, such as breech presentation, placenta previa, or prior myomectomy.

**Grobman:** Why did the authors decide to exclude women with prior cesareans? After all, induction of labor is not an absolute contraindication for women with this history.

**Linder:** I believe the authors wanted to eliminate the possibility that the overall outcomes could be influenced by this subgroup, making the results less generalizable for all multiparous women. For example, if there were complications that were related only to induction of labor in women with a scarred uterus, this association might make induction of labor appear more likely to have complications for the whole population. Alternatively, if physicians were avoiding labor inductions in women with prior cesareans, these patients—and their related complications—might be disproportionately represented in the spontaneously laboring group. Although a subgroup analysis could have tried to account for this potential confounding, another method to control for confounding is exclusion, which was the method of choice for this subgroup.

**Grobman:** I agree that it was a good decision to exclude these women, particularly given that the population of women with a prior cesarean section was small enough that any subgroup analysis would be severely limited.

**Grobman:** Was any other group of women excluded?

**Linder:** Yes, women who had premature rupture of membranes.

**Grobman:** Why do you think they excluded that population?

**Linder:** Once again, I think this population is different from multiparous women who arrive for an induction of labor but have no evidence that processes of parturition have begun. An argument could be made that rupture of membranes, even if premature, signifies that some process associated with delivery has started. Thus an outcome such as cesarean section may be the result of a different course in that group than in women who had undergone labor induction. Similarly, if patients with premature rupture of membranes had an increased frequency of infection and hemorrhage, this finding may be of no relevance for other women who are undergoing induction of labor. Again, these possibilities could have been assessed in subgroup analyses if the authors had chosen to include these patients, but the exclusion simplifies the final analysis.

**Grobman:** Can you touch upon the concept of external validity?

**Gerber:** Sure. External validity refers to the concept that the results of a study are generalizable to patients with similar characteristics who were not part of the actual study population—in this case, multiparous women undergoing labor.

**Grobman:** Could you please describe internal validity and contrast it with external validity?

**Facco:** A study with internal validity has been performed in such a way that the results are valid for the study population. Thus, a well-done study could have good internal validity, but if it is performed in a group of women with particular characteristics, it might only be valid for those women. Alternatively, if a study did not even have internal validity, the
conclusions would be questionable for anyone at all, including the subjects.

Grobman: Thank you for that comparison. What do you think about the external validity of this study?

Su: I think that their results are relatively generalizable. For example, they included 4 different community hospitals, and the women in the study population were fairly diverse with respect to their demographic characteristics.

Grobman: I agree that there were some aspects that enhance generalizability, such as including patients from community hospitals and not just tertiary care centers. One issue that we should at least consider, though, is the narrow geographic location, as well as the fact that the hospitals were all part of a single health system. There is at least the potential that some patterns of care would be specific to these locales and not generalizable to other institutions or other regions of the country. I think it is important to remember that external validity is enhanced by greater diversity of the population. Nevertheless, as you noted, the fact that the authors included multiple different hospitals would only increase the external validity.

Grobman: Does the study have sufficient internal validity?

Nelson: Ascertainment bias could have occurred in this study; it is often an issue for observational studies, particularly if they are retrospective. This type of bias can occur both at the time that the medical record is being produced, as well as when the data abstractors are collecting data for the study, especially if the outcome has some subjectivity. For example, someone who is doing medical record abstraction on a person who has been induced may be more likely to search for or code an event as a postpartum hemorrhage. Similarly, a provider who already has some sense that induction of labor is associated with hemorrhage may be more likely to label bleeding as a postpartum hemorrhage after an induction of labor. The first type of ascertainment bias can sometimes be limited if the abstractor who ascertains the outcome is blinded to the exposure. This type of blinding would not be possible for the second type of ascertainment bias we were talking about.

Grobman: What is confounding bias, and is it present in this study?

Gerber: Confounding occurs when an association between 2 factors is really due to a third factor linked to each of those first 2 factors. In the present study, for example, one should consider whether an association between induction and cesarean delivery was really due to a direct association, or whether women who were induced also had some other characteristic that made them more likely to have a cesarean section. Medical indications for induction, such as diabetes mellitus, may be such a factor; that is to say, women with diabetes are more likely to be induced and are more likely to have a cesarean section. Therefore, in these patients, one needs to determine whether there was truly an independent association between induction and cesarean section. In this study, 1 method used to assess the potential for confounding was performance of separate regression analyses for women who underwent medical and elective inductions. Also, multivariable regression is a technique that could have been used to account for other patient factors that may be different between the group of women who were induced and those who had a spontaneous labor.

Grobman: You are right to point out how potentially important bias can be introduced by confounding. We should always be aware of the potential for this type of bias in an observational study. Even when one is precise about accounting for potentially confounding factors, there is always the possibility that an unknown or unmeasurable factor may be different between groups, thereby affecting the results. This is 1 virtue of a randomized trial—in a randomized population, all factors other than the randomized factor should be equivalent between the different groups.

Nelson: In addition to stratification and regression, another method to deal with the possibility of confounding is the use of exclusion criteria. In this study, as we noted before, the authors have excluded women with a prior cesarean, and this effectively eliminates the possibility that the results would be affected by this subgroup.

Grobman: Is there any downside to using exclusion criteria?

Lindner: If you exclude many groups, or a factor that is present in many women, the study population can grow quite small and increase the possibility of type II error. Also, by not studying women with certain factors, one loses the ability to examine the association of those factors with the outcomes of interest.

Dugan: And, the more exclusions that are present, the more specific the population will be, so that there is a potential loss of external generalizability.

Grobman: In attempting to limit confounding bias, these authors turn to a type of regression called the random effects logistic regression. Could you tell us something about this choice?

Nelson: Well, a logistic regression was chosen because the outcomes of interest, such as cesarean section or hemorrhage, were dichotomous outcomes. The random effects technique was also used to account for the possibility that practice patterns of individual physicians could have affected the associations between induction of labor and the outcomes. For example, an individual physician could have higher rates of both induction and cesarean section. In that setting, it may not be true that induction is truly associated with cesarean; it may just be that a single physician is more likely to perform both. Essentially, there is the possibility that the data from each patient are not truly independent, but are correlated with 1 other factor—in this case, the identity of the physician. A random effects regression can be used to account for this correlation or clustering of data.

Conclusions

Grobman: What are the conclusions of this study?

Dugan: The article concludes that induction of a multiparous woman was associated with an increased risk of cesarean delivery, particularly among those women who required cervical ripening agents.
Groban: So, a multiparous woman who underwent an induction of labor had an increased risk of cesarean delivery compared to a multiparous woman who arrived in spontaneous labor.

Dugan: Yes. The authors also demonstrated that women who were induced had a greater risk of blood loss exceeding 500 mL, and they had a more prolonged stay in labor and delivery than those who spontaneously labored.

Groban: With regard to blood loss, I interesting finding was that induction with oxytocin alone was associated with a significantly greater risk of postpartum hemorrhage than induction with cervical ripening agents. This is counterintuitive. Why might that result have been obtained?

Facco: One reason might be that some women who received oxytocin actually had an unripe cervix but did not receive a cervical ripening agent. In a case like that, women might have required more prolonged oxytocin use and were at greater risk of hemorrhage. As the authors noted, they were not able to ascertain women’s cervical status; only whether they received a ripening agent, and thus the oxytocin-only group could actually be composed of women with varying degrees of cervical ripeness.

Groban: Could there be any other reason for this finding?

Facco: Yes, this could represent a type II error. Only 139 women received cervical ripening in this study. Consequently, when that subgroup alone is examined, whether in univariable or multivariable analyses, there is an increased probability that even if a true association exists, it will not be found to be statistically significant.

Groban: I think that is an important point, and it has relevance for the findings regarding cesarean delivery and medical and elective inductions. After reading this article, can you make a conclusion about whether or not multiparous women who are electively induced have an increased risk for cesarean delivery?

Julien: Looking at Table 3, it appears that there was a statistically significant increase in the odds of cesarean delivery among multiparous women who were induced for a medical indication. The chance of cesarean section also appears to have been increased for those women who are electively induced, according to the point estimate of the odds ratio. Nevertheless, this finding does not reach statistical significance, according to the 95% confidence interval that crosses 1. Once again, this may be due to a type II error related to the reduced sample size in the subgroup analysis.

Groban: Let’s consider for the moment only those women who have inductions for medical indications and an associated increase in odds for a cesarean section. Can you explain to me exactly what that odds ratio implies with regard to her reproductive outcome?

Facco: Sure. It indicates that compared to spontaneous labor, an induction of labor is associated with a greater probability for cesarean delivery. This could be important information when counseling patients, as it allows the health care provider to discuss the probability of certain outcomes for a woman who is proceeding with a labor induction. It is important for us to understand that even multiparous women have an increased chance of certain complications after labor induction. However, I think it is also important to note that this does not necessarily imply that a woman’s chance of having a cesarean section after labor induction is greater than it would be if she were to be expectantly managed. The alternative to an induction is not necessarily spontaneous labor; if a woman is not induced, she may not labor for several weeks or she may even need to be induced at a later gestational age. Thus, as the authors noted, their results should certainly keep us cognizant that the population of women in this study who were being induced may have had different outcomes than the population who was spontaneously laboring, and that these outcomes were particularly different for those who required cervical ripening and induction. But, as the authors also note, in order to be confident about the benefits or risks of induction for an individual woman, we will require further study through the use of different study designs, such as a randomized trial.

Dugan: I also want to note that when we think of increased risk, it is important to consider not just the odds ratio, but the difference in the actual frequencies. For example, the risk of cesarean section among women with spontaneous labor was 1.5%, so even a 2-to-3-fold increase in the chance of cesarean section in women who are induced will mean that their chance of vaginal delivery is still 95% or greater.

Groban: I think the concept of attributable risk is very important.

Groban: After all we have spoken about, do you think this study would change your clinical practice?

Lindner: I think the study further emphasizes that multiparous women, just like their nulliparous counterparts, should have the benefits and risks of medical intervention carefully weighed. I would hope this information would be incorporated into patient counseling, not just in regard to whether they should or should not undergo an induction of labor, but also with regard to the magnitude of expected outcomes if labor induction is undertaken.

Groban: Thank you. This article by Battista et al is very provocative, and I appreciate everybody’s willingness to evaluate it. I am sure it will generate a lot of interest and discussion in the future as well.

REFERENCES


Successful twin pregnancy after vaginal radical trachelectomy using transabdominal cervicoisthmic cerclage

Keun-Young Lee, MD; Hyun-Ah Jun, MD; Ju-Won Roh, MD; Ji-Eun Song, MD

A 32-year-old nulliparous woman was referred to her gynecologic oncologist for the management of an invasive cervical cancer incidentally detected by Pap smear at an examination while undergoing in vitro fertilization–embryo transfer (IVF-ET) procedure. Her infertility was secondary to premature ovarian failure. Otherwise, she was in good general health. Cervical conization documented a microinvasive adenocarcinoma of the cervix 1.5 mm in depth and 3 mm in width of stromal invasion. Histologically, the tumor was described as a poorly differentiated adenocarcinoma. The patient was counseled as to the options for management of cervical adenocarcinoma (FIGO stage IA1). Because of her intense desire to preserve fertility, a laparoscopic pelvic lymphadenectomy was performed followed by a vaginal radical trachelectomy (VRT). A prophylactic cerclage using a monofilament nonabsorbable polypropylene (1-0 Prolene; Ethicon, Somerville, NJ) suture was inserted transabdominally cervicoisthmic cerclage (TCIC) was performed for the prevention of cervical incompetence. There were no perioperative complications, and a postoperative scan revealed intact TCIC knots and a 1.5 cm length of the cervix. Subsequently, the patient was discharged home.

Follow-up serial scans showed stable cervical length but the presence of a complete placenta previa. She was hospitalized because of vaginal spotting at 29 3/7 weeks. Because her nonstress test showed uterine contractions, the patient was given magnesium sulfate intravenously and corticosteroids to improve fetal lung maturation. On day 10 of hospitalization (30 5/7 weeks), massive vaginal bleeding occurred abruptly. An emergency cesarean section was performed, and the patient gave birth to 1.41 kg and 1.51 kg female infants, both with 9/10 Apgar scores. The placenta was firmly attached to the uterus with poor uterine contraction despite intravenous oxytocin and methylergonovine administration. Uterine hemorrhage became profuse as delivery of the placenta was attempted. Immediate blood replacement therapy was performed and persistent bleeding necessitated prompt hysterectomy. The pathologic examination confirmed placenta increta and the absence of residual cancer. The twins were discharged alive and well; they continue to thrive.

COMMENT

There is no definite treatment for cervical incompetence occurring after VRT. Multiple pregnancy is already at higher risk of cervical incompetence, though both singleton and multiple pregnancy after VRT are associated with a clinically incompetent cervix and subsequent preterm delivery. The reported outcomes of twin pregnancies after VRT are disappointing. In the 6 largest published studies, there were 3 sets of twins in a total of 149 pregnancies. All 3 pregnancies ended at between 24-28 weeks.1 Therefore, some authors have recommended that prudence should dictate avoidance of multiple fetuses if at all possible.2 In the case reported here, the twins were delivered at 30 5/7 weeks’ gestation. The prolongation of pregnancy after TCIC was 126 days.

Although a permanent retention suture is typically placed in the cervix at the time of VRT, it appears that it alone is not sufficient for prolongation of gesta-
tion. Although the placement of another cerclage might be helpful, it often involves technical difficulties, because the “neo-cervix” after trachelectomy is fibrotic and markedly shortened. For successful cerclage, we elected an abdominal approach to perform TCIC because of the shortened “neo-cervix.”

TCIC is a technically demanding operation different from the Saling procedure, another procedure that can be performed during pregnancy to prevent complications after VRT. The purpose of the Saling procedure is to reduce the risk of chorioamnionitis by closing the vaginal mucosa over the cervical os after the first trimester. In contrast, the TCIC seeks to prevent cervical incompetence.3

We did not perform the Saling procedure because our patient showed no symptoms or signs of either preterm premature rupture of membrane or chorioamnionitis after TCIC, both common following VRT. Transvaginal sonography also confirmed a stable length of the cervix. If there had not been massive vaginal bleeding related to placenta previa, she may have carried her pregnancy even longer after TCIC.

A viable pregnancy resulting in live term or near term birth is the ultimate goal of women choosing VRT rather than hysterectomy. We report a successful outcome of twin pregnancy following VRT by preventing cervical incompetence using TCIC in the first trimester. TCIC should be considered as an option for maintaining twin pregnancy after VRT.

REFERENCES
Women in the reproductive age range can develop moderate to severe chronic kidney disease (CKD) in the setting of long-standing hypertension, diabetes mellitus, autoimmune disease, and primary glomerulopathy. There is growing evidence that women with advanced CKD who conceive and continue a pregnancy are at significant risk for adverse maternal and fetal outcomes. Patients with CKD are prone to disturbances of electrolyte, acid base, and fluid homeostasis. We report the case of a patient with advanced CKD who developed acute hyperkalemia in the setting of intrauterine fetal demise and uterine rupture. We hypothesize that the hyperkalemia was caused by potassium leak from the dead fetus and introduction of the potassium load into the maternal circulation as a result of uterine rupture.

**CASE REPORT**

A 37 year old African American woman, gravida 3, para 1, abortus 1 at 26 weeks of gestation was admitted to our hospital for abdominal pain. One day prior to admission, the patient noticed a loss of fetal movement and developed lower abdominal pain. The patient had nausea and had vomited twice since the onset of symptoms. The review of the systems was negative. The patient was diagnosed with hypertension 20 years ago. She had a history of CKD stage 4 (glomerular filtration rate 30 mL/min), gestational diabetes mellitus, and congestive heart failure (ejection fraction 30%). Her past surgical history was significant for 2 cesarean sections. The patient denied the use of alcohol, tobacco, or any discharge.

She was taking nifedipine, metoprolol, and furosemide in an outpatient setting. On physical exam the patient was afebrile, normotensive with a blood pressure of 125/75 mm Hg, tachycardic with a heart rate of 110 per minute, breathing 18 times a minute, and saturating 98% on room air. The pertinent findings on physical examination were a distended abdomen and diffusely tender abdomen on palpation. There were normoactive bowel sounds. Fetal movement or fetal heart sounds were not appreciated. Genital examination did not reveal vaginal blood or any discharge.

**LABORATORY TEST RESULTS**

Electrolytes included the following: sodium, 131 mEq/L; potassium, 6.1 mEq/L; chloride, 101 mEq/L; bicarbonate, 13 mEq/L; blood urea nitrogen 55 mEq/L; creatinine, 4.8 mEq/L; glucose, 237 mg/dL; calcium, 9.9 mEq/L; magnesium, 1.3 mEq/L; and phosphorus, 6.5 mEq/L. Complete blood count included the following: leucocytes, 22,000/mm³; hemoglobin, 8.4 g/dL; hematocrit, 26%; and platelets, 255,000/mm³. Creatine kinase was 221 U/liter; albumin, 3.2 g/dL; aspartate aminotransferase, 9 U/liter; alanine aminotransferase, 9 U/liter; alkaline phosphatase, 67 U/liter; gamma glutamyl transeptidase (GT), 32 U/liter; total bilirubin, 0.3 mg/dL; and lactate, 2.2 mmol/L. Urinalysis was pH 5.0, protein, 500 mg/dL; 100 mg/dL; trace ketones, no blood, and no casts. Arterial blood gas was pH 7.33, partial pressure of carbon dioxide (pCO₂) 22 mm Hg, and HCO₃ 14 mmol/L.

Electrocardiogram showed sinus tachycardia, peaked t-waves, and Q waves in the inferior leads. On real-time transabdominal sonography, there was no identifiable heart motion. Ultrasound of the kidney revealed 9.1 and 9.7 cm echogenic kidneys without hydronephrosis, consistent with medical renal disease. Despite medical management the serum potassium concentration increased from an admission value of 6.1 to 7.5 mmol/L (Figure) within 6 hours.

By using an intensive medical treatment with multiple drugs (Figure), the patient’s serum potassium concentration decreased to 5.2 mmol/L. During this time, the patient’s abdominal pain worsened, and she was noted to have a drop in her hemoglobin concentration from initially 8.4 to 6.4 g/dL. Laboratory values ruled out hemolysis. Blood transfusions were started, and the patient was taken to the operating room immediately. After general anesthesia and intubation, the peritoneum was opened and a ruptured uterus as well as 1 L of blood in the peritoneal cavity was found. During the procedure 7 blood transfusions were administered. Upon cesarian delivery, the dead fetus was noted to have peeling skin. Postoperatively serum potassium level remained within normal limits and the patient was discharged home after 8 days.

**COMMENT**

The medical management of pregnant women with advanced CKD can be a challenging scenario for clinicians. Women with CKD who conceive and continue a pregnancy are at significant risk for adverse maternal and fetal outcomes. Patients with CKD are prone to disturbances of electrolyte, acid base, and fluid homeostasis. We report a rare case of acute hyperkalemia in a patient with chronic kidney disease and intrauterine fetal demise. We propose that the hyperkalemia was due to a potassium load introduced into the maternal circulation as a result of intrauterine fetal demise and uterine rupture.

**Key words:** chronic kidney disease, fetal death, hyperkalemia, pregnancy, uterine rupture

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risk for adverse maternal and fetal outcomes.2,3

Patients with advanced CKD have impaired renal mechanisms to compensate for acute changes in fluid, electrolyte, and acid base changes. From a pathophysiologic standpoint, hyperkalemia can occur in 2 settings: external potassium intake or internal potassium redistribution. This patient did not receive any external potassium, nor did she receive any medication that could have contributed to the acute rise in her serum potassium concentration. Rhabdomyolysis, hemolysis, and lactic acidosis were ruled out by means of serum chemistries. Although the patient had chronic metabolic acidosis in the setting of advanced CKD, it does not explain her worsening hyperkalemia. After cesarean delivery, the patient’s serum potassium concentration normalized without further medical intervention. We hypothesize that the worsening hyperkalemia in this patient with advanced CKD was caused by potassium leak from intrauterine fetal demise into the maternal circulation by uterine rupture.

To our knowledge this is the first reported case of its kind. Further observations are needed to determine whether pregnant females with advanced CKD and intrauterine fetal death should undergo early termination of pregnancy to prevent hyperkalemia, which can be a life-threatening complication for the child-bearing mother.

REFERENCES