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Bacteria in the amniotic fluid without inflammation: early colonization vs. contamination

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Abstract

Objectives: Intra-amniotic infection, defined by the presence of microorganisms in the amniotic cavity, is often accompanied by intra-amniotic inflammation. Occasionally, laboratories report the growth of bacteria or the presence of microbial nucleic acids in amniotic fluid in the absence of intra-amniotic inflammation. This study was conducted to determine the clinical significance of the presence of bacteria in amniotic fluid samples in the absence of intra-amniotic inflammation.

Methods: A retrospective cross-sectional study included 360 patients with preterm labor and intact membranes who underwent transabdominal amniocentesis for evaluation of the microbial state of the amniotic cavity as well as intra-amniotic inflammation. Cultivation techniques were used to isolate microorganisms, and broad-range polymerase chain reaction coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) was utilized to detect the nucleic acids of bacteria, viruses, and fungi.

Results: Patients whose amniotic fluid samples evinced microorganisms but did not indicate inflammation had a similar perinatal outcome to those without microorganisms or inflammation [amniocentesis-to-delivery interval ($p=0.31$), spontaneous preterm birth before 34 weeks

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($p=0.83$), acute placental inflammatory lesions ($p=1$), and composite neonatal morbidity ($p=0.8$).

Conclusions: The isolation of microorganisms from a sample of amniotic fluid in the absence of intra-amniotic inflammation is indicative of a benign condition, which most likely represents contamination of the specimen during the collection procedure or laboratory processing rather than early colonization or infection.

Keywords: *Acinetobacter*; acute funisitis; acute histologic chorioamnionitis; amniocentesis; interleukin-6; intra-amniotic infection; intra-amniotic inflammation; microbial burden; preterm labor with intact membranes; *Propionibacterium acnes*.

Introduction

Infection is a major cause of morbidity and mortality for mothers and newborns [1–3] who represent relatively vulnerable hosts to the effects of microorganisms or microbial products (i.e. bacterial endotoxin). Therefore, the accurate identification of intra-amniotic infection has become important in patients at risk for adverse pregnancy outcome (preterm labor with intact membranes [4–13], preterm prelabor rupture of the membranes [14–25], clinical chorioamnionitis [26–29], idiopathic vaginal bleeding in the second or third trimester [30, 31], sonographic short cervix [32–34], cervical insufficiency [35]), and in patients who conceive despite having an intrauterine contraceptive device [36].

Amniotic fluid does not contain bacteria under normal circumstances [37–44]. However, microorganisms may gain access to the amniotic cavity through different pathways, of which the most frequent is an ascending route from the lower genital tract [1, 45–48]. Bacteria in the amniotic cavity, detectable by cultivation or molecular microbiologic techniques, may elicit a local inflammatory response [49–63], and this state is referred to as intra-amniotic infection. Persuasive evidence indicates that intra-amniotic infection is causally linked to spontaneous preterm labor and delivery [64–67], the development of acute histologic chorioamnionitis [65, 68–73] and funisitis [74, 75], a fetal inflammatory response [73, 76–85], and adverse perinatal outcomes [6, 65, 86–106]. Recent evidence suggests that maternal administration of antibiotics can eradicate intra-amniotic infection [32, 107–111].

The definition of intra-amniotic infection requires the presence of both microorganisms and an intra-amniotic inflammatory response [1, 45]. Occasionally, during clinical management, patients present with a situation for

which the laboratory reports bacterial growth or the presence of microbial nucleic acids in amniotic fluid in the absence of intra-amniotic inflammation [6, 13, 34, 92, 112–118]. The interpretation of this finding is a challenge. Are bacteria the result of contamination of amniotic fluid at the time of specimen collection/laboratory processing, or the result of early colonization by pathogenic bacteria before an inflammatory process is established? This conundrum haunts clinicians in virtually every specialty of medicine dealing with a presumably sterile specimen, such as cerebrospinal, pleural-pericardial, or amniotic fluid, or blood. Erroneous interpretation of a laboratory report can lead to devastating consequences in obstetrics, including iatrogenic preterm delivery. This study was conducted to determine the clinical significance of the presence of bacteria in amniotic fluid in the absence of intra-amniotic inflammation.

Materials and methods

Study population

This retrospective cohort study was conducted by searching the clinical database and bank of biological samples of Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch. Patients diagnosed with preterm labor and intact membranes at Hutzel Women's Hospital were included in the study when they met the following criteria: (1) a singleton gestation; (2) a transabdominal amniocentesis to assess the microbial state of the amniotic cavity between 20 and 35 weeks of gestation; (3) availability of amniotic fluid for the performance of molecular microbiologic studies; and (4) known pregnancy and neonatal outcomes. Patients were excluded from the study when they presented with (1) a rupture of the chorioamnionic membranes before amniotic fluid collection or (2) a chromosomal or structural fetal anomaly.

A transabdominal amniocentesis was offered to patients with the diagnosis of preterm labor and intact membranes at the discretion of the attending physician to identify the microbial status of the amniotic cavity. Women who agreed to undergo this procedure were asked to donate additional amniotic fluid and to allow the collection of clinical information for research purposes. The administration of antibiotics was left to the discretion of the physician.

All patients provided written informed consent prior to the procedure and the collection of samples. The use of biological specimens as well as clinical and ultrasound data for research purposes was approved by the Human Investigation Committee of Wayne State University.

Clinical definitions

Preterm labor was defined by the presence of at least two regular uterine contractions every 10 min associated with cervical changes in patients with a gestational age between 20 and 36 6/7 weeks. *Neonatal death* was defined as an infant death before 28 days of age [119].

Composite neonatal morbidity included two or more of the following: a 5-min Apgar score <7, bronchopulmonary dysplasia, respiratory distress syndrome, requirement for ventilation, necrotizing enterocolitis, intraventricular hemorrhage, retinopathy of prematurity, neonatal bacteremia, or neonatal intensive care unit admission.

Intra-amniotic inflammation was diagnosed when the amniotic fluid interleukin (IL)-6 concentration was ≥ 2.6 ng/mL [6, 120]. Based on the results of amniotic fluid culture, polymerase chain reaction with electrospray ionization mass spectrometry (PCR/ESI-MS) (Ibis® Technology-Athogen, Carlsbad, CA, USA) testing [20, 121–123], and the amniotic fluid IL-6 concentration, patients were classified into four subgroups: (1) *no intra-amniotic infection and/or inflammation*: negative amniotic fluid by both culture and PCR/ESI-MS in the absence of intra-amniotic inflammation (IL-6 <2.6 ng/mL); (2) *microorganisms in the amniotic fluid without intra-amniotic inflammation*: positive amniotic fluid by either culture or PCR/ESI-MS in the absence of intra-amniotic inflammation (IL-6 <2.6 ng/mL); (3) *sterile intra-amniotic inflammation*: negative amniotic fluid, by both culture and PCR/ESI-MS, in the presence of intra-amniotic inflammation (IL-6 ≥ 2.6 ng/mL); and (4) *intra-amniotic infection*: positive amniotic fluid, by either culture or PCR/ESI-MS, in the presence of intra-amniotic inflammation (IL-6 ≥ 2.6 ng/mL).

Sample collection

Amniotic fluid, upon collection, was immediately transported in a capped, sterile syringe to the clinical laboratory where it was cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas. Evaluations of the white blood cell (WBC) count [124], glucose concentration [125], and Gram stain [126] in amniotic fluid were also performed shortly after collection. Amniotic fluid not required for clinical assessment was centrifuged at $1,300 \times g$ for 10 min at 4 °C, shortly after amniocentesis, and the supernatant was aliquoted and stored at –80 °C until analysis.

Detection of microorganisms with cultivation and molecular microbiologic methods

Amniotic fluid was analyzed utilizing aerobic and anaerobic cultures, an assay for genital mycoplasmas, and broad-range real-time PCR/ESI-MS. The methods have been previously described in detail [11, 48, 127]. We included molecular microbiologic techniques for the detection of bacteria, fungi, and select viruses. PCR/ESI-MS identified 3,400 bacteria and 40 *Candida* spp., represented in the platform's signature database [127–129]. Fourteen primer pairs detected the following viruses: Human herpesvirus 1 (HHV-1), Human herpesvirus 2 (HHV-2), Human herpesvirus 3 (HHV-3), Human herpesvirus 4 (HHV-4), Human herpesvirus 5 (HHV-5), Human herpesvirus 8 (HHV-8), Human adenovirus, Human enteroviruses, BK polyomavirus, JC polyomavirus, and Human parvovirus B19 [130].

The microbial burden was assessed by calculating the genome equivalents per PCR well (GE/well). The microbial genome load per mL of amniotic fluid (GE/mL) is equal to the GE/well multiplied by 133.33. The sensitivity, or limit of detection (LOD), of PCR/ESI-MS for the detection of bacteria in the blood is, on average, 100 CFU/mL [95% confidence interval (CI), 6–600 CFU/mL] [129]. A comparison of detection limits between blood and amniotic fluid showed that the

assays have similar detection limits (100 CFU/mL) [11]. The LOD for the broad viral load in plasma ranges from 400 copies/mL to 6,600 copies/mL [131]. An LOD comparison of the samples of amniotic fluid and plasma was performed by spiking known amounts of a DNA virus (HHV-5) and an RNA virus (Human enterovirus) into these fluids. The LODs in amniotic fluid, similar to those in plasma, ranged from approximately 800 copies/mL to 1,600 copies/mL (depending upon the specific microorganism) [11].

Determination of IL-6 in amniotic fluid

IL-6 concentrations were determined to assess the presence and magnitude of the intra-amniotic inflammatory response, by using a sensitive and specific enzyme immunoassay obtained from R&D Systems (Minneapolis, MN, USA). The details of the assay, including sensitivity and the inter- and intra-assay coefficients of variation, have been previously reported [11, 127]. The cut-off value of 2.6 ng/mL has been previously reported for the diagnosis of intra-amniotic inflammation [11, 31, 127, 132].

Placental histopathologic examination

Placentas, collected in the Labor and Delivery Unit or Operating Room at Hutzel Women's Hospital of the Detroit Medical Center, were transferred to the clinical laboratory of the Perinatology Research Branch. Placental sampling was conducted in compliance with protocols of the Perinatology Research Branch, as previously described [133–138]. A minimum of 5 full-thickness sections of the chorionic plate, three sections of the umbilical cord, and three chorioamniotic membrane rolls, collected from each patient, were examined by placental pathologists who were blinded to the respective clinical histories and additional testing results. Acute inflammatory lesions of the placenta (acute chorioamnionitis and funisitis) were diagnosed according to established criteria, including staging and grading [136, 138, 139]. Severe acute placental inflammatory lesions are defined as stage 3 and/or grade 2 [136, 138].

Statistical analysis

The Kruskal–Wallis test, followed by the Mann–Whitney–Wilcoxon test for post-hoc analysis, was performed for the comparison of continuous variables among the subgroups. Categorical variables were compared by using the Chi-square test. To control the false discovery rate due to multiple comparisons, we used the Benjamini–Hochberg method for correction of nominal p-values. The analyses were conducted by using R language and environment for statistical computing (www.r-project.org). A heatmap, illustrating microbial burden, was generated by the ComplexHeatmap package in R, and the Euclidean distance was used for clustering after log-transformation of the data. Kaplan–Meier survival analysis and Cox proportional hazard regression models were used to compare the amniocentesis-to-delivery interval among the subgroups. Logistic regression was used to assess the association between microbial burden (above a given cut-off value) and the risk of preterm birth. The positive likelihood ratio [sensitivity/(1-specificity)] was determined

as a function of microbial burden. A p-value <0.05 was considered statistically significant.

Results

Characteristics of the study population

A total of 360 patients with preterm labor and intact membranes were included in this study. The demographic and clinical characteristics of the study population are displayed in Table 1. The median gestational age at amniocentesis was 28.7 weeks. The median gestational age at delivery was 33.6 weeks, and 14.4% (52/360) of the patients were delivered by cesarean section.

Table 1: Demographic and clinical characteristics of the study population.

Characteristics	Results (n=360)
Maternal age, years	23 (20–27)
Nulliparity	37.2% (134/360)
Smoking	23.6% (85/360)
Alcohol abuse	3.3% (12/359)
Pre-pregnancy body mass index, kg/m ²	24.4% (21.1–29.3)
Gestational age at amniocentesis, weeks	28.7 (24.9–32.0)
Gestational age at delivery, weeks	33.6 (28.4–36)
Cesarean delivery	14.4% (52/360)

Data are presented as median (interquartile range) or % (n/N).

The frequency of microorganisms in the amniotic fluid in the absence of inflammation

The frequency of microorganisms in amniotic fluid samples in the absence of intra-amniotic inflammation was 1.4% (5/360) by culture, 12.5% (45/360) by PCR/ESI-MS, and 13.3% (48/360) by using the combination of cultivation techniques and PCR/ESI-MS (either result – culture or PCR – was considered positive). The overall frequency of intra-amniotic inflammation in this cohort was 38% (136/360). In the presence of intra-amniotic inflammation, the frequency of microorganisms detected in amniotic fluid was 9.2% (33/360) by culture, 19.2% (69/360) by PCR/ESI-MS, and 19.4% (70/360) by using the combination of cultivation techniques and PCR/ESI-MS.

Based on the results of the amniotic fluid culture, PCR/ESI-MS, and amniotic fluid IL-6 concentration, patients were classified into four clinical subgroups: (1) 48.9% (176/360) did not have either intra-amniotic infection or intra-amniotic inflammation; (2) 13.3% (48/360) had microorganisms but without inflammation; (3) 18.3% (66/360) had sterile amniotic inflammation; and (4) 19.4% (70/360) had intra-amniotic infection.

Table 2 describes the results of the biomarkers of inflammation in amniotic fluid among the four subgroups. Most of the amniotic fluid samples (97%; 215/222) were collected before the administration of antibiotics, and there was no significant difference in the use of antibiotics before amniocentesis among the four subgroups. The

Table 2: Amniotic fluid analysis of four clinical subgroups according to results of amniotic fluid culture, PCR/ESI-MS, and amniotic fluid IL-6 concentrations in patients with preterm labor and intact membranes.

	No intra-amniotic inflammation/infection (n=176)	Microorganisms without intra-amniotic inflammation (n=48)	Sterile intra-amniotic inflammation (n=66)	Intra-amniotic infection (n=70)	p-Value
Gestational age at amniocentesis, weeks	30.1 (25.9–32.3)	29.3 (25.3–32.3)	27.6 (23.8–31.7)	26.4 (24.9–30.9)	0.02
Antibiotic use before amniocentesis	4.3% (4/94)	3.3% (1/30)	0% (0/43)	3.6% (2/55)	0.61
Positive amniotic fluid Gram stain	0% (0/174)	2.1% (1/48)	0% (0/65)	27.1% (19/70)	<0.001
Amniotic fluid WBC count, cells/mm ³	1 (0–7)	2 (0–8)	2 (0–10)	25 (2–430) ^{a,c,e}	<0.001
Amniotic fluid glucose, mg/dL	29 (23–33.5)	25 (20–31.5)	22 (18–27) ^b	13 (10–22) ^{a,c,e}	<0.001
Amniotic fluid interleukin-6, ng/mL	0.8 (0.4–1.2)	0.6 (0.3–1.2)	8.5 (4.4–17.5) ^{b,d}	75.4 (16.7–235.1) ^{a,c,e}	<0.001

Data are presented as median (interquartile range) or % (n/N). WBC, white blood cell; PCR/ESI-MS, polymerase chain reaction with electrospray ionization mass spectrometry. ^ap<0.05; No intra-amniotic inflammation/infection vs. Intra-amniotic infection. ^bp<0.05; No intra-amniotic inflammation/infection vs. Sterile intra-amniotic inflammation. ^cp<0.05; Microorganisms without inflammation vs. Intra-amniotic infection. ^dp<0.05; Microorganisms without inflammation vs. Sterile intra-amniotic inflammation. ^ep<0.05; Intra-amniotic infection vs. Sterile intra-amniotic inflammation.

Table 3: Microbiology of amniotic fluid in samples with positive cultures and/or PCR/ESI-MS according to the presence or absence of intra-amniotic inflammation.

Microorganisms without intra-amniotic inflammation						Microorganisms with intra-amniotic inflammation (intra-amniotic infection)					
Name of microorganisms	No. of culture-positive samples	No. of PCR-positive samples	No. of both-positive samples	Microbial burden (GE/well) ^a	Name of microorganisms	No. of culture-positive samples	No. of PCR-positive samples	No. of both-positive samples	Microbial burden (GE/well) ^a		
Bacteria and fungi	5	55	0	17 (10–38)	Bacteria and fungi	37	82	18	80 (20–178)		
<i>Propionibacterium acnes</i>	1	16	0	18 (14–31)	<i>Ureaplasma parvum</i>	0	8	0	573 (114–1,245)		
<i>Acinetobacter junii</i>	0	14	0	23 (18–27)	<i>Haemophilus influenzae</i>	2	2	2	158; 818		
<i>Staphylococcus aureus</i>	1	5	0	7 (5–8)	<i>Mycoplasma hominis</i>	3	5	2	446 (247–451)		
<i>Streptococcus viridans</i>	0	2	0	3; 11	<i>Streptococcus pneumonia</i>	0	1	0	286		
<i>Acidovorax temperans</i>	0	2	0	29; 54	<i>Ureaplasma urealyticum</i>	5	2	0	11; 272		
<i>Actinomyces odontolyticus</i>	0	1	0	3	<i>Staphylococcus aureus</i>	1	2	1	1; 201		
<i>Aeromonas caviae</i>	0	1	0	3	<i>Gardnerella vaginalis</i>	3	2	0	69; 171		
<i>Moraxella osloensis</i>	0	1	0	3	<i>Sneathia</i> spp.	3	12	3	168 (117–180)		
<i>Pantoea dispersa</i>	0	1	0	3	<i>Streptococcus agalactiae</i>	1	3	1	101; 142; 153		
<i>Streptococcus</i> spp.	0	1	0	3	<i>Escherichia coli</i>	1	1	1	149		
<i>Lactobacillus acidophilus</i>	0	1	0	5	<i>Candida albicans</i>	4	4	4	136 (45–337)		
<i>Streptococcus infantis</i>	0	1	0	6	<i>Fusobacterium nucleatum</i>	4	12	4	108 (32–120)		
<i>Haemophilus influenzae</i>	0	1	0	9	<i>Rhodococcus jostii</i>	0	1	0	58		
<i>Clostridium septicum</i>	0	1	0	12	<i>Pseudomonas mendocina</i>	0	1	0	51		
<i>Corynebacterium tuberculosis</i>	0	1	0	15	<i>Peptostreptococcus anaerobius</i>	1	1	0	50		
<i>Candida tropicalis</i>	0	1	0	35	<i>Kytococcus schroeteri</i>	0	1	0	48		
<i>Acinetobacter baumannii</i>	0	1	0	35	<i>Acinetobacter junii</i>	0	5	0	43 (15–61)		
<i>Staphylococcus arlettae</i>	0	1	0	43	<i>Streptococcus</i> spp.	1	1	0	36		
<i>Stenotrophomonas maltophilia</i>	0	1	0	45	<i>Streptobacillus moniliformis</i>	0	1	0	29		
<i>Pseudomonas fluorescens</i>	0	1	0	47	<i>Bacteroides fragilis</i>	0	1	0	21		
<i>Streptococcus agalactiae</i>	0	1	0	65	<i>Corynebacterium accolens</i>	0	1	0	19		
<i>Corynebacterium</i> spp.	1	0	0	–	<i>Propionibacterium acnes</i>	0	13	0	14 (12–20)		
<i>Ureaplasma urealyticum</i>	2	0	0	–	<i>Acinetobacter lwoffii</i>	0	1	0	14		
Viruses	0	19	0	17 (7–100)	<i>Pseudomonas entomophila</i>	0	1	0	10		
Human parvovirus B19	0	9	0	6 (4–10)	<i>Bacteroides ureolyticus</i>	3	0	0	0		
Human enterovirus	0	1	0	17	<i>Mobiluncus</i> spp.	2	0	0	–		
Human herpesvirus 2 (HHV-2)	0	2	0	90; 152	<i>Clostridium sporogenes</i>	1	0	0	–		
Human herpesvirus 5 (HHV-5)	0	7	0	109 (48–187)	<i>Staphylococcus capitis</i>	1	0	0	–		
					<i>Streptococcus anginosus</i>	1	0	0	–		
					Viruses	0	19	0	24 (15–78)		
					Human enterovirus	0	2	0	24; 1,000		
					Human herpesvirus 2 (HHV-2)	0	3	0	15; 67; 88		
					Human herpesvirus 5 (HHV-5)	0	6	0	34 (20–111)		
					Human parvovirus B19	0	7	0	15 (10–23)		
					Roseolovirus	0	1	0	20		

PCR/ESI-MS, polymerase chain reaction with electrospray ionization mass spectrometry. ^aData are presented as median (interquartile range) or each value.

Figure 1: Microbial burden in amniotic fluid between patients with intra-amniotic inflammation and those without intra-amniotic inflammation [median (IQR), 1.2 (1.0–1.6) vs. 1.9 (1.3–2.3) $\log_{10}$ (GE/well), $p < 0.005$]. The presence of intra-amniotic inflammation was defined as an elevated IL-6 (cut-off > 2.6 ng/mL).

distribution of the amniotic fluid WBC count and the concentrations of amniotic fluid IL-6 and amniotic fluid glucose varied significantly among the four subgroups (Kruskal–Wallis, $p < 0.001$ for all).

Microorganisms reported in amniotic fluid samples in the absence of intra-amniotic inflammation

Forty-eight amniotic fluid samples reported by the laboratory had microorganisms detected by culture or PCR but

no intra-amniotic inflammation: 35.4% (17/48) had two or more microorganisms and, overall, a total of 79 microorganisms (bacteria, $n=59$; fungus, $n=1$; and viruses, $n=19$) were identified (Table 3). *Propionibacterium acnes* ($n=17$) was the most frequent microorganism reported, followed by *Acinetobacter junii* ($n=14$). *P. acnes* was isolated by culture in only one patient [6% (1/17)] and, in the rest of the patients, positive results were attributable to PCR analysis [94% (16/17)]. All samples positive for *A. junii* were detected by PCR/ESI-MS (i.e. this organism was not detected by culture).

Among the 70 patients with intra-amniotic infection (positive for microorganisms and intra-amniotic inflammation), 47.1% (33/70) had two or more microorganisms and, overall, a total of 120 microorganisms (bacteria, $n=97$; fungus, $n=4$; and viruses, $n=19$) were identified (Table 3). Amniotic fluid samples from patients with intra-amniotic infection were dominated by *Sneathia* spp., *Fusobacterium nucleatum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Candida albicans*, which were not found in patients without intra-amniotic inflammation.

Microbial burden was low in amniotic fluid samples with microorganisms in the absence of intra-amniotic inflammation

The microbial burden was defined as the number of microorganisms in amniotic fluid and estimated by the number of gene copies per PCR well reaction (GE/well). Bacterial and fungal burdens were lower in amniotic fluid samples in the absence of intra-amniotic inflammation than in those with intra-amniotic inflammation [median (interquartile range (IQR), 17 (10–38) vs. 80 (20–178) GE/well, $p < 0.005$] (Figure 1). However, there was no significant difference in viral burden between those with or without inflammation [median (IQR), 17 (7–100) vs. 24 (15–78) GE/well, $p=0.34$].

Figure 2 is a heatmap illustrating microbial burden in the amniotic fluid as a function of the color (blue – low; red – high) according to the presence or absence of intra-amniotic inflammation. Amniotic fluid samples from patients with intra-amniotic infection were dominated by *U. parvum*, *M. hominis*, *Sneathia* spp., *C. albicans*, and *F. nucleatum* and exhibited high microbial burden [median (IQR), GE/well; *U. parvum*: 573 (114–1,245), *M. hominis*: 446 (247–451), *Sneathia* spp.: 168 (117–180), *C. albicans*: 136 (45–337), and *F. nucleatum*: 108 (32–120)]. Most patients with these microorganisms in amniotic fluid delivered within 7 days after amniocentesis [*U. parvum*: 75% (6/8),

Figure 2: Heatmap showing microbial taxa and microbial load in the amniotic fluid, $[\log_{10}(\text{GE}/\text{well} + 1)]$, according to PCR-based methods. High microbial burden is represented with color gradation which increases toward red, while low microbial burden tends toward blue, as shown in the color-key. The arrow indicates *Propionibacterium acnes*.

M. hominis: 100% (6/6), *Sneathia* spp.: 100% (12/12), *C. albicans*: 100% (4/4), *F. nucleatum*: 83% (10/12)] (Figure 2).

By contrast, *P. acnes* and *A. junii* were abundant in the samples of amniotic fluid without intra-amniotic inflammation and exhibited a low microbial burden [median (IQR), GE/well; *P. acnes*: 18 (14–31) and *A. junii*: 23 (18–27)]. *P. acnes* was also detected in the amniotic fluid samples with inflammation but consistently had a low microbial burden [median (IQR), 14 (12–20) GE/well] (Figure 2).

Pregnancy outcomes, acute placental inflammatory lesions, and neonatal outcomes

Table 4 shows the perinatal outcomes of the four subgroups: (1) patients reported by the laboratory to have microorganisms in the amniotic fluid but not intra-amniotic inflammation had similar outcomes as patients without either microorganisms or intra-amniotic

inflammation [spontaneous preterm birth <34 weeks of gestation ($p=1$), severe acute histologic chorioamnionitis ($p=0.76$), severe funisitis (necrotizing funisitis and/or intense umbilical vasculitis) ($p=1$), and composite neonatal morbidity ($p=0.8$)]; 2) patients with intra-amniotic infection, overall, had worse perinatal outcomes than those with microorganisms in the absence of inflammation [spontaneous preterm birth <34 weeks of gestation: 90% (63/70) vs. 31.2% (15/48), $p<0.001$; severe acute histologic chorioamnionitis: 55.7% (34/61) vs. 10.3% (4/39), $p<0.001$; and composite neonatal morbidity: 86.6% (58/67) vs. 33.3% (16/48), $p<0.001$] or those with sterile intra-amniotic inflammation [severe acute histologic chorioamnionitis: 55.7% (34/61) vs. 13.3% (8/60), $p<0.001$; funisitis [68.7% (46/67) vs. 27% (17/63), $p<0.001$]; and 3) patients with sterile intra-amniotic inflammation had worse perinatal outcomes than those with microorganisms in the absence of inflammation [spontaneous preterm birth <34 weeks of gestation: 83.3% (55/66) vs. 31.2% (15/48); composite neonatal morbidity: 76.6% (49/64) vs. 33.3% (16/48), $p<0.001$].

Table 4: Perinatal outcomes among the four subgroups of patients according to results of amniotic fluid culture, PCR/ESI-MS, and amniotic fluid IL-6 concentrations in patients with preterm labor and intact membranes.

	No intra-amniotic inflammation/infection (n=176)	Microorganisms without intra-amniotic inflammation (n=48)	Sterile intra-amniotic inflammation (n=66)	Intra-amniotic infection (n=70)	p-Value
Pregnancy outcomes					
Gestational age at delivery, weeks	35.3 (33.4–37.3)	34.9 (31.7–36.5)	29.9 (25.1–33.0) ^{c,e}	27.2 (25.2–31.9) ^{b,d}	<0.001
Preterm birth < 37 weeks	72.2% (127/176)	83.3% (40/48)	97% (64/66) ^{c,e}	97% (64/66) ^{b,d}	<0.001
Preterm birth < 34 weeks	31.2% (55/176)	37.5% (18/48)	84.8% (56/66) ^{c,e}	84.8% (56/66) ^{b,d}	<0.001
Spontaneous preterm birth < 37 weeks	66.5% (117/176)	70.8% (34/48)	95.5% (63/66) ^{c,e}	95.7% (67/70) ^{b,d}	<0.001
Spontaneous preterm birth < 34 weeks	30.1% (53/176)	31.2% (15/48)	83.3% (55/66) ^{c,e}	90% (63/70) ^{b,d}	<0.001
Interval amniocentesis to delivery, days	37.1 (19–63)	29.4 (12–57)	3.9 (1.4–10.4) ^{c,e,f}	1.4 (0.7–5) ^{b,d}	<0.001
Interval amniocentesis to delivery < 7 days	12.5% (22/176)	14.6% (7/48)	65.2% (43/66) ^{c,e}	82.9% (58/70) ^{b,d}	<0.001
Cesarean delivery	12.5% (22/176)	18.8% (9/48)	16.7% (11/66)	14.3% (10/70)	0.67
Fetal death	1.8% (3/164)	0% (0/40)	3.4% (2/59)	4.3% (3/70)	0.48
Acute placental inflammation					
Acute chorioamnionitis	34.2% (55/161)	34.1% (14/41)	60.3% (38/63) ^{c,e}	79.1% (53/67) ^{b,d}	<0.001
Severe lesions	33.5% (54/161)	31.8% (13/41)	60.3% (38/63) ^{c,e}	79.1% (53/67) ^{b,d}	<0.001
Stage 3: Necrotizing chorioamnionitis	6.5% (10/155)	10.3% (4/39)	13.3% (8/60) ^{c,f}	55.7% (34/61) ^{b,d}	<0.001
Grade 2: Subchorionic microabscess	4.3% (7/161)	7.3% (3/41)	9.5% (6/63) ^f	49.3% (33/67) ^{b,d}	<0.001
Funisitis	3.9% (6/153)	5.4% (2/37)	6.8% (4/59) ^f	31.5% (17/54) ^{b,d}	<0.001
Severe lesions	23.6% (38/161)	24.4% (10/41)	27% (17/63) ^f	68.7% (46/67) ^{b,d}	<0.001
Stage 3: Necrotizing funisitis	1.6% (2/127)	4.2% (1/24)	4.4% (2/45)	19.5% (8/41) ^b	<0.001
Grade 2: Intense umbilical vasculitis	0.6% (1/161)	2.4% (1/41)	1.6% (1/63)	10.4% (7/67) ^b	0.001
	0.8% (1/126)	0% (0/23)	4.4% (2/45)	7.7% (3/39)	0.08
Neonatal outcomes					
Birth weight, g	2,376 (1965–2,935)	2,142.5 (1,640–2,554) ^a	1,222.5 (743–1979) ^{c,e}	1,060 (815–1,660) ^{b,d}	<0.001
Male sex neonate	51.1% (89/174)	50% (24/48)	50% (33/66)	45.7% (32/70)	0.90
Neonatal death	3.1% (5/159)	0% (0/38)	16.4% (9/55) ^c	13.1% (8/61) ^{b,d}	<0.001
5-min Apgar score < 7	8.1% (14/172)	8.5% (4/47)	31.8% (21/66) ^{c,e}	37.7% (26/69) ^{b,d}	<0.001
Composite neonatal morbidity ^g	30.1% (52/173)	33.3% (16/48)	76.6% (49/64) ^{c,e}	86.6% (58/67) ^{b,d}	<0.001

Data are presented as median (interquartile range) or % (n/N) PCR/ESI-MS; polymerase chain reaction with electrospray ionization mass spectrometry. ^ap<0.05; no intra-amniotic inflammation/infection vs. microorganisms without inflammation. ^bp<0.05; No intra-amniotic inflammation/infection vs. intra-amniotic inflammation/infection. ^cp<0.05; no intra-amniotic inflammation/infection vs. sterile intra-amniotic inflammation. ^dp<0.05; microorganisms without inflammation vs. intra-amniotic infection. ^ep<0.05; microorganisms without inflammation vs. sterile intra-amniotic inflammation. ^fp<0.05; intra-amniotic infection vs. sterile intra-amniotic infection. ^gComposite neonatal morbidity includes more than one of the following: 5-min Apgar score < 7, bronchopulmonary dysplasia, respiratory distress syndrome, requirement for ventilation, necrotizing enterocolitis, intraventricular hemorrhage, retinopathy of prematurity, neonatal bacteremia, or neonatal intensive care unit admission.

Patients with bacteria in amniotic fluid but without intra-amniotic inflammation have a similar interval-to-delivery to those without either bacteria or intra-amniotic inflammation

Figure 3 displays the amniocentesis-to-delivery interval according to the presence or absence of microorganisms in amniotic fluid and intra-amniotic inflammation. Patients with microorganisms without intra-amniotic inflammation had a significantly longer amniocentesis-to-delivery interval than those with intra-amniotic inflammation regardless of the presence of microorganisms [median (IQR), 29.4 (12–57) vs. 1.4 (0.7–5) days, $p < 0.001$] or the absence of microorganisms [median (IQR), 29.4 (12–57) vs. 3.9 (1.4–10.4) days, $p < 0.001$]. There were no significant differences in the amniocentesis-to-delivery interval between patients with microorganisms in the absence of inflammation and those with amniotic fluid negative for microorganisms ($p = 0.31$).

Figure 3: Kaplan-Meier survival curves of amniocentesis-to-delivery interval (days) among four subgroups of patients according to the presence or absence of microorganisms in amniotic fluid and intra-amniotic inflammation.

Patients with bacteria in the amniotic fluid without intra-amniotic inflammation have a similar interval-to-delivery as those without bacteria or intra-amniotic inflammation ($p = 0.31$).

Discussion

Principal findings of the study

(1) The frequency of microorganisms in amniotic fluid without intra-amniotic inflammation was 1.4% (5/360) by

culture techniques, 12.5% (45/360) by PCR/ESI-MS, and 13.3% (48/360) by the combination of cultivation and PCR/ESI-MS; (2) the most frequently identified microorganisms in the 48 amniotic fluid specimens were *P. acnes*, followed by *A. junii*; (3) patients whose amniotic fluid samples had microorganisms but no indication of inflammation had a similar perinatal outcome to those in whom microorganisms were not found in the amniotic fluid [amniocentesis-to-delivery interval ($p = 0.31$), frequency of spontaneous preterm birth before 34 weeks of gestation ($p = 0.83$), acute placental inflammatory lesions ($p = 1$), and composite neonatal morbidity ($p = 0.8$)]; and (4) the microbial load of samples with bacteria reported by the laboratory in the absence of intra-amniotic inflammation was low. Therefore, we conclude that the presence of microorganisms in the absence of intra-amniotic inflammation most likely represents contamination at the time of specimen collection or laboratory processing and that it is a benign condition.

What is the difference? Intra-amniotic infection vs. colonization vs. contamination

When the culture of amniotic fluid obtained by amniocentesis is positive, the result could indicate one of three possibilities: infection, colonization, or contamination. **Intra-amniotic infection** is defined as the presence of microorganisms in the amniotic fluid, retrieved by amniocentesis, regardless of the presence or absence of clinical evidence of infection (e.g. fever, uterine tenderness, malodorous vaginal discharge, etc.) [1, 45], while **colonization of the amniotic cavity** refers to the presence of microorganisms in the amniotic fluid without any pathologic consequences [1, 45]. Microbes can colonize on a body surface, such as the skin, mouth, intestines, or airway, without causing disease in the person [140, 141]. However, amniotic fluid is normally sterile [37–42, 44, 142, 143], as is cerebrospinal fluid, or pleural or pericardial fluid. Thus, we have not used the term “colonization” of the amniotic cavity.

Given that the host immune response to intra-amniotic infection develops over time [66, 144–146], microorganisms may be able to **colonize the amniotic cavity** for a period of time without pathological consequences [147–150]. For example, an experiment based on a primate model showed that the number of *U. parvum* in amniotic fluid peaked at 3 days and uterine contractions peaked at 6–8 days after inoculation [146]. This finding suggests that microorganisms may exist in the amniotic cavity without clinical symptoms in the early phase of infection.

Moreover, clinical manifestations of microbial invasion of the amniotic cavity vary according to the virulence of the microorganisms [21, 65, 89, 116, 151–157], microbial burden [11, 20, 26, 64, 121, 158–160], and time frame of the acute inflammatory response [66, 146, 161].

Contamination occurs when microorganisms from an outside source are introduced into a sample [162]. For example, microorganisms normally present on the skin can gain access to amniotic fluid during amniocentesis or during the procedures required to prepare a specimen [20, 163, 164]. Indeed, contamination can originate from many sources including the laboratory environments [165, 166], plastic consumables [167], nucleic acid extraction kits [168–173], laboratory reagents [174–180], and cross-contamination from other samples [181, 182]. It is now well accepted that laboratory reagents and nucleic acid extraction kits harbor low levels of bacterial DNA [170, 183] similar to that found in soil or water samples [170, 172]. DNA contamination of reagents is unavoidable, given the ubiquity of microorganisms and the fact that many reagents are products of microbial processes and engineering [184].

Which bacteria are typical contaminants in amniotic fluid?

P. acnes was the most common microorganism isolated from amniotic fluid in the absence of intra-amniotic inflammation, and its presence should be considered suggestive of contamination rather than true infection. Several arguments support this view: (1) *P. acnes*, a commensal bacterium in the human skin microbiome [185, 186], is a common contaminant detected in cultures of blood and cerebrospinal fluid [187, 188]. Such skin bacteria can gain access to the amniotic fluid during an amniocentesis or through the procedures required to prepare the specimen [20, 163, 164]; (2) *Propionibacterium* spp. are reported as common contaminants present in the DNA extraction kits and other laboratory reagents [170, 171, 189–191]; in the current study, nearly all detection of *P. acnes* (97%; 31/32) came through the PCR method; and (3) all amniotic fluid samples positive with *P. acnes* yielded a consistently low bacterial burden regardless of the presence or absence of intra-amniotic inflammation.

Acidovorax temperans, *Pantoea dispersa*, *Staphylococcus arlettae*, and *Stenotrophomonas maltophilia* were detected in amniotic fluid samples with the absence of intra-amniotic inflammation. These microorganisms have rarely been reported to cause human infection and are found in laboratory environments [170, 189], reagents [170,

189], nebulizers [192], water dispensers [193], hemodialysis fluids [194], and intravenous fluids [194]. Therefore, in amniotic fluid, these microorganisms are likely to be contaminants.

A. junii was the second most common species of microorganism isolated from samples with the absence of intra-amniotic inflammation. *Acinetobacter* spp. are found in water and soil environments [195] and have previously been identified as contaminants [189, 196] in biology grade water [170], PCR reagents [170, 190], DNA extraction kits [170, 190], and air samples collected from a patient's room [197]. In the current study, any time *Acinetobacter* spp. (*Acinetobacter baumannii*, *A. junii*, and *Acinetobacter lwoffii*) were found, the microbial burden was low, which is also a characteristic of contamination. Although *Acinetobacter* spp. have been reported as potential pathogens causing nosocomial sepsis [198–200], preterm delivery [201, 202], acute chorioamnionitis, and a fetal inflammatory response [201, 202], questions always arise about whether these organisms are contaminants and whether the condition occurs as a sterile intra-amniotic inflammatory process or as organisms that escape detection by conventional methods. It is important to remember that, even in recent times, some bacteria have been difficult to identify for decades, including *Borrelia burgdorferi*, the organism responsible for Lyme disease [203], and *Helicobacter pylori* [204].

Bacteria likely to be pathogens in amniotic fluid

Microorganisms implicated as “true pathogens” in intra-amniotic infection include *Ureaplasma parvum*, *Mycoplasma hominis*, *Sneathia* spp., *Candida albicans*, *Fusobacterium nucleatum*, *Staphylococcus aureus*, *Gardnerella vaginalis*, *Haemophilus influenzae*, and *Streptococcus agalactiae* [20, 89, 116, 121, 151, 152, 158, 163, 205–213]. In the current study, these taxa were abundant in the amniotic fluid samples with intra-amniotic inflammation, as demonstrated in Figure 2, and their presence was associated with adverse pregnancy outcomes, including spontaneous preterm delivery, severe acute chorioamnionitis or funisitis, and a short interval-to-delivery.

Abundance of microorganisms to differentiate infection from contamination

Quantification of bacterial growth has been used to distinguish between contamination and true infection in

the clinical setting. For example, the difference between asymptomatic bacteriuria and contamination of a urine specimen is based on the number of colony-forming units in a urine specimen obtained via clean-catch. The presence of bacteria is considered to be clinically significant if there are more than 10^5 colony-forming units (CFU)/mL, and a lower number is thought to reflect contamination when the urine travels from the bladder to the container through the urethra, which normally contains bacteria [214].

In the past, quantitative cultivation-based microbiology methods have been used to assess the microbial burden in amniotic fluid. We have observed that patients with a higher microbial burden are more likely to have a positive Gram stain in amniotic fluid [4, 126, 215] or to present with preterm labor leading to preterm delivery [14].

Given that bacteria grow exponentially in amniotic fluid over time [66, 144–146], amniotic fluid samples with a true infection will have a much higher microbial burden in later stages of gestation than those that have been contaminated during collection and/or processing.

A low microbial burden in amniotic fluid assessed by PCR methods has been attributed to background DNA contamination in the extraction kit [42, 160], whereas a high microbial burden has been observed in patients who have intra-amniotic infection [160] with a strong intra-amniotic inflammatory response [11, 20, 26, 121, 160]. We reported that patients exhibiting a microbial burden higher than 17 GE/well had a higher frequency of intra-amniotic inflammation, acute histologic chorioamnionitis, and perinatal morbidity than those with a lower microbial burden, assessed by PCR [11].

In the current study, pathogenic bacteria in the amniotic fluid exhibited a high microbial burden [i.e. median (IQR), GE/well; *U. parvum*: 573 (114–1,245), *M. hominis*: 446 (247–451), *Sneathia* spp.: 168 (117–180), *C. albicans*: 136 (45–337), and *F. nucleatum*: 108 (32–120)], whereas bacteria considered as contaminants in amniotic fluid showed a lower microbial load [i.e. median (IQR), GE/well; *P. acne*: 17 (13–24) GE/well] regardless of the presence or absence of intra-amniotic inflammation. Moreover, the risk of preterm birth begins to increase exponentially when the microbial burden in amniotic fluid exceeds 17 GE/well (Figure 4). Therefore, we believe that a microbial burden, assessed with molecular microbiologic techniques, can assist in determining whether a positive result reflects contamination rather than a true infection.

Figure 4: Positive likelihood ratio for preterm birth before 34 weeks of gestation as a function of microbial burden [$\log_{10}$ (GE/well)]. The risk of preterm birth before 34 weeks begins to increase exponentially when the microbial burden (bacterial and fungal) exceeds $1.2 \log_{10}$ (GE/well). Viral invasion of the amniotic cavity was excluded from this analysis. In patients with several microorganisms, the calculation was based on the microorganism with the highest microbial burden.

Clinical significance of microorganisms in amniotic fluid without intra-amniotic inflammation: contamination or early infection?

Patients with microorganisms in amniotic fluid specimens (detected by either culture or molecular microbiologic techniques) without intra-amniotic inflammation have similar pregnancy outcomes to patients who did not have bacteria or inflammation. Therefore, we propose that finding bacteria in amniotic fluid in samples without intra-amniotic inflammation represents contamination. Consequently, clinical decisions, such as inducing labor or withholding treatment, given the suspicion of intra-amniotic infection does not seem to be justifiable.

Strengths and limitations

The major strengths of this study are emphasized as follows: (1) both cultivation and molecular microbiologic techniques were used to identify microorganisms in the samples of amniotic fluid collected by transabdominal amniocentesis from the amniotic cavity; therefore, the

diagnosis of microbial invasion was based on the use of state-of-the-art methodologies; (2) the assessment of intra-amniotic inflammation using the concentration of IL-6 in amniotic fluid; (3) the blinding of pathologists to obstetrical diagnoses and perinatal outcomes; and (4) the use of standardized protocols for placental examination.

The study also comprises the following limitations: (1) the duration of storage of the samples may have led to a degradation of the IL-6 concentration in amniotic fluid, which, in turn, may have yielded a lower concentration of the analytes as compared to the use of freshly collected and processed samples of amniotic fluid [216, 217]; (2) the lack of use of molecular markers to identify the presence of microorganisms in the extra-chorionic membranes, chorionic plate, and umbilical cord resulted in a lack of morphologic evidence of the location of microorganisms at different sites in the samples; and (3) the lack of use of metagenomics, strain culture, and/or strain-directed sequencing, which may be utilized to make the distinction between contamination and colonization.

Conclusions

The isolation of microorganisms or the detection of microbial nucleic acids from a sample of amniotic fluid, by cultivation and/or microbiologic molecular techniques in a clinical laboratory setting in the absence of intra-amniotic inflammation is a benign condition. Such a result most likely represents contamination of the specimen during the collection procedure or laboratory processing rather than early colonization or infection.

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References

1. Romero R, Mazor M. Infection and preterm labor. *Clin Obstet Gynecol* 1988;31:553–84.
2. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med* 2000;342:1500–7.
3. McClure EM, Goldenberg RL. Infection and stillbirth. *Semin Fetal Neonatal Med* 2009;14:182–9.
4. Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intra-amniotic infection in women with preterm labor and intact membranes. *Am J Obstet Gynecol* 1989; 161:817–24.
5. Skoll MA, Moretti ML, Sibai BM. The incidence of positive amniotic fluid cultures in patients preterm labor with intact membranes. *Am J Obstet Gynecol* 1989;161:813–6.
6. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 2001;185:1130–6.
7. Shim S, Yoon BH, Romero R, Shim J, Kim G, Jung H, et al. The clinical significance of detecting *Ureaplasma Urealyticum* by PCR in the amniotic fluid of patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 2003;187:S129.
8. Gomez R, Romero R, Nien JK, Chaiworapongsa T, Medina L, Kim YM, et al. A short cervix in women with preterm labor and intact membranes: a risk factor for microbial invasion of the amniotic cavity. *Am J Obstet Gynecol* 2005;192:678–89.
9. Espinoza J, Goncalves LF, Romero R, Nien JK, Stites S, Kim YM, et al. The prevalence and clinical significance of amniotic fluid 'sludge' in patients with preterm labor and intact membranes. *Ultrasound Obstet Gynecol* 2005;25:346–52.
10. Kim BJ, Romero R, Mi Lee S, Park CW, Shin Park J, Jun JK, et al. Clinical significance of oligohydramnios in patients with preterm labor and intact membranes. *J Perinat Med* 2011;39:131–6.
11. Romero R, Miranda J, Chaiworapongsa T, Chaemsaitong P, Gotsch F, Dong Z, et al. A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes. *Am J Reprod Immunol* 2014;71: 330–58.
12. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaitong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am J Reprod Immunol* 2014;72:458–74.
13. Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. *Am J Obstet Gynecol* 2014; 210:125.e1–15.
14. Romero R, Quintero R, Oyarzun E, Wu YK, Sabo V, Mazor M, et al. Intraamniotic infection and the onset of labor in preterm

- premature rupture of the membranes. *Am J Obstet Gynecol* 1988; 159:661–6.
15. Romero R, Ghidini A, Mazor M, Behnke E. Microbial invasion of the amniotic cavity in premature rupture of membranes. *Clin Obstet Gynecol* 1991;34:769–78.
 16. Averbuch B, Mazor M, Shoham-Vardi I, Chaim W, Vardi H, Horowitz S, et al. Intra-uterine infection in women with preterm premature rupture of membranes: maternal and neonatal characteristics. *Eur J Obstet Gynecol Reprod Biol* 1995;62:25–9.
 17. Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Nikolaitchouk N, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. *Acta Obstet Gynecol Scand* 2003;82:423–31.
 18. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* 2004;191:1339–45.
 19. Witt A, Berger A, Gruber CJ, Petricevic L, Apfalter P, Worda C, et al. Increased intrauterine frequency of *Ureaplasma urealyticum* in women with preterm labor and preterm premature rupture of the membranes and subsequent cesarean delivery. *Am J Obstet Gynecol* 2005;193:1663–9.
 20. DiGiulio DB, Romero R, Kusanovic JP, Gomez R, Kim CJ, Seok KS, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *Am J Reprod Immunol* 2010;64:38–57.
 21. Oh KJ, Lee KA, Sohn YK, Park CW, Hong JS, Romero R, et al. Intraamniotic infection with genital mycoplasmas exhibits a more intense inflammatory response than intraamniotic infection with other microorganisms in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* 2010;203: 211–8.
 22. Cobo T, Kacerovsky M, Palacio M, Hornychova H, Hougaard DM, Skogstrand K, et al. Intra-amniotic inflammatory response in subgroups of women with preterm prelabor rupture of the membranes. *PLoS One* 2012;7:e43677.
 23. Kacerovsky M, Musilova I, Khatibi A, Skogstrand K, Hougaard DM, Tambor V, et al. Intraamniotic inflammatory response to bacteria: analysis of multiple amniotic fluid proteins in women with preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2012;25:2014–9.
 24. Kacerovsky M, Musilova I, Andrys C, Hornychova H, Pliskova L, Kostal M, et al. Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. *Am J Obstet Gynecol* 2014;210:325.e1–10.
 25. Galaz J, Romero R, Slutsky R, Xu Y, Motomura K, Para R, et al. Cellular immune responses in amniotic fluid of women with preterm prelabor rupture of membranes. *J Perinat Med* 2020;48: 222–33.
 26. Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsathong P, Martinez A, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *J Perinat Med* 2015;43:19–36.
 27. Romero R, Chaemsathong P, Docheva N, Korzeniewski SJ, Kusanovic JP, Yoon BH, et al. Clinical chorioamnionitis at term VI: acute chorioamnionitis and funisitis according to the presence or absence of microorganisms and inflammation in the amniotic cavity. *J Perinat Med* 2016;44:33–51.
 28. Martinez-Varea A, Romero R, Xu Y, Miller D, Ahmed AI, Chaemsathong P, et al. Clinical chorioamnionitis at term VII: the amniotic fluid cellular immune response. *J Perinat Med* 2017;45: 523–38.
 29. Romero R, Pacora P, Kusanovic JP, Jung E, Panaitescu B, Maymon E, et al. Clinical chorioamnionitis at term X: microbiology, clinical signs, placental pathology, and neonatal bacteremia-implications for clinical care. *J Perinat Med* 2021;49: 275–98.
 30. Gomez R, Romero R, Nien JK, Medina L, Carstens M, Kim YM, et al. Idiopathic vaginal bleeding during pregnancy as the only clinical manifestation of intrauterine infection. *J Matern Fetal Neonat Med* 2005;18:31–7.
 31. Madan I, Romero R, Kusanovic JP, Mittal P, Chaiworapongsa T, Dong Z, et al. The frequency and clinical significance of intra-amniotic infection and/or inflammation in women with placenta previa and vaginal bleeding: an unexpected observation. *J Perinat Med* 2010;38:275–9.
 32. Hassan S, Romero R, Hendler I, Gomez R, Khalek N, Espinoza J, et al. A sonographic short cervix as the only clinical manifestation of intra-amniotic infection. *J Perinat Med* 2006; 34:13–9.
 33. Vaisbuch E, Hassan SS, Mazaki-Tovi S, Nhan-Chang CL, Kusanovic JP, Chaiworapongsa T, et al. Patients with an asymptomatic short cervix (≤ 15 mm) have a high rate of subclinical intraamniotic inflammation: implications for patient counseling. *Am J Obstet Gynecol* 2010;202:433. e1–8.
 34. Romero R, Miranda J, Chaiworapongsa T, Chaemsathong P, Gotsch F, Dong Z, et al. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. *J Matern Fetal Neonat Med* 2015;28:1343–59.
 35. Jung EJ, Romero R, Gomez-Lopez N, Paredes C, Diaz-Primera R, Hernandez-Andrade E, et al. Cervical insufficiency, amniotic fluid sludge, intra-amniotic infection, and maternal bacteremia: the need for a point-of-care test to assess inflammation and bacteria in amniotic fluid. *J Matern Fetal Neonatal Med* 2020;1–7. <https://doi.org/10.1080/14767058.2020.1863369>.
 36. Kim SK, Romero R, Kusanovic JP, Erez O, Vaisbuch E, Mazaki-Tovi S, et al. The prognosis of pregnancy conceived despite the presence of an intrauterine device (IUD). *J Perinat Med* 2010;38: 45–53.
 37. Prevedourakis CN, Strigou-Charalabis E, Kaskarelis DB. Bacterial invasion of amniotic cavity during pregnancy and labor. *Obstet Gynecol* 1971;37:459–61.
 38. Romero R, Norez J, Mazor M, Sepulveda W, Oyarzun E, Parra M, et al. Microbial invasion of the amniotic cavity during term labor. Prevalence and clinical significance. *J Reprod Med* 1993;38: 543–8.
 39. McLean LK, Chehab FF, Goldberg JD. Detection of viral deoxyribonucleic acid in the amniotic fluid of low-risk pregnancies by polymerase chain reaction. *Am J Obstet Gynecol* 1995;173:1282–6.
 40. Seong HS, Lee SE, Kang JH, Romero R, Yoon BH. The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor. *Am J Obstet Gynecol* 2008;199:375. e1–5.
 41. Rowlands S, Danielewski JA, Tabrizi SN, Walker SP, Garland SM. Microbial invasion of the amniotic cavity in midtrimester

- pregnancies using molecular microbiology. *Am J Obstet Gynecol* 2017;217:71.e1–5.
42. Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome* 2018;6:87.
 43. Reh binder EM, Lodrup Carlsen KC, Staff AC, Angell IL, Landro L, Hilde K, et al. Is amniotic fluid of women with uncomplicated term pregnancies free of bacteria? *Am J Obstet Gynecol* 2018;219: 289.e1–12.
 44. Liu Y, Li X, Zhu B, Zhao H, Ai Q, Tong Y, et al. Midtrimester amniotic fluid from healthy pregnancies has no microorganisms using multiple methods of microbiologic inquiry. *Am J Obstet Gynecol* 2020;223:248.e1–248.e21.
 45. Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, et al. Infection in the pathogenesis of preterm labor. *Semin Perinatol* 1988;12:262–79.
 46. Goldenberg RL, Andrews WW, Hauth JC. Choriodecidual infection and preterm birth. *Nutr Rev* 2002;60:S19–25.
 47. Suff N, Karda R, Diaz JA, Ng J, Baruteau J, Perocheau D, et al. Ascending vaginal infection using bioluminescent bacteria evokes intrauterine inflammation, preterm birth, and neonatal brain injury in pregnant mice. *Am J Pathol* 2018;188: 2164–76.
 48. Romero R, Gomez-Lopez N, Winters AD, Jung E, Shaman M, Bieda J, et al. Evidence that intra-amniotic infections are often the result of an ascending invasion: a molecular microbiological study. *J Perinat Med* 2019;47:915–31.
 49. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J Clin Invest* 1990;85:1392–400.
 50. Romero R, Gomez R, Galasso M, Munoz H, Acosta L, Yoon BH, et al. Macrophage inflammatory protein-1 α in term and preterm parturition: effect of microbial invasion of the amniotic cavity. *Am J Reprod Immunol* 1994;32:108–13.
 51. Fidel PL, Jr, Romero R, Wolf N, Cutright J, Ramirez M, Araneda H, et al. Systemic and local cytokine profiles in endotoxin-induced preterm parturition in mice. *Am J Obstet Gynecol* 1994;170: 1467–75.
 52. Dudley DJ, Hunter C, Varner MW, Mitchell MD. Elevation of amniotic fluid interleukin-4 concentrations in women with preterm labor and chorioamnionitis. *Am J Perinatol* 1996;13: 443–7.
 53. Maymon E, Ghezzi F, Edwin SS, Mazor M, Yoon BH, Gomez R, et al. The tumor necrosis factor alpha and its soluble receptor profile in term and preterm parturition. *Am J Obstet Gynecol* 1999;181: 1142–8.
 54. Chaiworapongsa T, Romero R, Espinoza J, Kim YM, Edwin S, Bujold E, et al. Macrophage migration inhibitory factor in patients with preterm parturition and microbial invasion of the amniotic cavity. *J Matern Fetal Neonatal Med* 2005;18:405–16.
 55. Esplin MS, Romero R, Chaiworapongsa T, Kim YM, Edwin S, Gomez R, et al. Monocyte chemotactic protein-1 is increased in the amniotic fluid of women who deliver preterm in the presence or absence of intra-amniotic infection. *J Matern Fetal Neonatal Med* 2005;17:365–73.
 56. Jacobsson B, Holst RM, Andersson B, Hagberg H. Monocyte chemotactic protein-2 and -3 in amniotic fluid: relationship to microbial invasion of the amniotic cavity, intra-amniotic inflammation and preterm delivery. *Acta Obstet Gynecol Scand* 2005;84:566–71.
 57. Gotsch F, Romero R, Kusanovic JP, Erez O, Espinoza J, Kim CJ, et al. The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic infection/inflammation, and spontaneous parturition at term: a role for interleukin-10. *J Matern Fetal Neonatal Med* 2008;21:529–47.
 58. Mittal P, Romero R, Kusanovic JP, Edwin SS, Gotsch F, Mazaki-Tovi S, et al. CXCL6 (granulocyte chemotactic protein-2): a novel chemokine involved in the innate immune response of the amniotic cavity. *Am J Reprod Immunol* 2008;60:246–57.
 59. Nhan-Chang CL, Romero R, Kusanovic JP, Gotsch F, Edwin SS, Erez O, et al. A role for CXCL13 (BCA-1) in pregnancy and intra-amniotic infection/inflammation. *J Matern Fetal Neonatal Med* 2008;21: 763–75.
 60. Marconi C, de Andrade Ramos BR, Peracoli JC, Donders GG, da Silva MG. Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. *Am J Reprod Immunol* 2011;65: 549–56.
 61. Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. *J Perinat Med* 2012;40:329–43.
 62. Gomez-Lopez N, Romero R, Leng Y, Xu Y, Slutsky R, Levenson D, et al. The origin of amniotic fluid monocytes/macrophages in women with intra-amniotic inflammation or infection. *J Perinat Med* 2019;47:822–40.
 63. Bhatti G, Romero R, Rice GE, Fitzgerald W, Pacora P, Gomez-Lopez N, et al. Compartmentalized profiling of amniotic fluid cytokines in women with preterm labor. *PLoS One* 2020;15:e0227881.
 64. Thiersch JB. Effect of lipopolysaccharides of Gram negative bacilli on the rat litter in utero. *Proc Soc Exp Biol Med* 1962;109:429–37.
 65. Dombroski RA, Woodard DS, Harper MJ, Gibbs RS. A rabbit model for bacteria-induced preterm pregnancy loss. *Am J Obstet Gynecol* 1990;163:1938–43.
 66. Gravett MG, Witkin SS, Haluska GJ, Edwards JL, Cook MJ, Novy MJ. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am J Obstet Gynecol* 1994;171:1660–7.
 67. Gomez-Lopez N, Romero R, Arenas-Hernandez M, Panaitescu B, Garcia-Flores V, Mial TN, et al. Intra-amniotic administration of lipopolysaccharide induces spontaneous preterm labor and birth in the absence of a body temperature change. *J Matern Fetal Neonat Med* 2018;31:439–46.
 68. Kallapur SG, Willet KE, Jobe AH, Ikegami M, Bachurski CJ. Intra-amniotic endotoxin: chorioamnionitis precedes lung maturation in preterm lambs. *Am J Physiol Lung Cell Mol Physiol* 2001;280: L527–36.
 69. Kramer BW, Moss TJ, Willet KE, Newnham JP, Sly PD, Kallapur SG, et al. Dose and time response after intraamniotic endotoxin in preterm lambs. *Am J Respir Crit Care Med* 2001;164:982–8.
 70. Moss TJ, Nitsos I, Newnham JP, Ikegami M, Jobe AH. Chorioamnionitis induced by subchorionic endotoxin infusion in sheep. *Am J Obstet Gynecol* 2003;189:1771–6.
 71. Kramer BW, Ikegami M, Moss TJ, Nitsos I, Newnham JP, Jobe AH. Endotoxin-induced chorioamnionitis modulates innate immunity of monocytes in preterm sheep. *Am J Respir Crit Care Med* 2005; 171:73–7.
 72. Berry CA, Nitsos I, Hillman NH, Pillow JJ, Polglase GR, Kramer BW, et al. Interleukin-1 in lipopolysaccharide induced

- chorioamnionitis in the fetal sheep. *Reprod Sci* 2011;18:1092–102.
73. Maxwell JR, Denson JL, Joste NE, Robinson S, Jantzie LL. Combined in utero hypoxia-ischemia and lipopolysaccharide administration in rats induces chorioamnionitis and a fetal inflammatory response syndrome. *Placenta* 2015;36:1378–84.
 74. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. *Am J Obstet Gynecol* 2000;183:1124–9.
 75. Watanabe T, Matsuda T, Hanita T, Okuyama K, Cho K, Kobayashi K, et al. Induction of necrotizing funisitis by fetal administration of intravenous granulocyte-colony stimulating factor and intra-amniotic endotoxin in premature fetal sheep. *Pediatr Res* 2007;62:670–3.
 76. Rounioja S, Rasanen J, Glumoff V, Ojaniemi M, Makikallio K, Hallman M. Intra-amniotic lipopolysaccharide leads to fetal cardiac dysfunction. A mouse model for fetal inflammatory response. *Cardiovasc Res* 2003;60:156–64.
 77. Cheah FC, Pillow JJ, Kramer BW, Polglase GR, Nitsos I, Newnham JP, et al. Airway inflammatory cell responses to intra-amniotic lipopolysaccharide in a sheep model of chorioamnionitis. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L384–93.
 78. Gavilanes AW, Strackx E, Kramer BW, Gantert M, Van den Hove D, Steinbusch H, et al. Chorioamnionitis induced by intraamniotic lipopolysaccharide resulted in an interval-dependent increase in central nervous system injury in the fetal sheep. *Am J Obstet Gynecol* 2009;200:437.e1–8.
 79. Kramer BW, Kallapur SG, Moss TJ, Nitsos I, Polglase GP, Newnham JP, et al. Modulation of fetal inflammatory response on exposure to lipopolysaccharide by chorioamnion, lung, or gut in sheep. *Am J Obstet Gynecol* 2010;202:77.e1–9.
 80. Kunzmann S, Glogger K, Been JV, Kallapur SG, Nitsos I, Moss TJ, et al. Thymic changes after chorioamnionitis induced by intraamniotic lipopolysaccharide in fetal sheep. *Am J Obstet Gynecol* 2010;202:476.e1–9.
 81. Bieghs V, Vlassaks E, Custers A, van Gorp PJ, Gijbels MJ, Bast A, et al. Chorioamnionitis induced hepatic inflammation and disturbed lipid metabolism in fetal sheep. *Pediatr Res* 2010;68:466–72.
 82. Strackx E, Sparnaaij MA, Vlassaks E, Jellema R, Kuypers E, Vles JS, et al. Lipopolysaccharide-induced chorioamnionitis causes acute inflammatory changes in the ovine central nervous system. *CNS Neurol Disord Drug Targets* 2015;14:77–84.
 83. Schmidt AF, Kannan PS, Chougnet CA, Danzer SC, Miller LA, Jobe AH, et al. Intra-amniotic LPS causes acute neuroinflammation in preterm rhesus macaques. *J Neuroinflamm* 2016;13:238.
 84. Nguyen DN, Thymann T, Goericke-Pesch SK, Ren S, Wei W, Skovgaard K, et al. Prenatal intra-amniotic endotoxin induces fetal gut and lung immune responses and postnatal systemic inflammation in preterm pigs. *Am J Pathol* 2018;188:2629–43.
 85. Jung E, Romero R, Yeo L, Diaz-Primera R, Marin-Concha J, Para R, et al. The fetal inflammatory response syndrome: the origins of a concept, pathophysiology, diagnosis, and obstetrical implications. *Semin Fetal Neonatal Med* 2020;25:101146.
 86. Leviton A. Preterm birth and cerebral palsy: is tumor necrosis factor the missing link? *Dev Med Child Neurol* 1993;35:553–8.
 87. Gravett MG, Haluska GJ, Cook MJ, Novy MJ. Fetal and maternal endocrine responses to experimental intrauterine infection in rhesus monkeys. *Am J Obstet Gynecol* 1996;174:1725–31. discussion 31–3.
 88. Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* 1997;177:19–26.
 89. Yoon BH, Chang JW, Romero R. Isolation of *Ureaplasma urealyticum* from the amniotic cavity and adverse outcome in preterm labor. *Obstet Gynecol* 1998;92:77–82.
 90. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. *Am J Obstet Gynecol* 2000;182:675–81.
 91. Nelson KB, Willoughby RE. Infection, inflammation and the risk of cerebral palsy. *Curr Opin Neurol* 2000;13:133–9.
 92. Hitti J, Tarczy-Hornoch P, Murphy J, Hillier SL, Aura J, Eschenbach DA. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. *Obstet Gynecol* 2001;98:1080–8.
 93. Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM. The role of infection in preterm labour and delivery. *Paediatr Perinat Epidemiol* 2001;15 (2 Suppl):41–56.
 94. Moon JB, Kim JC, Yoon BH, Romero R, Kim G, Oh SY, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. *J Perinat Med* 2002;30:301–6.
 95. Yoon BH, Park CW, Chaiworapongsa T. Intrauterine infection and the development of cerebral palsy. *BJOG* 2003;110:124 (20 Suppl)–7.
 96. Neufeld MD, Frigon C, Graham AS, Mueller BA. Maternal infection and risk of cerebral palsy in term and preterm infants. *J Perinatol* 2005;25:108–13.
 97. Shatrov JG, Birch SC, Lam LT, Quinlivan JA, McIntyre S, Mendz GL. Chorioamnionitis and cerebral palsy: a meta-analysis. *Obstet Gynecol* 2010;116:387–92.
 98. Kasper DC, Mechtler TP, Bohm J, Petricevic L, Gleiss A, Spargser J, et al. In utero exposure to *Ureaplasma* spp. is associated with increased rate of bronchopulmonary dysplasia and intraventricular hemorrhage in preterm infants. *J Perinat Med* 2011;39:331–6.
 99. Bhandari V, Buhimschi CS, Han CS, Lee SY, Pettker CM, Campbell KH, et al. Cord blood erythropoietin and interleukin-6 for prediction of intraventricular hemorrhage in the preterm neonate. *J Matern Fetal Neonatal Med* 2011;24:673–9.
 100. Korzeniewski SJ, Romero R, Cortez J, Pappas A, Schwartz AG, Kim CJ, et al. A “multi-hit” model of neonatal white matter injury: cumulative contributions of chronic placental inflammation, acute fetal inflammation and postnatal inflammatory events. *J Perinat Med* 2014;42:731–43.
 101. Kuban KC, O'Shea TM, Allred EN, Paneth N, Hirtz D, Fichorova RN, et al. Systemic inflammation and cerebral palsy risk in extremely preterm infants. *J Child Neurol* 2014;29:1692–8.
 102. Catov JM, Scifres CM, Caritis SN, Bertolet M, Larkin J, Parks WT. Neonatal outcomes following preterm birth classified according to placental features. *Am J Obstet Gynecol* 2017;216:411. e1–4.
 103. Oh KJ, Park JY, Lee J, Hong JS, Romero R, Yoon BH. The combined exposure to intra-amniotic inflammation and neonatal respiratory distress syndrome increases the risk of intraventricular hemorrhage in preterm neonates. *J Perinat Med* 2018;46:9–20.

104. Latino MA, Botta G, Badino C, Maria D, Petrozziello A, Sensini A, et al. Association between genital mycoplasmas, acute chorioamnionitis and fetal pneumonia in spontaneous abortions. *J Perinat Med* 2018;46:503–8.
105. Faro J, Romero R, Schwenkel G, Garcia-Flores V, Arenas-Hernandez M, Leng Y, et al. Intra-amniotic inflammation induces preterm birth by activating the NLRP3 inflammasome. *Biol Reprod* 2019;100:1290–305.
106. Peiris HN, Romero R, Vaswani K, Reed S, Gomez-Lopez N, Tarca AL, et al. Preterm labor is characterized by a high abundance of amniotic fluid prostaglandins in patients with intra-amniotic infection or sterile intra-amniotic inflammation. *J Matern Fetal Neonatal Med* 2019;1–16. <https://doi.org/10.1080/14767058.2019.1702953>.
107. Lee J, Romero R, Kim SM, Chaemsathong P, Yoon BH. A new antibiotic regimen treats and prevents intra-amniotic inflammation/infection in patients with preterm PROM. *J Matern Fetal Neonatal Med* 2016;29:2727–37.
108. Lee J, Romero R, Kim SM, Chaemsathong P, Park CW, Park JS, et al. A new anti-microbial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM. *J Matern Fetal Neonatal Med* 2016;29:707–20.
109. Yoon BH, Romero R, Park JY, Oh KJ, Lee J, Conde-Agudelo A, et al. Antibiotic administration can eradicate intra-amniotic infection or intra-amniotic inflammation in a subset of patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 2019;221:142.e1–22.
110. Oh KJ, Romero R, Park JY, Lee J, Conde-Agudelo A, Hong JS, et al. Evidence that antibiotic administration is effective in the treatment of a subset of patients with intra-amniotic infection/inflammation presenting with cervical insufficiency. *Am J Obstet Gynecol* 2019;221:140.e1–18.
111. Kacerovsky M, Romero R, Stepan M, Stranik J, Maly J, Pliskova L, et al. Antibiotic administration reduces the rate of intraamniotic inflammation in preterm prelabor rupture of the membranes. *Am J Obstet Gynecol* 2020;223:114.e1–20.
112. Coultrip LL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. *Am J Obstet Gynecol* 1994;171:901–11.
113. Hitti J, Riley DE, Krohn MA, Hillier SL, Agnew KJ, Krieger JN, et al. Broad-spectrum bacterial rDNA polymerase chain reaction assay for detecting amniotic fluid infection among women in premature labor. *Clin Infect Dis* 1997;24:1228–32.
114. Greci LS, Gilson GJ, Nevils B, Izquierdo LA, Qualls CR, Curet LB. Is amniotic fluid analysis the key to preterm labor? A model using interleukin-6 for predicting rapid delivery. *Am J Obstet Gynecol* 1998;179:172–8.
115. Angus SR, Segel SY, Hsu CD, Locksmith GJ, Clark P, Sammel MD, et al. Amniotic fluid matrix metalloproteinase-8 indicates intra-amniotic infection. *Am J Obstet Gynecol* 2001;185:1232–8.
116. Yoon BH, Romero R, Lim JH, Shim SS, Hong JS, Shim JY, et al. The clinical significance of detecting *Ureaplasma urealyticum* by the polymerase chain reaction in the amniotic fluid of patients with preterm labor. *Am J Obstet Gynecol* 2003;189:919–24.
117. Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Wennerholm UB, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women in preterm labor. *Acta Obstet Gynecol Scand* 2003;82:120–8.
118. Jacobsson B, Mattsby-Baltzer I, Hagberg H. Interleukin-6 and interleukin-8 in cervical and amniotic fluid: relationship to microbial invasion of the chorioamniotic membranes. *BJOG* 2005;112:719–24.
119. WHO. Neonatal and perinatal mortality: country, regional and global estimates. Geneva: World Health Organization; 2006.
120. Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, et al. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. *Am J Obstet Gynecol* 2007;197:292.e1–5.
121. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS One* 2008;3:e3056.
122. DiGiulio DB, Gervasi M, Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, et al. Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods. *J Perinat Med* 2010;38:503–13.
123. DiGiulio DB, Gervasi MT, Romero R, Vaisbuch E, Mazaki-Tovi S, Kusanovic JP, et al. Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses. *J Perinat Med* 2010;38:495–502.
124. Romero R, Quintero R, Nores J, Avila C, Mazor M, Hanaoka S, et al. Amniotic fluid white blood cell count: a rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery. *Am J Obstet Gynecol* 1991;165:821–30.
125. Romero R, Jimenez C, Lohda AK, Nores J, Hanaoka S, Avila C, et al. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. *Am J Obstet Gynecol* 1990;163:968–74.
126. Romero R, Emamian M, Quintero R, Wan M, Hobbins JC, Mazor M, et al. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. *Am J Obstet Gynecol* 1988;159:114–9.
127. Romero R, Miranda J, Chaemsathong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2015;28:1394–409.
128. Eckert DJ, Sampath R, Li H, Massire C, Matthews HE, Toleno D, et al. New technology for rapid molecular diagnosis of bloodstream infections. *Expert Rev Mol Diagn* 2010;10:399–415.
129. Metzgar D, Frinder M, Lovari R, Toleno D, Massire C, Blyn LB, et al. Broad-spectrum biosensor capable of detecting and identifying diverse bacterial and *Candida* species in blood. *J Clin Microbiol* 2013;51:2670–8.
130. A broad range of tests to meet your needs. Available from: www.athogen.com/consulting-services/microbial-tests.html.
131. Legoff J, Feghoul L, Mercier-Delarue S, Dalle JH, Scieux C, Cherot J, et al. Broad-range PCR-electrospray ionization mass spectrometry for detection and typing of adenovirus and other opportunistic viruses in stem cell transplant patients. *J Clin Microbiol* 2013;51:4186–92.
132. Romero R, Kadar N, Miranda J, Korzeniewski SJ, Schwartz AG, Chaemsathong P, et al. The diagnostic performance of the Mass Restricted (MR) score in the identification of microbial invasion of the amniotic cavity or intra-amniotic inflammation is not

- superior to amniotic fluid interleukin-6. *J Matern Fetal Neonatal Med* 2014;27:757–69.
133. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol* 2003;6:435–48.
 134. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation—a workshop report. *Placenta* 2005;26 (A Suppl):S114–7.
 135. Redline RW. Placental pathology: a systematic approach with clinical correlations. *Placenta* 2008;29 (A Suppl):S86–91.
 136. Kim CJ, Romero R, Chaemsaitong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol* 2015; 213 (4 Suppl):S29–52.
 137. Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, et al. Sampling and definitions of placental lesions: amsterdam placental workshop Group Consensus Statement. *Arch Pathol Lab Med* 2016;140:698–713.
 138. Romero R, Kim YM, Pacora P, Kim CJ, Benshalom-Tirosh N, Jaiman S, et al. The frequency and type of placental histologic lesions in term pregnancies with normal outcome. *J Perinat Med* 2018;46:613–30.
 139. Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, et al. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. *Mod Pathol* 2010;23:1000–11.
 140. Ford-Martin P. *Gale encyclopedia of medicine*. Detroit: The Gale Group Inc.; 2004.
 141. Dani A. Colonization and infection. *Cent European J Urol* 2014; 67:86–7.
 142. Rehbindler EM, Lodrup Carlsen KC, Staff AC, Angell IL, Landro L, Hilde K, et al. Is amniotic fluid of women with uncomplicated term pregnancies free of bacteria? *Am J Obstet Gynecol* 2018; 219:289.e1–289.e12.
 143. Burnham P, Gomez-Lopez N, Heyang M, Cheng AP, Lenz JS, Dadhania DM, et al. Separating the signal from the noise in metagenomic cell-free DNA sequencing. *Microbiome* 2020;8:18.
 144. Gravett MG, Adams KM, Sadowsky DW, Grosvenor AR, Witkin SS, Axthelm MK, et al. Immunomodulators plus antibiotics delay preterm delivery after experimental intraamniotic infection in a nonhuman primate model. *Am J Obstet Gynecol* 2007;197: 518.e1–8.
 145. Novy MJ, Duffy L, Axthelm MK, Sadowsky DW, Witkin SS, Gravett MG, et al. *Ureaplasma parvum* or *Mycoplasma hominis* as sole pathogens cause chorioamnionitis, preterm delivery, and fetal pneumonia in rhesus macaques. *Reprod Sci* 2009;16:56–70.
 146. Grigsby PL, Novy MJ, Sadowsky DW, Morgan TK, Long M, Acosta E, et al. Maternal azithromycin therapy for *Ureaplasma* intraamniotic infection delays preterm delivery and reduces fetal lung injury in a primate model. *Am J Obstet Gynecol* 2012; 207:475.e1–14.
 147. Mazor M, Chaim W, Shinwell ES, Glezerman M. Asymptomatic amniotic fluid invasion with *Candida albicans* in preterm premature rupture of membranes. Implications for obstetric and neonatal management. *Acta Obstet Gynecol Scand* 1993;72: 52–4.
 148. Mandar R, Li K, Ehrenberg A, Smidt I, Raukas E, Kask V, et al. Amniotic fluid microflora in asymptomatic women at mid-gestation. *Scand J Infect Dis* 2001;33:60–2.
 149. Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. Prevalence of viral DNA in amniotic fluid of low-risk pregnancies in the second trimester. *J Matern Fetal Neonatal Med* 2003;13: 381–4.
 150. Miller JL, Harman C, Weiner C, Baschat AA. Perinatal outcomes after second trimester detection of amniotic fluid viral genome in asymptomatic patients. *J Perinat Med* 2009;37:140–3.
 151. Yoon BH, Romero R, Park JS, Chang JW, Kim YA, Kim JC, et al. Microbial invasion of the amniotic cavity with *Ureaplasma urealyticum* is associated with a robust host response in fetal, amniotic, and maternal compartments. *Am J Obstet Gynecol* 1998;179:1254–60.
 152. Yoon BH, Romero R, Kim M, Kim EC, Kim T, Park JS, et al. Clinical implications of detection of *Ureaplasma urealyticum* in the amniotic cavity with the polymerase chain reaction. *Am J Obstet Gynecol* 2000;183:1130–7.
 153. Payne MS, Kemp MW, Kallapur SG, Kannan PS, Saito M, Miura Y, et al. Intrauterine *Candida albicans* infection elicits severe inflammation in fetal sheep. *Pediatr Res* 2014;75:716–22.
 154. Yoneda N, Yoneda S, Niimi H, Ueno T, Hayashi S, Ito M, et al. Polymicrobial amniotic fluid infection with mycoplasma/ureaplasma and other bacteria induces severe intra-amniotic inflammation associated with poor perinatal prognosis in preterm labor. *Am J Reprod Immunol* 2016;75:112–25.
 155. Sweeney EL, Kallapur SG, Meawad S, Gisslen T, Stephenson SA, Jobe AH, et al. *Ureaplasma* species multiple banded Antigen (MBA) variation is associated with the Severity of inflammation in vivo and in vitro in human placentae. *Front Cell Infect Microbiol* 2017;7:123.
 156. Revello R, Alcaide MJ, Abehsera D, Martin-Camean M, Sousa EFGM, Alonso-Luque B, et al. Prediction of chorioamnionitis in cases of intraamniotic infection by *Ureaplasma urealyticum* in women with very preterm premature rupture of membranes or preterm labour. *J Matern Fetal Neonatal Med* 2018;31: 1839–44.
 157. Oh KJ, Romero R, Park JY, Hong JS, Yoon BH. The earlier the gestational age, the greater the intensity of the intra-amniotic inflammatory response in women with preterm premature rupture of membranes and amniotic fluid infection by *Ureaplasma* species. *J Perinat Med* 2019;47:516–27.
 158. Jacobsson B, Aaltonen R, Rantakokko-Jalava K, Morken NH, Alanen A. Quantification of *Ureaplasma urealyticum* DNA in the amniotic fluid from patients in PTL and pPROM and its relation to inflammatory cytokine levels. *Acta Obstet Gynecol Scand* 2009; 88:63–70.
 159. Allen-Daniels MJ, Serrano MG, Pflugner LP, Fettweis JM, Prestosa MA, Koparde VN, et al. Identification of a gene in *Mycoplasma hominis* associated with preterm birth and microbial burden in intraamniotic infection. *Am J Obstet Gynecol* 2015;212:779.e1–13.
 160. Theis KR, Romero R, Motomura K, Galaz J, Winters AD, Pacora P, et al. Microbial burden and inflammasome activation in amniotic fluid of patients with preterm prelabor rupture of membranes. *J Perinat Med* 2020;48:115–31.
 161. McCall CE, Yoza B, Liu T, El Gazzar M. Gene-specific epigenetic regulation in serious infections with systemic inflammation. *J Innate Immun* 2010;2:395–405.
 162. Dargere S, Cormier H, Verdon R. Contaminants in blood cultures: importance, implications, interpretation and prevention. *Clin Microbiol Infect* 2018;24:964–9.

163. DiGiulio DB. Diversity of microbes in amniotic fluid. *Semin Fetal Neonatal Med* 2012;17:2–11.
164. Urushiyama D, Suda W, Ohnishi E, Araki R, Kiyoshima C, Kurakazu M, et al. Microbiome profile of the amniotic fluid as a predictive biomarker of perinatal outcome. *Sci Rep* 2017;7: 017–11699.
165. Willerslev E, Hansen AJ, Poinar HN. Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends Ecol Evol* 2004; 19:141–7.
166. Witt N, Rodger G, Vandesompele J, Benes V, Zumla A, Rook GA, et al. An assessment of air as a source of DNA contamination encountered when performing PCR. *J Biomol Tech* 2009;20: 236–40.
167. Motley ST, Picuri JM, Crowder CD, Minich JJ, Hofstadler SA, Eshoo MW. Improved multiple displacement amplification (MDA) and ultraclean reagents. *BMC Genom* 2014;15:443.
168. Dunn RR, Fierer N, Henley JB, Leff JW, Menninger HL. Home life: factors structuring the bacterial diversity found within and between homes. *PLoS One* 2013;8:e64133.
169. Naccache SN, Greninger AL, Lee D, Coffey LL, Phan T, Rein-Weston A, et al. The perils of pathogen discovery: origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. *J Virol* 2013;87:11966–77.
170. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol* 2014; 12:12.
171. Lauder AP, Roche AM, Sherrill-Mix S, Bailey A, Laughlin AL, Bittinger K, et al. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome* 2016;4:29.
172. Glassing A, Dowd SE, Galanduk S, Davis B, Chiodini RJ. Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. *Gut Pathog* 2016;8:12.
173. Weyrich LS, Duchene S, Soubrier J, Arriola L, Llamas B, Breen J, et al. Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. *Nature* 2017;544:357–61.
174. McFeters GA, Broadaway SC, Pyle BH, Egozy Y. Distribution of bacteria within operating laboratory water purification systems. *Appl Environ Microbiol* 1993;59:1410–5.
175. Tanner MA, Goebel BM, Dojka MA, Pace NR. Specific ribosomal DNA sequences from diverse environmental settings correlate with experimental contaminants. *Appl Environ Microbiol* 1998; 64:3110–3.
176. Nogami T, Ohto T, Kawaguchi O, Zaitzu Y, Sasaki S. Estimation of bacterial contamination in ultrapure water: application of the anti-DNA antibody. *Anal Chem* 1998;70:5296–301.
177. McAlister MB, Kulakov LA, O'Hanlon JF, Larkin MJ, Ogden KL. Survival and nutritional requirements of three bacteria isolated from ultrapure water. *J Ind Microbiol Biotechnol* 2002;29:75–82.
178. Grah N, Olofsson M, Ellnebo-Svedlund K, Monstein HJ, Jonasson J. Identification of mixed bacterial DNA contamination in broad-range PCR amplification of 16S rDNA V1 and V3 variable regions by pyrosequencing of cloned amplicons. *FEMS Microbiol Lett* 2003;219:87–91.
179. Barton HA, Taylor NM, Lubbers BR, Pemberton AC. DNA extraction from low-biomass carbonate rock: an improved method with reduced contamination and the low-biomass contaminant database. *J Microbiol Methods* 2006;66:21–31.
180. Shen H, Rogelj S, Kieft TL. Sensitive, real-time PCR detects low-levels of contamination by *Legionella pneumophila* in commercial reagents. *Mol Cell Probes* 2006;20:147–53.
181. Seitz V, Schaper S, Droge A, Lenze D, Hummel M, Hennig S. A new method to prevent carry-over contaminations in two-step PCR NGS library preparations. *Nucleic Acids Res* 2015;43: e135.
182. Ballenghien M, Faivre N, Galtier N. Patterns of cross-contamination in a multispecies population genomic project: detection, quantification, impact, and solutions. *BMC Biol* 2017; 15:25.
183. Theis KR, Romero R, Winters AD, Greenberg JM, Gomez-Lopez N, Alhousseini A, et al. Does the human placenta delivered at term have a microbiota? Results of cultivation, quantitative real-time PCR, 16S rRNA gene sequencing, and metagenomics. *Am J Obstet Gynecol* 2019;220:267.e1–39.
184. Kim D, Hofstaedter CE, Zhao C, Mattei L, Tanes C, Clarke E, et al. Optimizing methods and dodging pitfalls in microbiome research. *Microbiome* 2017;5:52.
185. Brook I, Edith HF. Infections caused by *Propionibacterium* species. *Rev Infect Dis* 1991;13:819–22.
186. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep* 2016; 6:13.
187. Portillo ME, Corvec S, Borens O, Trampuz A. *Propionibacterium* acnes: an underestimated pathogen in implant-associated infections. *BioMed Res Int* 2013;2013:804391.
188. Gharamti AA, Kanafani ZA. *Cutibacterium* (formerly *Propionibacterium*) acnes infections associated with implantable devices. *Expert Rev Anti Infect Ther* 2017;15: 1083–94.
189. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in low microbial biomass microbiome studies: issues and recommendations. *Trends Microbiol* 2019; 27:105–17.
190. Weyrich LS, Farrer AG, Eisenhofer R, Arriola LA, Young J, Selway CA, et al. Laboratory contamination over time during low-biomass sample analysis. *Mol Ecol Resour* 2019;19:982–96.
191. Theis KR, Romero R, Greenberg JM, Winters AD, Garcia-Flores V, Motomura K, et al. No consistent evidence for microbiota in murine placental and fetal tissues. *mSphere* 2020;5. <https://doi.org/10.1128/mSphere.00933-19>.
192. Denton M, Rajgopal A, Mooney L, Qureshi A, Kerr KG, Keer V, et al. *Stenotrophomonas maltophilia* contamination of nebulizers used to deliver aerosolized therapy to inpatients with cystic fibrosis. *J Hosp Infect* 2003;55:180–3.
193. Furuhashi K, Ishizaki N, Fukuyama M. Bacterial contamination in cold water samples obtained from water dispensers. *Biocontrol Sci* 2015;20:147–51.
194. Gomila M, Gascó J, Busquets A, Gil J, Bernabeu R, Buades JM, et al. Identification of culturable bacteria present in haemodialysis water and fluid. *FEMS Microbiol Ecol* 2005;52: 101–14.
195. Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M. Reservoirs of non-baumannii *Acinetobacter* species. *Front Microbiol* 2016;7: 49.
196. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp: an overview. *Microbes Environ* 2011;26:101–12.

197. Shimose LA, Doi Y, Bonomo RA, De Pascale D, Viau RA, Cleary T, et al. Contamination of Ambient air with *Acinetobacter baumannii* on Consecutive inpatient days. *J Clin Microbiol* 2015; 53:2346–8.
198. Shete VB, Ghadage DP, Muley VA, Bhore AV. *Acinetobacter* septicemia in neonates admitted to intensive care units. *J Lab Physicians* 2009;1:73–6.
199. Mittal S, Sharma M, Yadav A, Bala K, Chaudhary U. *Acinetobacter lwoffii* an emerging pathogen in neonatal ICU. *Infect Disord Drug Targets* 2015;15:184–8.
200. Gramatniece A, Silamikelis I, Zahare I, Urtans V, Zahare I, Dimina E, et al. Control of *Acinetobacter baumannii* outbreak in the neonatal intensive care unit in Latvia: whole-genome sequencing powered investigation and closure of the ward. *Antimicrob Resist Infect Contr* 2019;8:84.
201. Aivazova V, Kainer F, Friesse K, Mylonas I. *Acinetobacter baumannii* infection during pregnancy and puerperium. *Arch Gynecol Obstet* 2010;281:171–4.
202. He M, Kostadinov S, Gundogan F, Struminsky J, Pinar H, Sung CJ. Pregnancy and perinatal outcomes associated with *Acinetobacter baumannii* infection. *AJP Rep* 2013;3:51–6.
203. Marques AR. Laboratory diagnosis of Lyme disease: advances and challenges. *Infect Dis Clin North Am* 2015;29:295–307.
204. Talebi Bezmin Abadi A. Diagnosis of *Helicobacter pylori* using invasive and noninvasive approaches. *J Pathog* 2018;2018: 9064952.
205. Shute KM, Kimber RG. *Haemophilus influenzae* intra-amniotic infection with intact membranes. *J Am Board Fam Pract* 1994;7: 335–41.
206. Ben-David Y, Hallak M, Evans MI, Abramovici H. Amnionitis and premature delivery with intact amniotic membranes involving *Staphylococcus aureus*. A case report. *J Reprod Med* 1995;40: 485–6.
207. Jalava J, Mantymaa ML, Ekblad U, Toivanen P, Skurnik M, Lassila O, et al. Bacterial 16S rDNA polymerase chain reaction in the detection of intra-amniotic infection. *Br J Obstet Gynaecol* 1996; 103:664–9.
208. Negishi H, Matsuda T, Okuyama K, Sutoh S, Fujioka Y, Fujimoto S. *Staphylococcus aureus* causing chorioamnionitis and fetal death with intact membranes at term. A case report. *J Reprod Med* 1998; 43:397–400.
209. Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of *Ureaplasma urealyticum* in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. *J Infect Dis* 2003;187:518–21.
210. Perni SC, Vardhana S, Korneeva I, Tuttle SL, Paraskevas LR, Chasen ST, et al. *Mycoplasma hominis* and *Ureaplasma urealyticum* in midtrimester amniotic fluid: association with amniotic fluid cytokine levels and pregnancy outcome. *Am J Obstet Gynecol* 2004;191:1382–6.
211. 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.
212. Musilova I, Pliskova L, Kutova R, Jacobsson B, Paterova P, Kacerovsky M. *Streptococcus agalactiae* in pregnancies complicated by preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2016;29:1036–40.
213. Ouseph MM, Krigman H, He M. *Streptococcus pneumoniae*-an uncommon but noteworthy cause of intrauterine fetal demise and acute necrotizing funisitis. *Fetal Pediatr Pathol* 2019;38: 352–8.
214. Kass EH. Bacteriuria and the diagnosis of infections of the urinary tract; with observations on the use of methionine as a urinary antiseptic. *AMA Arch Intern Med* 1957;100: 709–14.
215. Romero R, Yoon BH, Mazor M, Gomez R, Gonzalez R, Diamond MP, et al. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and Gram stain in the detection of microbial invasion in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* 1993;169:839–51.
216. Spong CY, Ghidini A, Ossandon M, Walker CN, Pezzullo JC. Are the cytokines interleukin-6 and angiogenin stable in frozen amniotic fluid?. *Am J Obstet Gynecol* 1998;178:783–6.
217. Porter AE, Auth J, Prince M, Ghidini A, Brenneman DE, Spong CY. Optimization of cytokine stability in stored amniotic fluid. *Am J Obstet Gynecol* 2001;185:459–62.