3. Koonings PP, Grimes DA. Adnexal torsion in postmenopausal women. Obstet Gynecol 1989;73:11–2.
4. Eitan R, Galoyan N, Zuckerman B, Shaya M, Shen O, Beller U. The risk of malignancy in postmenopausal women presenting with adenxal torsion. Gynecol Oncol 2007;106:211–4.
5. McCulloch B. Takotsubo cardiomyopathy. AACN Advanced Critical Care 2009;20:194–200.
6. Tsai TT, Nallamothu BK, Prasad A, Saint S, Bates ER. A change of heart. N Engl J Med 2009;361:1010–6.
7. Prasad A, Lerman A, Rihal CS. Apical ballooning syndrome (Tako-Tsubo or stress cardiomyopathy): a mimic of acute myocardial infarction. Am Heart J 2008;155:408–17.
8. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999;340:1801–11.

Term Stillbirth Caused by Oral *Fusobacterium nucleatum*

Yiping W. Han, PhD, Yann Fardini, PhD, Casey Chen, DDS, PhD, Karla G. Iacampo, MD, Victoria A. Peraino, Jaime M. Shamonki, MD, and Raymond W. Redline, MD

BACKGROUND: Intrauterine infection is a recognized cause of adverse pregnancy outcome, but the source of infection is often undetermined. We report a case of stillbirth caused by *Fusobacterium nucleatum* that originated in the mother's mouth.

CASE: A woman with pregnancy-associated gingivitis experienced an upper respiratory tract infection at term, followed by stillbirth a few days later. *F. nucleatum* was isolated from the placenta and the fetus. Examination of different microbial floras from the mother identified the same clone in her subgingival plaque but not in the supragingival plaque, vagina, or rectum.

CONCLUSION: *F. nucleatum* may have translocated from the mother's mouth to the uterus when the immune system was weakened during the respiratory infection.

From the Department of Periodontics, School of Dental Medicine, Department of Pathology, School of Medicine, Case Western Reserve University, Cleveland, Ohio; the Division of Primary Oral Health Care, School of Dentistry, University of Southern California, Los Angeles, Los Angeles, California; and the Departments of Obstetrics and Gynecology and Pathology, Saint John's Health Center, Santa Monica, California.

Supported in part by National Institute of Dental and Craniofacial Research grants RO1 DE 14924, KO2 DE 16102, and R21 DE171650 (Y.W.H.).

The authors thank Dr. Floyd Dewhirst for consultation on the use of the Human Oral Microbiome Database.

Presented at the Gordon Research Conference on Periodontal Diseases, August 2–7, 2009, New London, New Hampshire.

Corresponding author: Yiping W. Han, PhD, Department of Periodontics, School of Dental Medicine, Case Western Reserve University, Cleveland, OH 44106-4905; e-mail: yiping.han@case.edu.

Financial Disclosure
The authors did not report any potential conflicts of interest.

ISSN: 0029-7844/10

This case sheds light on patient management for those with pregnancy-associated gingivitis.
(Obstet Gynecol 2010;115:442–5)

Stillbirth is a significant public health concern, accounting for 60% of perinatal deaths. Infections account for 10–25% of all stillbirths. In this report, we present a case of unusual term stillbirth caused by *Fusobacterium nucleatum*, a gram-negative anaerobic bacterium prevalent in intrauterine infection but not associated with stillbirth before. We demonstrate that this organism may have originated from the mother's oral cavity.

CASE

A 35-year-old primigravid Asian woman reporting decreased fetal movement was admitted to Saint John's Health Center at 39 5/7 weeks gestation. Up to the day of hospitalization, the patient had received routine prenatal care and the pregnancy had been uncomplicated with the exception of a two-vessel umbilical cord found by ultrasonography. Subsequent serial ultrasonograms revealed no other anatomical abnormalities. On the day of admission, the patient reported that she had last felt the fetus move at approximately 5:00 that morning. The mother had been mildly ill with an upper respiratory tract infection for the previous 3 days, running a low-grade fever of 37.8°C. There was no history of amniotic fluid leakage, bleeding, or abnormal uterine contractions. At admission, absence of fetal heartbeat was confirmed by ultrasonography. The membrane was ruptured artificially by the obstetrician, who noted slightly bloody and strongly foul-smelling amniotic fluid. Over the next several hours, the patient's labor progressed without difficulty, and a significantly malodorous stillborn female fetus weighing 3,323 g was delivered vaginally early the next morning. Titers for toxoplasmosis, other viruses, rubella, cytomegalovirus, and herpes simplex viruses, and parvovirus drawn before delivery were negative.

The placenta was relatively small (fifth percentile for 39 weeks of gestation) and had a single umbilical artery. Acute chorioamnionitis with umbilical phlebitis, chorionic vasculitis, and foci of recent nonocclusive chorionic vessel thrombosis were noted. Unusual features of the chorioamnionitis in this case included an unusually severe acute deciduitis with foci of decidual necrosis in the placental

membranes and a single focus of acute deciduitis in the decidua basalis (Fig. 1). Gram-negative bacilli were observed in the amnion and subchorion by Gram stain, and placental culture was positive for *F. nucleatum*.

The mother consented to a complete autopsy of the fetus. Minimal maceration and autolysis of organs were observed, consistent with fetal demise less than 12 hours before delivery. No congenital anomalies were noted, and the fetus was appropriate for gestational age by autopsy measurements. There was diffuse venous congestion. Organ weights were unremarkable, with the exception of the lungs, which were twice the normal weight and showed histologic evidence of massive intraalveolar hemorrhage. Giemsa stain revealed multiple foci of filamentous bacteria in the bronchi, with no accompanying inflammatory response. Filamentous bacteria were also seen in large numbers in the stomach without transit to the colon, suggesting relatively recent acquisition. *F. nucleatum* was isolated from both the lung and the stomach as pure culture using the routine culturing methods in the clinical microbiology laboratory at Saint John's Health Center.

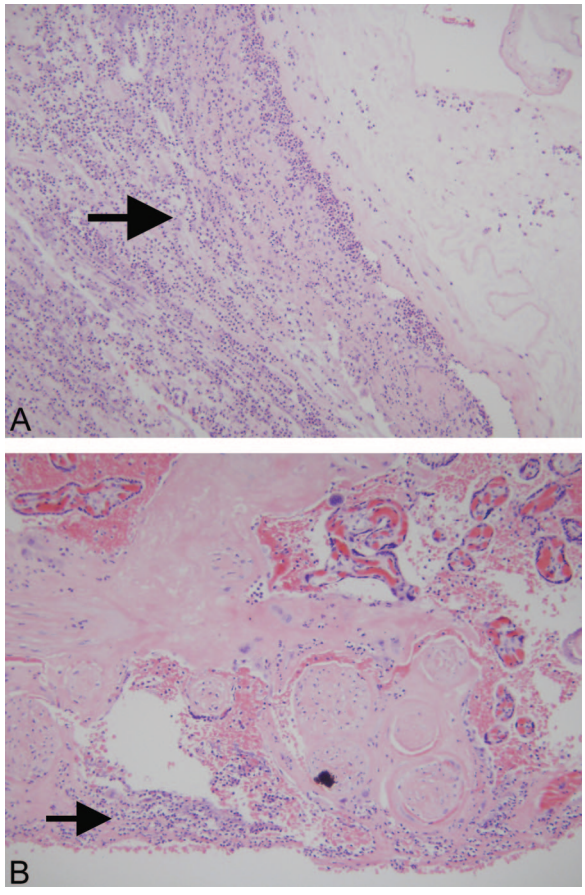

Fig. 1. Accentuated decidual inflammatory response in acute chorioamnionitis caused by *Fusobacterium nucleatum*. **A.** Decidua capsularis (hematoxylin-eosin [H&E] stain; × 200, original magnification): there is an unusually extensive and intense neutrophilic infiltrate in the decidua capsularis (*arrow*). **B.** Decidua basalis (H&E; × 200, original magnification): focus of acute deciduitis in the decidua basalis underlying the intervillous space (*arrow*). These foci are rarely seen in typical cases of acute chorioamnionitis. *Han. Term Stillbirth Caused By* F. nucleatum. *Obstet Gynecol 2010.*

The patient reported excessive gum bleeding during her pregnancy. After the identification of *F. nucleatum* in the stillborn fetus, a full-mouth periodontal examination was performed at 3 weeks postpartum, revealing minimal gingival inflammation and no signs of periodontitis. Thus, the patient may have had pregnancy-associated gingivitis.

To investigate the possible source of *F. nucleatum* infection, vaginal, introitus, and rectal swabs and supragingival and subgingival plaque samples were collected from the mother. The bacteria isolated from the stillborn fetus were designated *F. nucleatum* strain 708. The central part of the region corresponding to the 16S–23S rRNA genes was amplified by polymerase chain reaction (PCR) using universal primers as described previously.[1] Based on the amplified sequence, *Fusobacterium*-specific primers were designed and used to analyze the following samples collected from the patient: pooled full-mouth supragingival plaque; pooled full-mouth subgingival plaque; and introitus, vaginal, and rectal swabs.

Using universal primers, bacterial DNA was amplified from all samples, indicating adequate sample collection at different sites. When *Fusobacterium*-specific primers were used, bacterial DNA was amplified only from the supragingival and subgingival plaque samples but not from the other samples (Table 1). Clone libraries were generated using the *Fusobacterium*-specific PCR amplicons. A group of 10–24 random clones from each library was sequenced. The 16S rRNA portion of each sequence was used with the basic local alignment search tool against the Human Oral Microbiome Database for species identification (Table 1). *F. nucleatum* 708 belonged to subspecies *animalis* oral taxon 420. Two of the 24 clones from the subgingival library also belonged to this taxon (Table 1). Furthermore, one of these two clones matched *F. nucleatum* 708. No *F. nucleatum* subspecies *animalis* oral taxon 420 was identified among the 19 clones analyzed from the supragingival plaque library. Considered together, the results of PCR and clone analysis indicate that *F. nucleatum* was present in the mother's subgingival flora but absent from her supragingival, vaginal, and rectal floras.

COMMENT

A PubMed search in November 2009 using the key words "stillbirth," "*Fusobacterium*," and "oral bacteria" revealed no prior cases of oral bacteria causing stillbirth. Studies from our group and others have demonstrated that *F. nucleatum* is one of the most prevalent species in intrauterine infection, predominantly identified in cases of preterm birth.[2,3] Although intrauterine infection of *F. nucleatum* has long been sus-

Table 1. Identification of *Fusobacterium* Species in the Maternal Samples

Samples	Identification*	No. of Clones Analyzed*
Fusobacterium nucleatum 708	*F. nucleatum* ss *animalis* oral taxon 420	10
Subgingival plaque	*F. nucleatum* ss *animalis* oral taxon 420	2†
	F. nucleatum ss *polymorphum* oral taxon 202	5
	F. nucleatum ss *vincentii* oral taxon 200	17
Supragingival plaque	*F. naviforme* oral taxon 200	2
	F. nucleatum ss *polymorphum* oral taxon 202	14
	F. nucleatum ss *vincentii* oral taxon 200	3
Introitus swab	—‡	
Vaginal swab	—‡	
Rectal swab	—‡	

ss, subspecies.

* 16S rDNA clone libraries were generated for *F. nucleuatm* 708, subgingival plaque, and supragingival plaque, respectively, by cloning the *Fusobacterium*-specific amplicons. Randomly selected clones were sequenced and compared against the Human Oral Microbiome Database using the basic local alignment search tool. The taxon was identified if the sequence identify was more than 98.9%.

† One of these two clones had 100% sequence match in the 16S rRNA region with *F. nucleatum* 708.

‡ No fusobacterial DNA was amplified from these samples.

pected to originate from the oral cavity, this hypothesis has not been proven in humans until now. In this unusual case of term stillbirth, *F. nucleatum* was found in the mother's oral cavity, but not in her vaginal or rectal floras. A total of four *Fusobacteria* taxa were identified from the subgingival and supragingival plaque samples, among which three were *F. nucleatum* taxa. These results indicate that *F. nucleatum* is part of the normal flora in the mother's mouth. No *F. nucleatum* was detected in the mother's vaginal or rectum samples, thus an ascending route of infection was unlikely.

Evidence is accumulating that oral bacteria play a significant yet previously unrecognized role in intrauterine infection leading to preterm birth.[1,2] As one of the major microbiomes in the human body, the oral flora serves as a microbial reservoir. Part of the reason that the role of oral bacteria was underestimated may be because the majority of oral species are uncultivated and thus cannot be detected by the routine culturing methods used by the hospital microbiology laboratories. For instance, an uncultivated oral species, *Bergeyella*, which had never been identified in intrauterine infection, has been detected repeatedly in amniotic fluid associated with preterm birth using 16S rRNA gene-based culture-independent technology.[1,2] Likewise, we speculate that a certain portion of the unexplained stillbirths may be caused by previously unrecognized oral bacteria translocated to the uterus independent of the ascending vaginal route. Further systematic analysis is needed to determine the prevalence of oral bacteria in stillbirth.

How do oral bacteria invade into the uterus? Previous studies in animals have provided clues. Hematogenous injection of orally related *F. nucleatum* into pregnant mice resulted in specific bacterial colonization in the placenta causing localized inflammation.[4,5] The bacteria first colonized in the mouse decidua by crossing the endothelium, followed by proliferation and spreading to fetal membranes, amniotic fluid, and the fetus, and eventually causing fetal demise.[4] The pattern of infection is similar to the case reported here. It is plausible that *F. nucleatum* translocated from the mother's mouth hematogenously. Supporting this notion is the mother's report of excessive gum bleeding during pregnancy. This is indictive of pregnancy-associated gingivitis (ie, plaque-induced inflammation of the gingiva), which has been reported to be present among at least three quarters of the pregnant population.[6] Such a condition would increase transient bacteremia. The upper respiratory tract infection might have weakened the mother's immune system enough to provide a window of escape for the bacteria to colonize in the uterus. In the murine model described above, *F. nucleatum* was cleared completely from the maternal circulation after 24 hours of injection. However, once colonized in the immune-privileged placenta, the bacteria proliferated quickly and caused fetal death within 3 days.[4] This time frame coincides with the time the mother became ill before fetal demise.

Pathologic findings in this case support the above scenario. Acute chorioamnionitis was stage 2, a pattern estimated to occur after approximately 12–24 hours of infection. The placenta was small for gestational age and had foci of laminar necrosis in the decidua, which may have allowed small numbers of decidual bacteria to grow more readily. There was an

unusual amount of membrane acute deciduitis and a focus of acute deciduitis in the decidua basalis. Both findings are unusual in ascending infections, and the latter was a prominent feature of the hematogenous murine model described above. Autopsy findings suggest relatively recent and sudden death, possibly caused by endotoxemia, as evidenced by marked venous congestion, massive intraalveolar hemorrhage, and the lack of an inflammatory response to infection.

Periodontal disease has been recognized as a risk factor for adverse pregnancy outcome. However, the efficacy of periodontal treatment on birth outcome has been inconsistent.[7,8] Interestingly, the Lopez trial, which focused on treating pregnancy-associated gingivitis, reported that the incidence of preterm/low birth weight in the group receiving periodontal and maintenance therapies was reduced to 2.14% from 6.71% in the control group, with an odds ratio of 3.26. After adjustment of several known risk factors, an odds ratio of 2.76 (95% confidence interval, 1.29–5.88; P=.008) was obtained. The authors concluded that gingivitis was an independent risk factor for preterm/low birth weight, a finding consistent with the case reported here.[7] This case sheds light on patient management for those with pregnancy-associated gingivitis who also contract additional infections. Prophylactic antibiotic therapy may be considered in the presence of multiple infections to prevent prolonged bacteremia and potential hematogenous translocation of the oral bacteria into the uterus.

REFERENCES

1. Han YW, Ikegami A, Bissada NF, Herbst M, Redline RW, Ashmead GG. Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J Clin Microbiol 2006;44:1475–83.
2. Han YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. J Clin Microbiol 2009; 47:38–47.
3. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol 1992;79:351–7.
4. Han YW, Redline RW, Li M, Yin L, Hill GB, McCormick TS. Fusobacterium nucleatum induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. Infect Immun 2004;72:2272–9.
5. Liu H, Redline RW, Han YW. Fusobacterium nucleatum induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. J Immunol 2007;179: 2501–8.
6. Arafat AH. Periodontal status during pregnancy. J Periodontol 1974;45:641–3.
7. Lopez NJ, Da Silva I, Ipinza J, Gutierrez J. Periodontal therapy reduces the rate of preterm low birth weight in women with pregnancy-associated gingivitis. J Periodontol 2005;76: 2144–53.
8. Michalowicz BS, Hodges JS, DiAngelis AJ, Lupo VR, Novak MJ, Ferguson JE, et al. Treatment of periodontal disease and the risk of preterm birth. N Engl J Med 2006;355:1885–94.