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ORIGINAL ARTICLE

A new anti-microbial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM

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Abstract

Objective: Antibiotic administration is a standard practice in preterm premature rupture of membranes (PROM). Specific anti-microbial agents often include ampicillin and/or erythromycin. Anaerobes and genital mycoplasmas are frequently involved in preterm PROM, but are not adequately covered by antibiotics routinely used in clinical practice. Our objective was to compare outcomes of PROM treated with standard antibiotic administration versus a new combination more effective against these bacteria.

Study design: A retrospective study compared perinatal outcomes in 314 patients with PROM <34 weeks receiving anti-microbial regimen 1 (ampicillin and/or cephalosporins; $n = 195$, 1993–2003) versus regimen 2 (ceftriaxone, clarithromycin and metronidazole; $n = 119$, 2003–2012). Intra-amniotic infection/inflammation was assessed by positive amniotic fluid culture and/or an elevated amniotic fluid MMP-8 concentration (>23 ng/mL).

Results: (1) Patients treated with regimen 2 had a longer median antibiotic-to-delivery interval than those with regimen 1 [median (interquartile range) 23 d (10–51 d) versus 12 d (5–52 d), $p < 0.01$]; (2) patients who received regimen 2 had lower rates of acute histologic chorioamnionitis (50.5% versus 66.7%, $p < 0.05$) and funisitis (13.9% versus 42.9%, $p < 0.001$) than those who had received regimen 1; (3) the rates of intra-ventricular hemorrhage (IVH) and cerebral palsy (CP) were significantly lower in patients allocated to regimen 2 than regimen 1 (IVH: 2.1% versus 19.0%, $p < 0.001$ and CP: 0% versus 5.7%, $p < 0.05$); and (4) subgroup analysis showed that regimen 2 improved perinatal outcomes in pregnancies with intra-amniotic infection/inflammation, but not in those without intra-amniotic infection/inflammation (after adjusting for gestational age and antenatal corticosteroid administration).

Conclusion: A new antibiotic combination consisting of ceftriaxone, clarithromycin, and metronidazole prolonged the latency period, reduced acute histologic chorioamnionitis/funisitis, and improved neonatal outcomes in patients with preterm PROM. These findings suggest that the combination of anti-microbial agents (ceftriaxone, clarithromycin, and metronidazole) may improve perinatal outcome in preterm PROM.

Keywords

Ceftriaxone, cerebral palsy, clarithromycin, intra-amniotic infection, intra-amniotic inflammation, intra-ventricular hemorrhage, metronidazole, preterm birth

History

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Introduction

Preterm premature rupture of membranes (PROM) complicates 2% of all pregnancies [1–5] and accounts for approximately one-third of all cases of preterm birth [6]. Preterm PROM has been the subject of several clinical and epidemiologic studies [7–25], and is considered one of the ''great obstetrical syndromes'' [26–28] responsible for spontaneous preterm birth [26,29,30]. Microbial invasion of the amniotic cavity (MIAC) is detected in approximately 30% of affected patients when using cultivation techniques [31–54], and in 50% when using a combination of cultivation and molecular techniques [55–60]. Studies in which serial

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amniocenteses have been performed in patients with preterm PROM indicate that the prevalence of MIAC increases over time, and reaches approximately 75% when patients with preterm PROM begin preterm labor [36]. Thus, infection of the amniotic cavity seems to play a role in both the genesis of preterm PROM [31–64] and the onset of preterm labor [36]. Hence, the standard of care is to administer antibiotics to patients with preterm PROM with the goal of treating or preventing ascending infection [1,65–81].

Randomized clinical trials [82–96], as well as several systematic reviews and meta-analyses [2,97–100], show that anti-microbial agents can prolong the latency period [2,83–89,91–94,97,100] and reduce the rates of clinical chorioamnionitis [2,22,83,86,89,90,92,94,97], neonatal morbidity (including neonatal infection) [2,82–84,86,90–92,94,97,98], major cerebral abnormalities identified by ultrasound, [2,83,92,94] and the need for surfactant administration and oxygen therapy [2,84,92,94]. While several antibiotic regimens have been proposed for preterm PROM, the combination of ampicillin/amoxicillin with erythromycin is among the most widely used [92,101]. Yet, a long-term follow-up study of infants included in the ORACLE trial showed that there was no demonstrable benefit or harm at age 7 with antibiotic administration to patients with preterm PROM [102]. Thus, questions remain about the optimal care for women presenting with preterm PROM.

Ampicillin and erythromycin are inadequate in eradicating many of the organisms detected in preterm PROM. This has been attributed to the limited transplacental passage, and hence suboptimal anti-microbial activity in the amniotic fluid (only 3% of erythromycin [103,104] and 2.6% of azithromycin [105] cross the placenta). Clarithromycin, a semi-synthetic macrolide, on the other hand, has a much higher rate of transplacental passage than erythromycin, and is effective for the treatment of genital mycoplasmas [106,107]. This has led to the proposal that clarithromycin could be useful in the setting of preterm PROM [106]. Other anti-microbial agents that might be helpful include metronidazole, which is particularly effective against anaerobic bacteria [108–110] involved in bacterial vaginosis [111–139] and preterm PROM [55,57,119,140–142], and ceftriaxone, which has a long half-life and has enhanced coverage for Gram-negative bacteria [143–145].

Based on our previous studies about the microbiology of preterm PROM, the reports about the enhanced placental transfer of clarithromycin [106,107], the difficulty in eradicating *Ureaplasmas* [43,57,171–180], with erythromycin [146–150], and evidence that frequently used anti-microbial agents do not eradicate or prevent MIAC in preterm PROM [151], we changed the clinical management of preterm PROM at our center to include the use of a combination of ceftriaxone, clarithromycin and metronidazole. Herein, we explore whether women who presented with preterm PROM after this change in practice had better or worse pregnancy outcomes than those who received the prior regimen (ampicillin or cephalosporins were the most commonly used).

Materials and methods

Study design

This was a retrospective cohort study which included women with singleton gestations admitted to the Seoul National University Hospital between January 1993 and June 2012 with the diagnosis of preterm PROM (<34 weeks of gestation). Amniocentesis is routinely offered for microbiologic studies and assessment of fetal lung maturity to all patients with the diagnosis of preterm PROM. The inclusion criteria were: (1) antenatal antibiotic treatment for at least 24 h, (2) availability of perinatal outcomes, and (3) availability of amniotic fluid obtained by transabdominal amniocentesis for microbiologic studies. Amniocenteses were performed after written informed consent was obtained. Rupture of membranes (ROM) was diagnosed by a previous history of watery vaginal discharge and a combination of the following tests: confirming leakage of amniotic fluid from cervical os, vaginal pooling of amniotic fluid, and a positive nitrazine test through a sterile speculum examination. The Institutional Review Board of the Seoul National University Hospital approved the collection and the use of samples and clinical information for research purposes. The Seoul National University Hospital has a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

Antibiotics

Between January 1993 and August 2003, patients ($n = 195$) received ampicillin and/or cephalosporins. Some patients ($n = 33$) received erythromycin ($n = 23$), metronidazole ($n = 15$), azithromycin ($n = 8$) or gentamicin ($n = 1$) in addition to ampicillin or cephalosporins [this regimen will be considered number 1 for the purpose of this manuscript ($n = 195$)]. Between September 2003 and June 2012, the anti-microbial regimen included ceftriaxone 1 g (intravenous) every 24 h, clarithromycin 500 mg (oral) every 12 h, and metronidazole (intravenous) 500 mg every 8 h, routinely administered to the patients with preterm PROM [regimen 2 ($n = 119$)]. Antibiotics were administered until the patient delivered or there was no evidence of amniotic fluid leakage, with the exception of metronidazole, which was administered for a maximum of 4 weeks. The study population was divided into two groups according to the antibiotic regimen (1 versus 2). Group B streptococcus (GBS) screening and intra-partum treatment has never been used in our institution because neonatal GBS sepsis is extremely rare.

Amniotic fluid

Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas using methods previously described [40,152–154]. An aliquot of amniotic fluid was examined in a hemocytometer chamber to determine the white blood cell count. Amniotic fluid not used for diagnostic tests was centrifuged and stored at $-80\,^{\circ}C$.

Diagnosis of intra-amniotic infection/inflammation, acute histologic chorioamnionitis, funisitis, and perinatal outcomes

Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 (MMP-8) concentration (>23 ng/mL), as previously reported [43,155–161]. The concentration of MMP-8 was measured in stored

amniotic fluid using a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Bucks, UK), according to the manufacturer's instructions. The sensitivity of the test is 0.3 ng/mL while the intra- and inter-assay coefficients of variation are less than 10%. Intra-amniotic infection/inflammation was defined as a positive amniotic fluid culture and/or an elevated amniotic fluid MMP-8 concentration (>23 ng/mL) [43,155–161].

The diagnosis of acute histologic chorioamnionitis was made on the basis of the presence of acute inflammatory changes in the examination of the extra-placental chorioamniotic membrane roll and/or chorionic plate of the placenta [162–164]. Funisitis was diagnosed as the neutrophil infiltration into the umbilical vessel walls or Wharton's jelly with the criteria reported previously [156,163–167]. Perinatal outcomes included pregnancy outcomes such as impending spontaneous preterm delivery and spontaneous preterm delivery, and neonatal outcomes such as respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), proven congenital neonatal sepsis, necrotizing enterocolitis (NEC), intra-ventricular hemorrhage (IVH), peri-ventricular leukomalacia (PVL), and cerebral palsy (CP). Briefly, RDS was diagnosed as the presence of respiratory distress, an increased oxygen requirement ($FiO_2 > 0.4$), and diagnostic radiological and laboratory findings in the absence of evidence of any other causes of respiratory distress [168]. BPD was diagnosed using the criteria of the National Institute of Child Health Workshop definition, i.e., the treatment with oxygen >21% for at least 28 d, and also diagnosed in the presence of typical findings at autopsy [154]. Congenital neonatal sepsis was diagnosed in the presence of a positive-blood culture result within 72 h of delivery [165]. NEC was diagnosed in the presence of abdominal distension and feeding intolerance (vomiting or increased gastric residual) for at least 24 h with clear evidence of intramural air, perforation, and meconium plug syndrome by radiological examination, or definite surgical or autopsy findings of NEC [162]. IVH was diagnosed by ultrasonographic examination or magnetic resonance imaging (MRI) of the neonatal head (≥Grade II) [169]. PVL was diagnosed as the presence of cystic lesions within the peri-ventricular white matter by ultrasonographic examination or MRI. CP was diagnosed in the presence of definite abnormalities on the neurodevelopmental assessment (i.e., abnormalities of developmental milestones, posture evaluated by the Vojta method and reflex) and persistent abnormalities of muscle tone [169,170]. Composite neonatal morbidity was defined when one or more neonatal outcomes including RDS, BPD, congenital neonatal sepsis, NEC, and IVH were diagnosed.

Statistical analysis

For continuous variables, median and interquartile were calculated and Mann–Whitney U tests were employed. For categorical variables, frequencies and percentages were calculated and the χ^2 test or Fisher's exact test were used. The generalized Wilcoxon test for survival analysis was used to compare the antibiotic-to-delivery interval between groups. Patients who were delivered because of maternal–fetal indications had their antibiotic-to-delivery interval considered as censored observations, with a censoring time equal to the antibiotic-to-delivery interval. To adjust for the gestational age at the time of the initiation of antibiotics therapy and intra-amniotic infection/inflammation, logistic regression analysis and Cox proportional hazards modeling were used. Subgroup analysis according to the presence or absence of intra-amniotic infection/inflammation was also conducted to evaluate the efficacy of prophylactic antibiotic administration in the absence of intra-amniotic infection/inflammation and therapeutic antibiotic use in the presence of intra-amniotic infection/inflammation. Statistical analyses were conducted using SPSS Version 19.0 (SPSS Inc., Chicago, IL). All p values were two-sided and a p value of <0.05 was considered statistically significant.

Results

Characteristics of the study population

Three hundred and fourteen patients with preterm PROM were included in this study. Table 1 shows the demographic and clinical characteristics of the study population. The median gestational age at the time of the initiation of antibiotic therapy and amniocentesis was lower in patients who received antibiotic regimen 2 (ceftriaxone, clarithromycin, and metronidazole) than in those who received regimen 1 ($p < 0.001$, for each). Patients who received regimen 2 had a higher rate of intra-amniotic infection/inflammation and a higher median amniotic fluid MMP-8 concentration than in those who received regimen 1 ($p < 0.001$ for each).

Pregnancy and neonatal outcomes

Patients treated with regimen 2 had a significantly longer median antibiotic-to-delivery interval than those who received regimen 1 [median (interquartile) 23 d (10–51 d) versus 12 d (5–52 d), $p < 0.01$] (see Figure 1). For this analysis, 99 patients who delivered because of maternal and/or fetal indications had this interval censored. Multivariate survival analysis demonstrated that the antibiotic-to-delivery interval in patients who received regimen 2 was significantly longer than in those treated with regimen 1 after adjusting for gestational age at the time of the initiation of antibiotics therapy, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (hazards ratio 0.69, 95% confidence interval (CI) 0.49–0.96; $p < 0.05$).

Table 2 shows pregnancy outcomes according to antibiotic regimen. The rate of spontaneous preterm delivery within 7 d after initiation of anti-microbial therapy was lower in patients who received regimen 2 than in those who received regimen 1 [20.2% (21/104) versus 43.6% (78/179), $p < 0.001$], which remained significant after adjusting for gestational age at the time of the initiation of antibiotic treatment, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (OR 0.40, 95% CI 0.21–0.77, $p < 0.01$). There was no difference in the rate of spontaneous preterm delivery before 34 weeks between the two groups. However, the rate of spontaneous preterm delivery before 32 weeks in patients who received regimen 2 was significantly lower than in those who received regimen 1 after adjusting for

Table 1. Demographics and clinical characteristics of the study population.

	Regimen 1 group* ($n = 195$)	Regimen 2 group† ($n = 119$)	p value
Maternal age (year)‡	30 (27–33)	32 (29–36)	<0.001
Nulliparity (%)	47.2 (92/195)	54.6 (65/119)	NS
Prior preterm birth (%)	14.9 (29/195)	11.8 (14/119)	NS
Amniotic fluid MMP-8 > 23 ng/mL (%)	41.0 (80/195)	61.3 (73/119)	<0.001
Positive amniotic fluid culture (%)	23.1 (45/195)	28.7 (33/115)	NS
Amniotic fluid MMP-8 (ng/mL)‡	7.1 (0.9–161.0)	46.3 (7.9–242.7)	<0.001
Intra-amniotic infection/inflammation (%)	45.6 (89/195)	66.4 (79/119)	<0.001
Gestational age at the time of the initiation of antibiotic treatment (weeks)‡	30.1 (27.1–32.6)	27.7 (23.4–31.6)	<0.001
Gestational age at amniocentesis (weeks)‡	30.3 (27.1–32.7)	28.0 (24.0–31.6)	<0.001
Antenatal corticosteroids administration (%)	71.5 (133/186)	84.0 (100/119)	<0.05

MMP-8, matrix metalloproteinase-8; NS, not significant.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole
‡Median (interquartile).

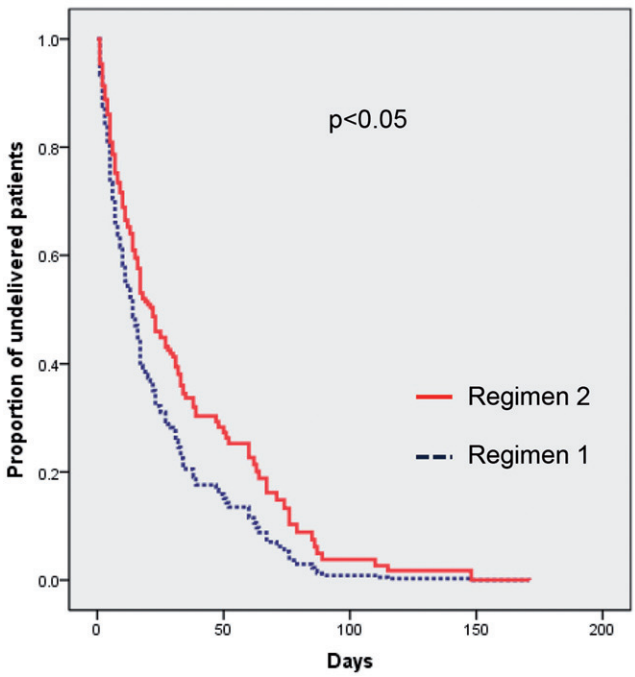

Figure 1. Survival analysis of the antibiotic-to-delivery interval according to the antibiotic regimen. Patients who received regimen 2 (ceftriaxone, clarithromycin, and metronidazole) had a significantly longer median antibiotics-to-delivery interval than those who received regimen 1 (ampicillin and/or cephalosporins, in some patients other antibiotics were combined with them.) [median (interquartile) 23 d (10–51 d) versus 12 d (5–52 d), $p < 0.01$], which remained significant after adjusting for gestational age at the time of the initiation of antibiotic therapy, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (hazards ratio 0.69, 95% (CI) 0.49–0.96; $p < 0.05$ by Cox proportional hazards model analysis).

confounding factors (OR 0.36, 95% CI 0.15–0.86, $p < 0.05$). Of note, acute histologic chorioamnionitis and funisitis were less frequent in patients treated with regimen 2 than in those treated with regimen 1 before and after controlling confounding factors (for acute histologic chorioamnionitis: OR 0.30, 95% CI 0.16–0.57; for funisitis OR 0.11; 95% CI 0.05–0.25) ($p < 0.001$, for each).

Table 3 displays neonatal outcomes according to antibiotic regimen. Patients who received regimen 2 had a lower rate of IVH than those who received regimen 1 after adjusting for confounding factors including gestational age at the time of the initiation of antibiotic treatment, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (OR 0.08, 95% CI 0.02–0.35, $p = 0.001$). The rates of PVL and composite neonatal morbidity in patients who received regimen 2 tend to be lower than in those receiving regimen 1 after adjusting for confounding factors (for PVL: OR 0.11, 95% CI 0.01–1.05, $p = 0.055$; for composite neonatal morbidity: OR 0.59, 95% CI 0.31–1.11, $p = 0.099$). There was no case of CP (0/96) among infants who had received regimen 2, while CP was diagnosed in 5.7% (9/157) of those whose mothers received regimen 1 ($p < 0.05$).

Intra-amniotic infection/inflammation and antibiotic regimen

As intra-amniotic infection/inflammation is one of the most important and well-established risk factors for adverse pregnancy and neonatal outcomes, subgroup analysis according to the presence or absence of intra-amniotic infection/inflammation was performed. Tables 4 and 5 show pregnancy and neonatal outcomes in patients with and without intra-amniotic infection/inflammation. Among patients without intra-amniotic infection/inflammation, there were no differences in outcomes between the two antibiotic regimens.

In contrast, among patients with intra-amniotic infection/inflammation, the rates of spontaneous preterm delivery within 14 d, 7 d, and 2 d after initiation of antibiotic treatment were significantly lower in those receiving regimen 2 than in those receiving regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration (for within 14 d: OR 0.17, 95% CI 0.07–0.41; for within 7 d: OR 0.22, 95% CI 0.09–0.50; for within 2 d: OR 0.24, 95% CI 0.08–0.69) ($p < 0.01$, for each). The rates of spontaneous preterm delivery before 34 weeks and 32 weeks of gestation were also lower in patients treated with regimen 2 than in those treated with regimen 1 after adjusting for confounding factors (before 34 weeks: OR 0.18, 95% CI 0.05–0.62; before 32 weeks: OR 0.11, 95% CI 0.03–0.44) ($p < 0.01$, for each). Acute histologic chorioamnionitis and funisitis were observed less frequently in patients receiving regimen 2 than in those receiving regimen 1 (for acute histologic chorioamnionitis: OR 0.08, 95% CI 0.03–

Table 2. Pregnancy outcomes of the study population according to the regimen of antibiotics used.

	Regimen 1 group* ($n = 195$)	Regimen 2 group† ($n = 119$)	p value	p value‡ and odds ratio (OR)
Gestational age at delivery (weeks)§	33.0 (29.9–34.6)	31.4 (27.0–33.9)	<0.001	
Cesarean delivery (%)	32.8 (62/189)	33.0 (38/115)	NS	
Birthweight (g)§	1970 (1380–2410)	1640 (980–2110)	<0.001	
Baby male (%)	62.0 (114/184)	59.6 (68/114)	NS	
Spontaneous preterm delivery (%)				
≤14 d	59.2 (100/169)	39.1 (36/92)	<0.01	<0.05 (OR 0.45)
≤7 d	43.6 (78/179)	20.2 (21/104)	<0.001	<0.01 (OR 0.40)
≤2 d	16.8 (32/190)	8.8 (10/114)	<0.05	NS (OR 0.58)
Spontaneous preterm delivery (%)				
<37 weeks	82.1 (128/156)	95.8 (69/72)	0.005	0.066 (OR 3.63)
<34 weeks	60.1 (101/168)	66.7 (58/87)	NS	NS (OR 0.74)
<32 weeks¶	57.0 (65/114)	56.9 (37/65)	NS	<0.05 (OR 0.36)
Acute histologic chorioamnionitis (%)	66.7 (102/153)	50.5 (49/97)	<0.05	<0.001 (OR 0.30)
Funisitis (%)	42.9 (66/154)	13.9 (14/101)	<0.001	<0.001 (OR 0.11)

NS, not significant.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole.
‡p value after adjusting for gestational age at the time of the initiation of antibiotic treatment, intra-amniotic infection/inflammation, and antenatal corticosteroids administration.
§Median (interquartile).
¶Among cases who were admitted due to preterm PROM before 32 weeks of gestation.

Table 3. Neonatal outcomes of the study population according to the regimen of antibiotics used.

	Regimen 1 group* ($n = 195$)	Regimen 2 group† ($n = 119$)	p value	p value‡ and odds ratio (OR) and 95% CI
Respiratory distress syndrome (%)	11.0 (19/173)	15.2 (15/99)	NS	NS (OR 0.64, 95% CI: 0.28–1.50)
Bronchopulmonary dysplasia (%)	12.7 (21/165)	28.1 (27/96)	<0.01	NS (OR 0.75, 95% CI: 0.31–1.77)
Congenital neonatal sepsis, proven (%)	4.6 (8/174)	1.0 (1/99)	NS	NS (OR 0.30, 95% CI: 0.03–2.52)
Intra-ventricular hemorrhage (%)	19.0 (32/168)	2.1 (2/97)	<0.001	0.001 (OR 0.08, 95% CI: 0.02–0.35)
Peri-ventricular leukomalacia (%)	6.1 (10/165)	1.0 (1/98)	0.058	0.055 (OR 0.11, 95% CI: 0.01–1.05)
Necrotizing enterocolitis (%)	3.5 (6/171)	9.2 (9/98)	0.051	NS (OR 1.96, 95% CI: 0.6–6.47)
Composite neonatal morbidity (%)	35.1 (60/171)	36.7 (36/98)	NS	0.099 (OR 0.59, 95% CI: 0.31–1.11)
Cerebral palsy (%)	5.7 (9/157)	0.0 (0/96)	<0.05	NA
Survival (%)	88.4 (160/181)	82.6 (95/115)	NS	NS (OR 2.01, 95% CI: 0.78–5.16)

NA, not applicable; NS, not significant; CI: confidence interval.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole.
‡p value after adjusting for gestational age at the time of the initiation of antibiotics treatment, intra-amniotic infection/inflammation, and antenatal corticosteroids administration.

0.27; for funisitis OR 0.08, 95% CI 0.03–0.20) ($p < 0.001$, for each). Patients who received antibiotic regimen 2 had lower rates of IVH, PVL, and composite neonatal morbidity and a higher survival rate than those who had received regimen 1 after adjusting for confounding factors, including gestational age at the time of the initiation antibiotic treatment and antenatal corticosteroid administration (for IVH: OR 0.06, 95% CI 0.01–0.31; for PVL: OR 0.07, 95% CI 95% CI 0.01–0.85; for composite neonatal morbidity: OR 0.24, 95% CI 0.01–0.62; for survival: OR 3.63, 95% CI 1.12–11.79) ($p < 0.05$, for each). There was no case of CP (0/59) in those who received regimen 2, while CP was diagnosed in 11.1% (7/63) of those who received regimen 1 ($p < 0.05$).

A significantly longer antibiotic-to-delivery interval was observed in patients with intra-amniotic infection/inflammation who received antibiotic regimen 2 than in those who received antibiotic regimen 1 (see Figure 2) [median (interquartile) 29 d (10–60 d) versus 5 d (2–14 d), $p < 0.001$], but not in patients without intra-amniotic infection/inflammation ($p > 0.4$). Multivariate survival analysis also demonstrated that the antibiotic-to-delivery interval in patients who received antibiotic regimen 2 was significantly longer than in those who received antibiotic regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration, only in the context of intra-amniotic infection/inflammation (hazards ratio 0.41, 95% CI 0.27–0.63; $p < 0.001$).

Discussion

Principal findings of this study

The administration of ceftriaxone, clarithromycin, and metronidazole (regimen 2), when compared to that of regimen 1 (mainly ampicillin and/or cephalosporins), in patients with preterm PROM was associated with: (1) a significantly longer latency period and lower rates of spontaneous preterm delivery within 7 d; (2) lower rates of acute histologic

Table 4. Perinatal outcomes of patients without intra-amniotic infection/inflammation according to the regimen of antibiotics used.

	Regimen 1 group* ($n = 106$)	Regimen 2 group† ($n = 40$)	p value	p value‡ and odds ratio (OR) and 95% CI
Gestational age at amniocentesis (weeks)§	31.4 (28.6–32.8)	31.2 (28.3–32.9)	NS	
Gestational age at the time of the initiation of antibiotics treatment (weeks)§	31.4 (28.5–32.8)	31.2 (28.3–32.9)	NS	
Gestational age at delivery (weeks)§	34.3 (32.9–37.2)	33.5 (31.8–34.1)	<0.01	
Spontaneous preterm delivery (%)				
<37 weeks	67.1 (57/85)	92.3 (24/26)	<0.05	<0.05 (OR 5.80, 95% CI: 1.16–29)
<34 weeks	37.1 (36/97)	48.5 (16/33)	NS	NS (OR 1.41, 95% CI: 0.62–3.20)
<32 weeks¶	23.2 (13/56)	28.6 (6/21)	NS	NS (OR 0.97, 95% CI: 0.29–3.24)
Spontaneous delivery (%)				
≤14 d	40.6 (39/96)	43.8 (14/32)	NS	NS (OR 1.33, 95% CI: 0.52–3.37)
≤7 d	29.0 (29/100)	25.0 (9/36)	NS	NS (OR 0.99, 95% CI: 0.37–2.62)
≤2 d	6.6 (7/106)	12.8 (5/39)	NS	0.076 (OR 3.96, 95% CI: 0.86-18.16)
Acute histologic chorioamnionitis (%)	44.6 (37/83)	38.2 (13/34)	NS	NS (OR 0.74, 95% CI: 0.32–1.74)
Funisitis (%)	22.6 (19/84)	11.1 (4/36)	NS	0.059 (OR 0.32, 95% CI: 0.10–1.05)
Respiratory distress syndrome (%)	8.2 (8/98)	10.5 (4/38)	NS	NS (OR 0.75, 95% CI: 0.18–3.15)
Bronchopulmonary dysplasia (%)	4.1 (4/97)	7.9 (3/38)	NS	NS (OR 1.11, 95% CI: 0.19–6.50)
Congenital neonatal sepsis, proven (%)	4.1 (4/98)	0.0 (0/38)	NS	NA
Intra-ventricular hemorrhage (%)	8.3 (8/96)	0.0 (0/37)	NS	NA
Peri-ventricular leukomalacia (%)	2.1 (2/96)	0.0 (0/38)	NS	NA
Necrotizing enterocolitis (%)	2.1 (2/97)	10.5 (4/38)	0.053	NS (OR 3.76, 95% CI: 0.64–22.09)
Composite neonatal morbidity (%)	18.4 (18/98)	23.7 (9/38)	NS	NS (OR 1.24, 95% CI: 0.48–3.22)
Cerebral palsy (%)	2.1 (2/94)	0.0 (0/37)	NS	NA
Survival (%)	96.0 (96/100)	94.9 (37/39)	NS	NS (OR 0.70, 95% CI: 0.10–5.02)

NA, not applicable; NS, not significant; CI: confidence interval.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole.
‡p value after adjusting for gestational age at the time of the initiation of antibiotics treatment and antenatal corticosteroids administration.
§Median (interquartile).
¶Among cases who were admitted due to preterm PROM before 32 weeks of gestation.

Table 5. Perinatal outcomes of patients with intra-amniotic infection/inflammation according to the regimen of antibiotics used.

	Regimen 1 group* ($n = 89$)	Regimen 2 group† ($n = 79$)	p value	p value‡ and odds ratio (OR) and 95% CI
Gestational age at amniocentesis (weeks)§	29.4 (26.5–32.0)	26.1 (23.0–28.9)	<0.001	
Gestational age at the time of the initiation of antibiotics treatment (weeks)§	28.9 (26.1–32.0)	25.1 (22.6–28.4)	<0.001	
Gestational age at delivery (weeks)§	30.4 (27.5–32.9)	28.6 (25.4–32.6)	NS	
Spontaneous preterm delivery (%)				
<37 weeks	100.0 (71/71)	97.8 (45/46)	NS	NA
<34 weeks	91.5 (65/71)	77.8 (42/54)	<0.05	<0.01 (OR 0.18, 95% CI: 0.05–0.62)
<32 weeks¶	89.7 (52/58)	70.5 (31/44)	<0.05	<0.01 (OR 0.11, 95% CI: 0.03–0.44)
Spontaneous delivery (%)				
≤14 d	83.6 (61/73)	36.7 (22/60)	<0.001	<0.001 (OR 0.17, 95% CI: 0.07–0.41)
≤7 d	62.0 (49/79)	17.6 (12/68)	<0.001	<0.001 (OR 0.22, 95% CI: 0.09–0.50)
≤2 d	29.8 (25/84)	6.7 (5/75)	<0.001	<0.01 (OR 0.24, 95% CI: 0.08–0.69)
Acute histologic chorioamnionitis (%)	92.9 (65/70)	57.1 (36/63)	<0.001	<0.001 (OR 0.08, 95% CI: 0.03–0.27)
Funisitis (%)	67.1 (47/70)	15.4 (10/65)	<0.001	<0.001 (OR 0.08, 95% CI: 0.03–0.20)
Respiratory distress syndrome (%)	14.7 (11/75)	18.0 (11/61)	NS	NS (OR 0.43, 95% CI: 0.14–1.36)
Bronchopulmonary dysplasia (%)	25.0 (17/68)	41.4 (24/58)	0.05	NS (OR 0.46, 95% CI: 0.16–1.36)
Congenital neonatal sepsis, proven (%)	5.3 (4/76)	1.6 (1/61)	NS	NS (OR 0.67, 95% CI: 0.06–7.95)
Intra-ventricular hemorrhage (%)	33.3 (24/72)	3.3 (2/60)	<0.001	0.001 (OR 0.06, 95% CI: 0.01–0.31)
Peri-ventricular leukomalacia (%)	11.6 (8/69)	1.7 (1/60)	<0.05	<0.05 (OR 0.07, 95% CI: 0.01–0.85)
Necrotizing enterocolitis (%)	5.4 (4/74)	8.3 (5/60)	NS	NS (OR 1.09, 95% CI: 0.22–5.52)
Composite neonatal morbidity (%)	57.5 (42/73)	45.0 (27/60)	NS	<0.01 (OR 0.24, 95% CI: 0.10–0.62)
Cerebral palsy (%)	11.1 (7/63)	0.0 (0/59)	<0.05	NA
Survival (%)	79.0 (64/81)	76.3 (58/76)	NS	<0.05 (OR 3.63, 95% CI: 1.12–11.79)

NA, not applicable; NS, not significant.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole.
‡p value after adjusting for gestational age at the time of the initiation of antibiotics treatment and antenatal corticosteroids administration.
§Median (interquartile).
¶Among cases who were admitted due to preterm PROM before 32 weeks of gestation,

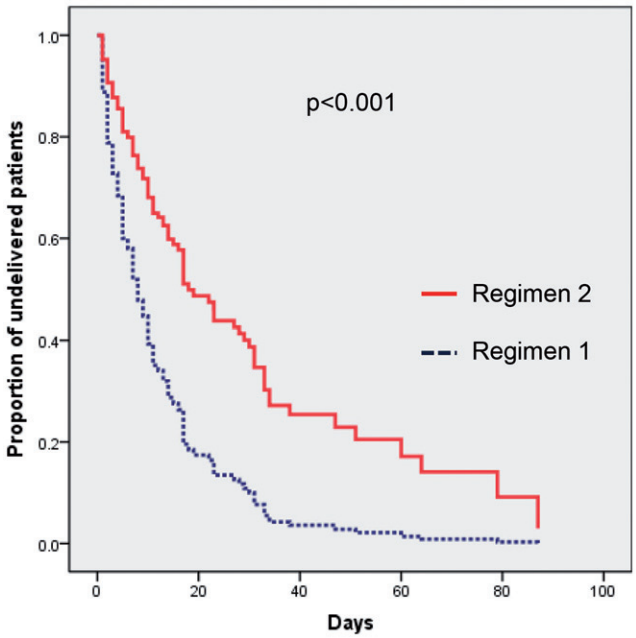

Figure 2. Survival analysis of the antibiotic-to-delivery interval according to the antibiotic regimen in patients with intra-amniotic infection/inflammation at the time of amniocentesis. A significant longer antibiotic-to-delivery interval was observed in patients with intra-amniotic infection/inflammation who received antibiotic regimen 2 (ceftriaxone, clarithromycin, and metronidazole) than in those who received antibiotic regimen 1 (ampicillin and/or cephalosporins, in some patients other antibiotics were combined with them) [median (interquartile) 29 d (10–60 d) versus 5 d (2–14 d), $p < 0.001$]. Multivariate survival analysis using the Cox proportional hazards model analysis also demonstrated the longer antibiotic-to-delivery interval in patients who received antibiotic regimen 2 than in those who received regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration (hazards ratio 0.41, 95% CI 0.27–0.63; $p < 0.001$).

chorioamnionitis and funisitis; and (3) lower rates of IVH and CP. Among patients with the diagnosis of intra-amniotic infection/inflammation, the administration of antibiotic regimen 2, but not regimen 1, was associated with a longer duration of the latency period, lower rates of spontaneous preterm delivery within 14 d, 7 d, and 48 h, acute histologic chorioamnionitis and funisitis, and improved neonatal outcome (IVH, PVL, composite neonatal morbidity and CP). However, this effect was not demonstrable in patients without evidence of intra-amniotic infection/inflammation.

Microorganisms involved in preterm PROM

Thirty to 50 percent of patients with preterm PROM have MIAC, detected using a combination of cultivation [31–54] and molecular microbiologic techniques [55–60]. The organisms involved included Tenericutes such as *Ureaplasma* [43,57,171–180], *Mycoplasma hominis* [45,57,171,178,181–185], Firmicutes (e.g. *Streptococcus agalactieae*, *GBS*, *Streptococcus salivarius*, *Streptococcus pneumonia*, *Enterococcus faecalis*, or *Lactobacillus species*), Fusobacteria (e.g. *Fusobacterium nucleatum*, *Sneathia sp.*), Bacteroidetes (e.g. *Bacteroides fragilis*, *Bacteroides sp.*, *Prevotella sp.*), Proteobacteria (*Haemophilus sp.*), Actinobacteria (e.g. *Gardnerella vaginalis*, *Bifidobacterium sp*, *Rothia sp.*), and Candida sp. [55,57,186]. Polymicrobial infections, which were present in 30% of cases, and fungi were found in 5% of cases [55,57]. Given the broad range of microorganisms involved and the difficulty in identifying the microorganism in most patients, broad anti-microbial coverage has been used in clinical trials, as well as in practice [101,187–189].

Antibiotic administration in patients with preterm PROM: randomized clinical trials, systematic reviews, and meta-analyses

A Cochrane review including 22 randomized controlled trials of antibiotic administration to patients with preterm PROM concluded that this intervention prolonged the latency period, neonatal infection, abnormal sonographic cerebral findings, reduced respiratory morbidities, and the need for oxygen and surfactant, and also reduced the frequency of clinical chorioamnionitis [2]. However, most patients comprising these meta-analyses came from two trials. The first, by Mercer et al. [92], used a combination of intravenous ampicillin and erythromycin for 2 d, followed by oral amoxicillin and erythromycin for 5 d. The second trial, by Kenyon et al. [94], used a factorial design with four treatment arms including oral anti-microbials for 10 d (the arms were: placebo, oral erythromycin, amoxicillin-clavulanic acid, or the combination of erythromycin and amoxicillin-clavulanic acid). Patients receiving erythromycin and/or amoxicillin-clavulanic acid remained undelivered at 48 h more frequently than patients in the placebo group. However, the use of amoxicillin-clavulanic acid did not yield additional benefits (i.e. reduction in neonatal morbidity) observed in patients allocated to receive erythromycin [94]. In the study of Kenyon et al., but not in that by Mercer et al., NEC was more frequent among patients receiving amoxicillin-clavulanic acid [94]. The findings of the study of Mercer et al. [92] and those of Kenyon et al. [94] have informed practice in the United States and Europe. A 7-year follow-up study of the ORACLE trial has not shown a benefit of antibiotic administration in terms of CP, behavioral problems, or childhood death [102]. Therefore, the benefits of antibiotic administration are demonstrable only in short-term outcomes so far.

The rationale for a new antibiotic regimen in preterm PROM

Isolated reports document that the eradication of MIAC in preterm PROM is possible [190–194]. However, in a study in which amniocentesis was performed before and after the administration of antibiotics, these agents did not eradicate or prevent subsequent intra-amniotic infection [151]. Potential explanations for these observations are that some antibiotics have poor transplacental passage (i.e. erythromycin) [103,104] and that infections may develop from organisms not adequately covered by the anti-microbials [146–150]. For example, a recent study indicated that 80% of genital mycoplasmas are resistant to erythromycin: this is largely the case for *Ureaplasma parvum*, the most common biovar in the amniotic fluid of our patients [195]. This was the impetus for considering a new antibiotic regimen in our practice. Oral clarithromycin was chosen because of its efficacy against

mycoplasmas and greater transplacental passage than erythromycin and azithromycin [103,104]. Indeed, clarithromycin has a lower minimal inhibitory concentration (MIC) for *Ureaplasma* than erythromycin [196,197] that has greater anti-microbial properties against *Ureaplasma* [198]. A previous study demonstrated that the bioavailability of clarithromycin after oral administration is sufficient for adequate anti-microbial activity [199,200]. Moreover, the concentration of an active metabolite, 14-hydroxyclarithromycin, in plasma is greater after oral administration than following intravenous infusion [199]. Intravenous metronidazole was included because of its powerful effect against anaerobic bacteria frequently present in preterm PROM [108,109]. Intravenous administration was chosen over oral administration, hoping to decrease the likelihood of gastrointestinal side effects associated with oral use [108]. A third-generation cephalosporin, intravenous ceftriaxone, was included to enhance coverage of aerobic organisms such as *Streptococcus sp.*, *Haemophilus sp*, and beta-lactamase-producing strains of *Hemophilus sp*. [143–145]. Moreover, ceftriaxone readily crosses the placenta and can be found in umbilical cord blood, amniotic fluid, and the placenta. In these biological fluids and tissue, the concentrations achieved are sufficient for anti-microbial effects to be obtained [201]. Antibiotics were administered until delivery, unless the patient remained undelivered for 4 weeks (in which case metronidazole was discontinued because of the concern for adverse events). The rationale for continuing antibiotics until delivery was that intra-amniotic infection can occur despite short-term antibiotic administration [151]. The practice at the Seoul National University Hospital had been to administer antibiotics from the time of preterm PROM until delivery. Thus, the main difference between regimens 1 and 2 was the spectrum of antibiotic coverage rather than the duration of treatment.

An expanded anti-microbial spectrum of treatment resulted in improved pregnancy and neonatal outcomes

The results of the current study indicated that the new antibiotic regimen was associated with a longer latency period, lower rates of spontaneous preterm delivery within 48 h, 7 d, and 14 d as well as improvement of neonatal outcomes and a reduction of the rates of acute histologic chorioamnionitis and funisitis. This benefit occurred in patients with demonstrable intra-amniotic infection/inflammation at the beginning of the therapy, but not in patients without this condition. Our findings are in contrast to those previously reported, indicating that a combination of amoxicillin and erythromycin administered for a short period of time did not reduce the frequency of acute histologic chorioamnionitis [96,202,203]. Of interest, in a randomized clinical trial in which the efficacy of cefazolin, cefazolin plus erythromycin, or cefazolin plus clarithromycin for 7 d was compared, the use of cefazolin with clarithromycin was associated with a reduction in the percentage of patients with severe funisitis. The lack of difference in neonatal outcomes in that study could be attributed to the sample size, as there were less than 36 patients per group [96].

A likely explanation for the beneficial effects of the new antibiotic regimen is the broad anti-microbial coverage. However, clarithromycin, which has immunomodulatory properties, has been used in experimental sepsis and acute pyelonephritis [204–210], and it was shown to inhibit the production of proinflammatory factors by human mononuclear cells [211–213]. The combination of ampicillin and erythromycin in patients with preterm PROM was previously shown to decrease the maternal concentration of granulocyte colony stimulating factor (G-CSF) but not of intercellular adhesion molecule (ICAM-1), interleukin-6 (IL-6), IL-10, and tumor necrosis factor-alpha (TNF-α) [189]. However, antibiotic administration did not decrease umbilical concentrations of cytokines or adhesion molecules [189]. Further studies are required to determine if the new anti-microbial combination can decrease the production of pro-inflammatory cytokines. The reduction in the frequency of funisitis suggests that this is likely to be the case.

Duration of anti-microbial therapy

The duration of anti-microbial therapy in preterm PROM has varied among studies [95,214,215]. Mercer et al. administered parenteral antibiotics for 2 d followed by oral administration for 5 d (total of 7 d) [92]. Kenyon et al. used the oral route for 10 d of treatment [94]. The results of these two studies are largely consistent on the short-term benefits for prolongation of the latency period, and reduction in neonatal infection rates.

The duration of anti-microbial therapy for most clinical infections is largely arbitrary and generally for 7 d. This has been the case for urinary tract infections [216–220], pyelonephritis [221–226], and endometritis [227–230], However, recent studies have shown that a shorter duration of antibiotics in pregnant and non-pregnant subjects has similar efficacy and potential benefit in terms of compliance and cost [231–234]. Therefore, investigators have attempted to address whether the benefits of anti-microbial therapy can be achieved with a shorter duration. Segel et al. [214] compared the efficacy of the antibiotic administration for 3 d versus 7 d of intravenous ampicillin in patients with preterm PROM between 24 and 33 weeks of gestation. For the first 48 h, all patients received parenteral ampicillin (2 g IV every 6 h), then were randomized to oral therapy with ampicillin 500 mg per oral every 6 h for 3 d or 7 d. There were no statistically significant differences in achieving a 7-d latency period (RR 0.83; 95% 0.51–1.38) or in the rate of clinical chorioamnionitis, endometritis, or composite neonatal morbidity [214]. Subsequently, Lewis et al. [95] reported the result of a prospective randomized trial in which patients were allocated to receive either 3 d or 7 d of ampicillin plus sulbactam (intravenous 3 g every 6 h for either 12 or 28 doses) in patients with preterm PROM (24–34 weeks; $n = 84$ patients). There were no differences in the latency period between the two groups or the frequency of neonatal complications [95]. Collectively, these studies raised the question of whether 7 d of treatment is required. However, the statistical power to detect the difference is a potential limitation.

The other question about the duration of anti-microbial therapy is whether longer treatment can result in a beneficial effect. If the goal of treatment is to control the growth of microorganisms in the amniotic cavity or to prevent secondary intra-amniotic infection, then a longer duration of therapy has

potential benefits. A previous study indicated that when treatment with an anti-microbial agent was administered for a total of 7 d (ampicillin and erythromycin) or 10–14 d (ceftriaxone, clindamycin, and erythromycin), microbial eradication did not occur in a large number of patients and inflammation developed in 32% of cases that did not receive it at the time of the first amniocentesis [151]. This raised the question of whether a longer duration of anti-microbial therapy may be more efficacious. The practice at the Seoul National University Hospital for the last 25 years has been to administer the anti-microbial agent until delivery has occurred rather than stopping at 7 d or 10 d. Therefore, a unique feature of the current study is that antibiotics were administered for an extended period of time much longer than that conventionally used in the United States or Europe. Since the duration of antibiotic treatment was similar for regimens 1 and 2, this could not account for differences observed in the outcome of this study. Exposure to antibiotics could be associated with a short-term individual effect or community-wide consequences such as the emergence of resistant organisms similar to those that have been identified after the widespread utilization of GBS prophylaxis [235–237]. Our hospital did not detect an increase in the frequency of resistant organisms in the newborn intensive care unit or in the newborns treated in this study.

Strengths and limitations

The strength of this study is that it is the only systematic examination of the effect of a new antibiotic regimen in preterm PROM at a single institution in which the anti-microbial agents were used for an extended period of time rather than a standard short-term administration. Moreover, we were able to assess the intra-amniotic inflammatory state of the patients enrolled in the study, which allowed us to detect that anti-microbial agents prolong pregnancy and improve neonatal outcomes in patients with (but not without) demonstrable intra-amniotic infection/inflammation.

Several limitations must be acknowledged. First, this was not a randomized controlled trial, which is the gold standard to test interventions in clinical medicine. Second, the use of a historical control group has the potential to introduce bias, particularly in outcomes that could change over time. This may affect some neonatal endpoints, but would not affect the duration of the latency period or the rate of acute histologic chorioamnionitis and funisitis. It is noteworthy that the median gestational age at the time of the administration of antibiotics to patients who received regimen 1 was significantly higher than that of patients who received regimen 2 (30.1 weeks versus 27.7 weeks; $p < 0.001$). Interestingly, the frequency of intra-amniotic infection/inflammation was also greater in patients who received regimen 2 than in those who received regimen 1.

It is difficult to determine which antibiotics among ceftriaxone, clarithromycin, and metronidazole were responsible for better perinatal outcomes in regimen 2 compared to regimen 1. However, the combination of those three antibiotics in the current study, having anti-microbial activity against most microorganisms found in the amniotic fluid of patients with preterm PROM and the greatest anti-microbial potency against genital mycoplasmas, could improve prenatal outcomes through the eradication of pre-existing intra-amniotic infection/inflammation, the prevention of *de novo* intra-amniotic infection/inflammation and the reduction of the frequency of funisitis, a histologic hallmark of fetal inflammatory response syndrome [166].

Conclusions

Antibiotic treatment with intravenous ceftriaxone, metronidazole, and oral clarithromycin (regimen 2) was associated with the prolongation of the duration of pregnancy after preterm PROM, reduce acute histologic chorioamnionitis and acute funisitis, as well as the rate of adverse neonatal outcomes compared to regimen 1. The findings herein justify further studies to determine the optimal anti-microbial regimen and duration of treatment in patients with preterm PROM.

Declaration of interest

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References

1. Mercer B. Antibiotics in the management of PROM and preterm labor. Obstet Gynecol Clin North Am 2012;39:65–76.
2. Kenyon S, Boulvain M, Neilson JP. Antibiotics for preterm rupture of membranes. Cochrane Database Syst Rev 2013;12:CD001058.
3. Parry S, Strauss III J. Premature rupture of the fetal membranes. N Engl J Med 1998;338:663–70.
4. Romero R, Yeo L, Gotsch F, et al. Prelabor rupture of the membranes. In: Winn H, Chervanak F, Romero R, eds. Clinical maternal-fetal medicine online, 2nd ed. London (UK): Informa Healthcare; 2011:1–24.
5. Santolaya-Forgas J, Romero R, Espinoza J, et al. Prelabour rupture of the membranes. In: Reece E, Hobbins J, eds. Clinical obstetrics the fetus & mothers, 3rd ed. Malden (MA): Blackwell; 2008:1130–88.
6. Goldenberg R, Culhane J, Iams J, et al. Epidemiology and causes of preterm birth. Lancet 2008;371:75–84.
7. Lebherz T, Hellman L, Madding R, et al. Double-blind study of premature rupture of the membranes: a report of 1,896 cases. Am J Obstet Gynecol 1963;87:218–25.
8. Sacks M, Baker TH. Spontaneous premature rupture of the membranes: a prospective study. Am J Obstet Gynecol 1967;97: 888–93.
9. Gunn G, Mishell Jr D, Morton DG. Premature rupture of the fetal membranes: a review. Am J Obstet Gynecol 1970;106:469–83.
10. Premature rupture of the membranes. Br Med J 1979;1:1165–6.
11. Gibbs R, Blanco JD. Premature rupture of the membranes. Obstet Gynecol 1982;60:671–9.
12. Ismail A, Lahiri S. Management of prelabour rupture of membranes (PROM) at term. J Perinat Med 2013;41:647–9.
13. Practice bulletins No. 139: premature rupture of membranes. Obstet Gynecol 2013;122:918–30.
14. Eschenbach D. Reply to: Ismail AQT, Lahiri S. Management of prelabor rupture of membranes (PROM) at term. J Perinat Med 2013;41:653–5.
15. Grunebaum A. Reply to ''Management of prelabour rupture of membranes (PROM) at term''. J Perinat Med 2013;41:651–2.

16. Ramsauer B, Vidaeff A, Hosli I, et al. The diagnosis of rupture of fetal membranes (ROM): a meta-analysis. J Perinat Med 2013;41: 233–40.
17. Gelber S, Brent E, Varrey A, et al. Equivalence of erythromycin and azithromycin for treatment of PPROM (abstract 690). Am J Obstet Gynecol 2013;208:S291.
18. Pintucci A, Meregalli V, Colombo P, et al. Premature rupture of membranes at term in low risk women: how long should we wait in the ''latent phase''? J Perinat Med 2014;42:189–96.
19. Wong L, Holmgren C, Silver R, et al. Outcomes of expectantly managed pregnancies with multiple gestations and preterm premature rupture of membranes prior to 26 weeks. Am J Obstet Gynecol 2015;212:215 e1–9.
20. Manuck T, Varner MW. Neonatal and early childhood outcomes following early vs later preterm premature rupture of membranes. Am J Obstet Gynecol 2014;211:308 e1–6.
21. Mehari K, Kneitel A, Langen E. Antibiotic administration in periviable premature rupture of membrane (abstract 532). Am J Obstet Gynecol 2014;210:S262.
22. Lee J, Kang M, Kim E, et al. Effect of triple antibiotics on amniotic fluid infection/inflammation: an inter-era comparison of 20 years in patients with preterm PROM (abstract 475). Am J Obstet Gynecol (Supplement) 2014;210:S238.
23. Saccone G, Berghella V. Antibiotic prophylaxis for term or near-term premature rupture of membranes: metaanalysis of randomized trials. Am J Obstet Gynecol 2014 Dec 30. [Epub ahead of print].
24. Kacerovsky M, Musilova I, Hornychova H, et al. Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes. Am J Obstet Gynecol 2014;211:385 e1–9.
25. Wong L, Holmgren C, Silver R, et al. Outcomes of expectantly managed pregnancies with multiple gestations and preterm premature rupture of membranes prior to 26 weeks. Am J Obstet Gynecol 2015;212:215 e211–19.
26. Romero R, Espinoza J, Kusanovic J, et al. The preterm parturition syndrome. BJOG 2006;113:17–42.
27. Romero R. Prenatal medicine: the child is the father of the man. 1996. J Matern Fetal Neonatal Med 2009;22:636–9.
28. Di Renzo GC. The great obstetrical syndromes. J Matern Fetal Neonatal Med 2009;22:633–5.
29. Romero R, Lockwood CJ. Pathogenesis of spontaneous preterm labor. In: Creasy R, Resnik R, Iams J, eds. Creasy and Resnik's maternal-fetal medicine: principles and practice. Philadelphia (PA): Elsevier; 2009:521–43.
30. Romero R, Dey S, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014;345:760–5.
31. Garite T, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol 1982;59:539–45.
32. Cotton D, Hill L, Strassner H, et al. Use of amniocentesis in preterm gestation with ruptured membranes. Obstet Gynecol 1984; 63:38–43.
33. Zlatnik F, Cruikshank D, Petzold C, et al. Amniocentesis in the identification of inapparent infection in preterm patients with premature rupture of the membranes. J Reprod Med 1984;29: 656–60.
34. Broekhuizen F, Gilman M, Hamilton PR. Amniocentesis for gram stain and culture in preterm premature rupture of the membranes. Obstet Gynecol 1985;66:316–21.
35. Feinstein S, Vintzileos A, Lodeiro J, et al. Amniocentesis with premature rupture of membranes. Obstet Gynecol 1986;68: 147–52.
36. Romero R, Quintero R, Oyarzun E, et al. Intraamniotic infection and the onset of labor in preterm premature rupture of the membranes. Am J Obstet Gynecol 1988;159:661–6.
37. Dudley J, Malcolm G, Ellwood D. Amniocentesis in the management of preterm premature rupture of the membranes. Aust NZ J Obstet Gynaecol 1991;31:331–6.
38. Romero R, Yoon B, Mazor M, 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.
39. Font G, Gauthier D, Meyer W, et al. Catalase activity as a predictor of amniotic fluid culture results in preterm labor or premature rupture of membranes. Obstet Gynecol 1995;85:656–8.
40. Yoon B, Jun J, Park K, et al. Serum C-reactive protein, white blood cell count, and amniotic fluid white blood cell count in women with preterm premature rupture of membranes. Obstet Gynecol 1996;88: 1034–40.
41. Blackwell S, Berry SM. Role of amniocentesis for the diagnosis of subclinical intra-amniotic infection in preterm premature rupture of the membranes. Curr Opin Obstet Gynecol 1999;11: 541–7.
42. Romero R, Espinoza J, Chaiworapongsa T, et al. Infection and prematurity and the role of preventive strategies. Semin Neonatol 2002;7:259–74.
43. Shim S, Romero R, Hong J, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol 2004;191:1339–45.
44. Romero R, Espinoza J, Goncalves L, et al. The role of inflammation and infection in preterm birth. Semin Reprod Med 2007;25:21–39.
45. Oh K, Lee K, Sohn Y, 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 e211–18.
46. Lee S, Romero R, Lee S, et al. Amniotic fluid volume in intra-amniotic inflammation with and without culture-proven amniotic fluid infection in preterm premature rupture of membranes. J Perinat Med 2010;38:39–44.
47. Cobo T, Palacio M, Martinez-Terron M, et al. Clinical and inflammatory markers in amniotic fluid as predictors of adverse outcomes in preterm premature rupture of membranes. Am J Obstet Gynecol 2011;205:126 e121–8.
48. Kacerovsky M, Musilova I, Khatibi A, 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;25:2014–19.
49. Kacerovsky M, Andrys C, Hornychova H, et al. Amniotic fluid soluble Toll-like receptor 4 in pregnancies complicated by preterm prelabor rupture of the membranes. J Matern Fetal Neonatal Med 2012;25:1148–55.
50. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. Semin Fetal Neonatal Med 2012;17:12–19.
51. Kacerovsky M, Andrys C, Drahosova M, et al. Soluble toll-like receptor 1 family members in the amniotic fluid of women with preterm prelabor rupture of the membranes. J Matern Fetal Neonatal Med 2012;25:1699–704.
52. Kacerovsky M, Cobo T, Andrys C, et al. The fetal inflammatory response in subgroups of women with preterm prelabor rupture of the membranes. J Matern Fetal Neonatal Med 2013;26:795–801.
53. Romero R, Chaemsaithong P, Korzeniewski S, et al. A point of care test for interleukin-6 in amniotic fluid in preterm prelabor rupture of membranes: a step toward the early treatment of acute intra-amniotic inflammation/infection. J Maternal Fetal Neonatal Med 2014 (submitted).
54. Combs C, Gravett M, Garite T, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol 2014;210:125 e121–5 e115.
55. DiGiulio D, Romero R, Kusanovic J, 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.
56. Kacerovsky M, Pliskova L, Bolehovska R, et al. The microbial load with genital mycoplasmas correlates with the degree of histologic chorioamnionitis in preterm PROM. Am J Obstet Gynecol 2011; 205:213 e211–17.
57. Romero R, Miranda J, Chaemsaithong P, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2014 Sep 29. [Epub ahead of print]. doi:10.3109/14767058.2014.958463.
58. Kacerovsky M, Menon R, Drahosova M, et al. Amniotic fluid nucleosome in pregnancies complicated by preterm prelabor rupture of the membranes. J Matern Fetal Neonatal Med 2014;27: 155–61.
59. Fortner K, Grotegut C, Ransom C, et al. Bacteria localization and chorion thinning among preterm premature rupture of membranes. PLoS One 2014;9:e83338.
60. Kacerovsky M, Musilova I, Andrys C, 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 e321–5 e310.
61. Kim K, Romero R, Park H, 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 e291–5.
62. Tsiartas P, Kacerovsky M, Musilova I, et al. The association between histological chorioamnionitis, funisitis and neonatal outcome in women with preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2013;26:1332–6.
63. Bastek J, Weber A, McShea M, et al. Prenatal inflammation is associated with adverse neonatal outcomes. Am J Obstet Gynecol 2014;210:450.e1–10.
64. Armstrong-Wells J, Donnelly M, Post M, et al. Inflammatory predictors of neurologic disability after preterm premature rupture of membranes. Am J Obstet Gynecol 2015;212:212 e1–9.
65. Simhan H, Canavan TP. Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG 2005;112: 32–7.
66. van der Ham D, Nijhuis J, Mol B, et al. Induction of labour versus expectant management in women with preterm prelabour rupture of membranes between 34 and 37 weeks (the PPROMEXIL-trial). BMC Pregn Childbirth 2007;7:11.
67. Manuck T, Maclean C, Silver R, et al. Preterm premature rupture of membranes: does the duration of latency influence perinatal outcomes? Am J Obstet Gynecol 2009;201:414 e411–16.
68. Waters T, Mercer BM. The management of preterm premature rupture of the membranes near the limit of fetal viability. Am J Obstet Gynecol 2009;201:230–40.
69. Singh K, Mercer B. Antibiotics after preterm premature rupture of the membranes. Clin Obstet Gynecol 2011;54:344–50.
70. Faksh A, Wax J, Lucas F, et al. Preterm premature rupture of membranes $\geq$ 32 weeks' gestation: impact of revised practice guidelines. Am J Obstet Gynecol 2011;205:340 e341–5.
71. Melamed N, Ben-Haroush A, Pardo J, et al. Expectant management of preterm premature rupture of membranes: is it all about gestational age? Am J Obstet Gynecol 2011;204:48 e41–8.
72. Nold C, Hussain N, Smith K, et al. Optimal time for delivery with preterm premature rupture of membranes from 32 to 36 6/7 weeks. J Matern Fetal Neonatal Med 2011;24:933–5.
73. Di Renzo G, Roura L, Facchinetti F, et al. Guidelines for the management of spontaneous preterm labor: identification of spontaneous preterm labor, diagnosis of preterm premature rupture of membranes, and preventive tools for preterm birth. J Matern Fetal Neonatal Med 2011;24:659–67.
74. Grigsby P, Novy M, Sadowsky D, 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 e471–5 e414.
75. van der Ham D, van der Heyden J, Opmeer B, et al. Management of late-preterm premature rupture of membranes: the PPROMEXIL-2 trial. Am J Obstet Gynecol 2012;207:276.e1–10.
76. Rutanen EM. Comment on: guidelines for the management of spontaneous preterm labor: identification of spontaneous preterm labor, diagnosis of preterm premature rupture of membranes and preventive tools for preterm birth. J Matern Fetal Neonatal Med 2012;25:546–9 (author reply 547–8).
77. Mousiolis A, Papantoniou N, Mesogitis S, et al. Optimum mode of delivery in gestations complicated by preterm premature rupture of the membranes. J Matern Fetal Neonatal Med 2012;25: 1044–9.
78. Canzoneri B, Grotegut C, Swamy G, et al. Maternal serum interleukin-6 levels predict impending funisitis in preterm premature rupture of membranes after completion of antibiotics. J Matern Fetal Neonatal Med 2012;25:1329–32.
79. Hunter T, Byrnes M, Nathan E, et al. Factors influencing survival in pre-viable preterm premature rupture of membranes. J Matern Fetal Neonatal Med 2012;25:1755–61.
80. Dagklis T, Petousis S, Margioula-Siarkou C, et al. Parameters affecting latency period in PPROM cases: a 10-year experience of a single institution. J Matern Fetal Neonatal Med 2013;26:1455–8.
81. Dudenhausen JW. Primary prevention of preterm birth. J Perinat Med 2014;42:431–3.
82. Amon E, Lewis S, Sibai B, et al. Ampicillin prophylaxis in preterm premature rupture of the membranes: a prospective randomized study. Am J Obstet Gynecol 1988;159:539–43.
83. Johnston M, Sanchez-Ramos L, Vaughn A, et al. Antibiotic therapy in preterm premature rupture of membranes: a randomized, prospective, double-blind trial. Am J Obstet Gynecol 1990;163:743–7.
84. McGregor J, French J, Seo K. Antimicrobial therapy in preterm premature rupture of membranes: results of a prospective, double-blind, placebo-controlled trial of erythromycin. Am J Obstet Gynecol 1991;165:632–40.
85. Christmas J, Cox S, Andrews W, et al. Expectant management of preterm ruptured membranes: effects of antimicrobial therapy. Obstet Gynecol 1992;80:759–62.
86. Kurki T, Hallman M, Zilliacus R, et al. Premature rupture of the membranes: effect of penicillin prophylaxis and long-term outcome of the children. Am J Perinatol 1992;9:11–16.
87. Mercer B, Moretti M, Prevost R, et al. Erythromycin therapy in preterm premature rupture of the membranes: a prospective, randomized trial of 220 patients. Am J Obstet Gynecol 1992;166: 794–802.
88. Lockwood C, Costigan K, Ghidini A, et al. Double-blind: placebo-controlled trial of piperacillin prophylaxis in preterm membrane rupture. Am J Obstet Gynecol 1993;169:970–6.
89. Owen J, Groome L, Hauth JC. Randomized trial of prophylactic antibiotic therapy after preterm amnion rupture. Am J Obstet Gynecol 1993;169:976–81.
90. Ernest J, Givner LB. A prospective, randomized, placebo-controlled trial of penicillin in preterm premature rupture of membranes. Am J Obstet Gynecol 1994;170:516–21.
91. Grable I, Garcia P, Perry D, et al. Group B Streptococcus and preterm premature rupture of membranes: a randomized, double-blind clinical trial of antepartum ampicillin. Am J Obstet Gynecol 1996;175:1036–42.
92. Mercer B, Miodovnik M, Thurnau G, et al. Antibiotic therapy for reduction of infant morbidity after preterm premature rupture of the membranes. A randomized controlled trial. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. JAMA 1997;278:989–95.
93. Magwali T, Chipato T, Majoko F, et al. Prophylactic augmentin in prelabor preterm rupture of the membranes. Int J Gynaecol Obstet 1999;65:261–5.
94. Kenyon S, Taylor D, Tarnow-Mordi W. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. Lancet 2001;357:979–88.
95. Lewis D, Adair C, Robichaux A, et al. Antibiotic therapy in preterm premature rupture of membranes: are seven days necessary? A preliminary, randomized clinical trial. Am J Obstet Gynecol 2003;188:1413–6 (discussion 1416–17).
96. Kwak H, Shin M, Cha H, et al. The efficacy of cefazolin plus macrolide (erythromycin or clarithromycin) versus cefazolin alone in neonatal morbidity and placental inflammation for women with preterm premature rupture of membranes. Placenta 2013;34: 346–52.
97. August Fuhr N, Becker C, van Baalen A, et al. Antibiotic therapy for preterm premature rupture of membranes – results of a multicenter study. J Perinat Med 2006;34:203–6.
98. Hutzal C, Boyle E, Kenyon S, et al. Use of antibiotics for the treatment of preterm parturition and prevention of neonatal morbidity: a metaanalysis. Am J Obstet Gynecol 2008;199:620 e621–8.
99. Lamont RF. Antibiotics used in women at risk of preterm birth. Am J Obstet Gynecol 2008;199:583–4.
100. Ehsanipoor R, Chung J, Clock C, et al. A retrospective review of ampicillin-sulbactam and amoxicillin + clavulanate vs cefazolin/cephalexin and erythromycin in the setting of preterm premature rupture of membranes: maternal and neonatal outcomes. Am J Obstet Gynecol 2008;198:e54–6.
101. ACOG Practice Bulletin No. 80. Premature rupture of membranes. Clinical management guidelines for obstetrician-gynecologists. Obstet Gynecol 2007;109:1007–19.
102. Kenyon S, Pike K, Jones D, et al. Childhood outcomes after prescription of antibiotics to pregnant women with preterm rupture of the membranes: 7-year follow-up of the ORACLE I trial. Lancet 2008;372:1310–18.

103. Philipson A, Sabath L, Charles D. Transplacental passage of erythromycin and clindamycin. N Engl J Med 1973;288: 1219–21.
104. Heikkinen T, Laine K, Neuvonen P, et al. The transplacental transfer of the macrolide antibiotics erythromycin, roxithromycin and azithromycin. BJOG 2000;107:770–5.
105. Ramsey P, Vaules M, Vasdev G, et al. Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol 2003; 188:714–18.
106. Witt A, Sommer E, Cichna M, et al. Placental passage of clarithromycin surpasses other macrolide antibiotics. Am J Obstet Gynecol 2003;188:816–19.
107. Park H, Ahn B, Kwan Jun J. Placental transfer of clarithromycin in human pregnancies with preterm premature rupture of membranes. J Perinat Med 2012;40:641–6.
108. Freeman C, Klutman N, Lamp KC. Metronidazole. A therapeutic review and update. Drugs 1997;54:679–708.
109. Brook I, Wexler H, Goldstein EJ. Antianaerobic antimicrobials: spectrum and susceptibility testing. Clin Microbiol Rev 2013;26: 526–46.
110. Novak A, Rubic Z, Dogas V, et al. Antimicrobial susceptibility of clinically isolated anaerobic bacteria in a University Hospital Center Split, Croatia in 2013. Anaerobe 2015;31:31–6.
111. Sobel JD. Bacterial vaginosis – an ecologic mystery. Ann Intern Med 1989;111:551–3.
112. Sobel JD. Bacterial vaginosis. Br J Clin Pract Suppl 1990;71:65–9.
113. Cook R, Redondo-Lopez V, Schmitt C, et al. Clinical, microbiological, and biochemical factors in recurrent bacterial vaginosis. J Clin Microbiol 1992;30:870–7.
114. Joesoef M, Schmid G, Hillier SL. Bacterial vaginosis: review of treatment options and potential clinical indications for therapy. Clin Infect Dis 1999;28:S57–65.
115. Sobel JD. Bacterial vaginosis. Ann Rev Med 2000;51:349–56.
116. Donders G, Vereecken A, Bosmans E, et al. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG 2002;109:34–43.
117. Haggerty C, Hillier S, Bass D, et al. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. Clin Infect Dis 2004;39:990–5.
118. Ferris M, Masztal A, Aldridge K, et al. Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. BMC Infect Dis 2004;4:5.
119. Romero R, Chaiworapongsa T, Kuivaniemi H, et al. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. Am J Obstet Gynecol 2004;190:1509–19.
120. Hillier SL. The complexity of microbial diversity in bacterial vaginosis. N Engl J Med 2005;353:1886–7.
121. Lamont R, Taylor-Robinson D. Review of the accuracy of various diagnostic tests for bacterial vaginosis to predict preterm birth (Honest et al., BJOG, May 2004). BJOG 2005;112:259–60 (author reply 260–1).
122. Fredricks D, Fiedler T, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005; 353:1899–911.
123. Austin M, Meyn L, Hillier SL. Susceptibility of vaginal bacteria to metronidazole and tinidazole. Anaerobe 2006;12:227–30.
124. Donders GG. Definition and classification of abnormal vaginal flora. Best Pract Res Clin Obstet Gynaecol 2007;21: 355–73.
125. Brotman R, Ravel J. Ready or not: the molecular diagnosis of bacterial vaginosis. Clin Infect Dis 2008;47:44–6.
126. Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment. Expert Rev Anti Infect Ther 2009;7: 1109–24.
127. Donders G. Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: a review. Obstet Gynecol Surv 2010;65:462–73.
128. Lamont R, Taylor-Robinson D. The role of bacterial vaginosis, aerobic vaginitis, abnormal vaginal flora and the risk of preterm birth. BJOG 2010;117:119–20 (author reply 120–1).
129. Zhou X, Brotman R, Gajer P, et al. Recent advances in understanding the microbiology of the female reproductive tract and the causes of premature birth. Infect Dis Obstet Gynecol 2010; 2010:737425.
130. Marrazzo J, Martin D, Watts D, et al. Bacterial vaginosis: identifying research gaps proceedings of a workshop sponsored by DHHS/NIH/NIAID. Sex Transm Dis 2010;37:732–44.
131. Lamont R, Sobel J, Akins R, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG 2011;118:533–49.
132. Turovskiy Y, Sutyak Noll K, Chikindas ML. The aetiology of bacterial vaginosis. J Appl Microbiol 2011;110:1105–28.
133. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 2011;108: 4680–7.
134. Donders GG. The prevalence of bacterial vaginosis and aerobic vaginitis in young Finish women. APMIS 2011;119:224–5 (author reply 226).
135. Ma B, Forney L, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol 2012;66:371–89.
136. Brocklehurst P, Gordon A, Heatley E, et al. Antibiotics for treating bacterial vaginosis in pregnancy. Cochrane Database Syst Rev 2013;1:CD000262.
137. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol 2013;209:505–23.
138. Donders G, Zodzika J, Rezeberga D. Treatment of bacterial vaginosis: what we have and what we miss. Expert Opin Pharmacother 2014;15:645–57.
139. Fredricks D, Fiedler T, Thomas K, et al. Changes in vaginal bacterial concentrations with intravaginal metronidazole therapy for bacterial vaginosis as assessed by quantitative PCR. J Clin Microbiol 2014;52:3137.
140. Gomez L, Sammel M, Appleby D, et al. Evidence of a gene-environment interaction that predisposes to spontaneous preterm birth: a role for asymptomatic bacterial vaginosis and DNA variants in genes that control the inflammatory response. Am J Obstet Gynecol 2010;202:386 e381–6.
141. Mancuso M, Figueroa D, Szychowski J, et al. Midtrimester bacterial vaginosis and cervical length in women at risk for preterm birth. Am J Obstet Gynecol 2011;204:342 e341–5.
142. Foxman B, Wen A, Srinivasan U, et al. Mycoplasma, bacterial vaginosis-associated bacteria BVAB3, race, and risk of preterm birth in a high-risk cohort. Am J Obstet Gynecol 2014;210:226 e221–7.
143. Adu A, Armour CL. Drug utilisation review (DUR) of the third generation cephalosporins. Focus on ceftriaxone, ceftazidime and cefotaxime. Drugs 1995;50:423–39.
144. Klein N, Cunha BA. Third-generation cephalosporins. Med Clin North Am 1995;79:705–19.
145. Lamb H, Ormrod D, Scott L, et al. Ceftriaxone: an update of its use in the management of community-acquired and nosocomial infections. Drugs 2002;62:1041–89.
146. Kechagia N, Bersimis S, Chatzipanagiotou S. Incidence and antimicrobial susceptibilities of genital mycoplasmas in outpatient women with clinical vaginitis in Athens, Greece. J Antimicrob Chemother 2008;62:122–5.
147. Dongya M, Wencheng X, Xiaobo M, et al. Transition mutations in 23S rRNA account for acquired resistance to macrolides in *Ureaplasma urealyticum*. Microb Drug Resist 2008;14:183–6.
148. Bayraktar M, Ozerol I, Gucluer N, et al. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. Int J Infect Dis 2010;14:e90–5.
149. Xiao L, Crabb D, Duffy L, et al. Mutations in ribosomal proteins and ribosomal RNA confer macrolide resistance in human *Ureaplasma* spp. Int J Antimicrob Agents 2011;37:377–9.
150. Redelinghuys M, Ehlers M, Dreyer A, et al. Antimicrobial susceptibility patterns of *Ureaplasma* species and *Mycoplasma hominis* in pregnant women. BMC Infect Dis 2014;14:171.
151. Gomez R, Romero R, Nien J, et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. J Matern Fetal Neonatal Med 2007;20:167–73.
152. Yoon B, Romero R, Lim J, 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.
153. Yoon B, Yang S, Jun J, et al. Maternal blood C-reactive protein, white blood cell count, and temperature in preterm labor: a

comparison with amniotic fluid white blood cell count. Obstet Gynecol 1996;87:231–7.
154. Lee J, Oh K, Yang H, et al. The importance of intra-amniotic inflammation in the subsequent development of atypical chronic lung disease. J Matern Fetal Neonatal Med 2009;22:917–23.
155. Maymon E, Romero R, Pacora P, et al. Human neutrophil collagenase (matrix metalloproteinase 8) in parturition, premature rupture of the membranes, and intrauterine infection. Am J Obstet Gynecol 2000;183:94–9.
156. Park J, Romero R, Yoon B, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. Am J Obstet Gynecol 2001;185:1156–61.
157. Yoon B, Oh S, Romero R, et al. An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol 2001;185:1162–7.
158. Moon J, Kim J, Yoon B, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. J Perinat Med 2002;30:301–6.
159. Nien J, Yoon B, Espinoza J, et al. A rapid MMP-8 bedside test for the detection of intra-amniotic inflammation identifies patients at risk for imminent preterm delivery. Am J Obstet Gynecol 2006; 195:1025–30.
160. Park C, Yoon B, Kim S, et al. The frequency and clinical significance of intra-amniotic inflammation defined as an elevated amniotic fluid matrix metalloproteinase-8 in patients with preterm labor and low amniotic fluid white blood cell counts. Obstet Gynecol Sci 2013;56:167–75.
161. Kim S, Romero R, Park J, et al. The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis in preterm gestation. J Matern Fetal Neonatal Med 2014 Oct 20. [Epub ahead of print]. doi:10.3109/14767058.2014.961009.
162. Yoon B, Romero R, Kim C, et al. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. Am J Obstet Gynecol 1995;172:960–70.
163. Redline R, Heller D, Keating S, et al. Placental diagnostic criteria and clinical correlation – a workshop report. Placenta 2005;26: S114–17.
164. Redline RW. Inflammatory responses in the placenta and umbilical cord. Semin Fetal Neonatal Med 2006;11:296–301.
165. Yoon B, Romero R, Park J, 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.
166. Pacora P, Chaiworapongsa T, Maymon E, et al. Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. J Matern Fetal Neonatal Med 2002;11:18–25.
167. Yoon B, Romero R, Shim J, et al. C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. J Matern Fetal Neonatal Med 2003;14:85–90.
168. Lee J, Oh K, Park C, et al. The presence of funisitis is associated with a decreased risk for the development of neonatal respiratory distress syndrome. Placenta 2011;32:235–40.
169. Yoon B, Jun J, Romero R, 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.
170. Yoon B, Romero R, Park J, 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.
171. Cassell G, Davis R, Waites K, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16–20 weeks of gestation: potential effect on outcome of pregnancy. Sex Transm Dis 1983;10:294–302.
172. Gauthier D, Meyer W, Bieniarz A. Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes. Am J Obstet Gynecol 1991;165:1105–10.
173. Romero R, Mazor M, Morrotti R, et al. Infection and labor: VII. Microbial invasion of the amniotic cavity in spontaneous rupture of membranes at term. Am J Obstet Gynecol 1992;166: 129–33.
174. Gray D, Robinson H, Malone J, et al. Adverse outcome in pregnancy following amniotic fluid isolation of *Ureaplasma urealyticum*. Prenat Diagn 1992;12:111–17.
175. Horowitz S, Mazor M, Romero R, et al. Infection of the amniotic cavity with *Ureaplasma urealyticum* in the midtrimester of pregnancy. J Reprod Med 1995;40:375–9.
176. Yoon B, Romero R, Park J, 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.
177. Yoon B, Romero R, Kim M, 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.
178. Perni S, Vardhana S, Korneeva I, 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.
179. Olomu I, Hecht J, Onderdonk A, et al. Perinatal correlates of *Ureaplasma urealyticum* in placenta parenchyma of singleton pregnancies that end before 28 weeks of gestation. Pediatrics 2009;123:1329–36.
180. Jacobsson B, Aaltonen R, Rantakokko-Jalava K, et al. 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.
181. Jacobsson B, Mattsby-Baltzer I, Andersch B, 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.
182. Kacerovsky M, Pavlovsky M, Tosner J. Preterm premature rupture of the membranes and genital mycoplasmas. Acta Medica (Hradec Kralove) 2009;52:117–20.
183. Capoccia R, Greub G, Baud D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. Curr Opin Infect Dis 2013;26:231–40.
184. Kwak D, Hwang H, Kwon J, et al. Co-infection with vaginal *Ureaplasma urealyticum* and *Mycoplasma hominis* increases adverse pregnancy outcomes in patients with preterm labor or preterm premature rupture of membranes. J Matern Fetal Neonatal Med 2014;27:333–7.
185. Allen-Daniels M, Serrano M, Pflugner L, et al. Identification of a gene in *Mycoplasma hominis* associated with preterm birth and microbial burden in intra-amniotic infection. Am J Obstet Gynecol 2015 Jan 28. [Epub ahead of print].
186. Romero R, Reece E, Duff G, et al. Prenatal diagnosis of *Candida albicans* chorioamnionitis. Am J Perinatol 1985;2:121–2.
187. Mercer B, Goldenberg R, Das A, et al. What we have learned regarding antibiotic therapy for the reduction of infant morbidity after preterm premature rupture of the membranes. Semin Perinatol 2003;27:217–30.
188. Mercer BM. Preterm premature rupture of the membranes. Obstet Gynecol 2003;101:178–93.
189. Mercer B, Crouse D, Goldenberg R, et al. The antibiotic treatment of PPROM study: systemic maternal and fetal markers and perinatal outcomes. Am J Obstet Gynecol. 2012;206:145 e141–9.
190. Romero R, Scioscia A, Edberg S, et al. Use of parenteral antibiotic therapy to eradicate bacterial colonization of amniotic fluid in premature rupture of membranes. Obstet Gynecol 1986;67: 15S–17S.
191. Romero R, Hagay Z, Nores J, et al. Eradication of *Ureaplasma urealyticum* from the amniotic fluid with transplacental antibiotic treatment. Am J Obstet Gynecol 1992;166:618–20.
192. Mazor M, Chaim W, Horowitz S, et al. Successful treatment of preterm labour by eradication of *Ureaplasma urealyticum* with erythromycin. Arch Gynecol Obstet 1993;253:215–18.
193. Mazor M, Chaim W, Meirovitz M, et al. Eradication of viridans streptococci from the amniotic cavity by parenteral antibiotic administration: a case report. J Reprod Med 1995;40:820–2.
194. Smorgick N, Frenkel E, Zaidenstein R, et al. Antibiotic treatment of intra-amniotic infection with *Ureaplasma urealyticum:* a case report and literature review. Fetal Diagn Ther 2007;22:90–3.

195. Kim M, Kim G, Romero R, et al. Biovar diversity of *Ureaplasma urealyticum* in amniotic fluid: distribution, intrauterine inflammatory response and pregnancy outcomes. J Perinat Med 2003;31: 146–52.
196. Samra Z, Rosenberg S, Dan M. Susceptibility of *Ureaplasma urealyticum* to tetracycline, doxycycline, erythromycin, roxithromycin, clarithromycin, azithromycin, levofloxacin and moxifloxacin. J Chemother 2011;23:77–9.
197. Park H, Kim S, Huh H, et al. Prevalence and antibiotics susceptibilities of the *Ureaplasma* species isolated from asymptomatic pregnant women (abstract 163). Am J Obstet Gynecol (Supplement) 2015;212:S97.
198. Duffy L, Crabb D, Searcey K, et al. Comparative potency of gemifloxacin, new quinolones, macrolides, tetracycline and clindamycin against *Mycoplasma* spp. J Antimicrob Chemother 2000; 45:29–33.
199. Chu S, Deaton R, Cavanaugh J. Absolute bioavailability of clarithromycin after oral administration in humans. Antimicrob Agents Chemother 1992;36:1147–50.
200. Rodvold KA. Clinical pharmacokinetics of clarithromycin. Clin Pharmacokinet 1999;37:385–98.
201. Kafetzis D, Brater D, Fanourgakis J, et al. Ceftriaxone distribution between maternal blood and fetal blood and tissues at parturition and between blood and milk postpartum. Antimicrob Agents Chemother 1983;23:870–3.
202. Hauth JC. The NICHD-MFMU antibiotic treatment of pPROM study: correlation with acute placental inflammation and perinatal mortality. Am J Obstet Gynecol 1997;176:S53.
203. Bendon R, Faye-Petersen O, Pavlova Z, et al. Fetal membrane histology in preterm premature rupture of membranes: comparison to controls, and between antibiotic and placebo treatment. The National Institute of Child Health and Human Development Maternal Fetal Medicine Units Network, Bethesda, MD, USA. Pediatr Dev Pathol 1999;2:552–8.
204. Tsai W, Standiford TJ. Immunomodulatory effects of macrolides in the lung: lessons from in-vitro and in-vivo models. Curr Pharm Des 2004;10:3081–93.
205. Giamarellos-Bourboulis E, Adamis T, Laoutaris G, et al. Immunomodulatory clarithromycin treatment of experimental sepsis and acute pyelonephritis caused by multidrug-resistant *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2004; 48:93–9.
206. Giamarellos-Bourboulis E, Adamis T, Sabracos L, et al. Clarithromycin: immunomodulatory therapy of experimental sepsis and acute pyelonephritis by *Escherichia coli*. Scand J Infect Dis 2005;37:48–54.
207. Giamarellos-Bourboulis E, Baziaka F, Antonopoulou A, et al. Clarithromycin co-administered with amikacin attenuates systemic inflammation in experimental sepsis with *Escherichia coli*. Int J Antimicrob Agents 2005;25:168–72.
208. Baziaka F, Giamarellos-Bourboulis E, Raftogiannis M, et al. Immunomodulatory effect of three-day continuous administration of clarithromycin for experimental sepsis due to multidrug-resistant *Pseudomonas aeruginosa*. J Chemother 2008;20:63–8.
209. Giamarellos-Bourboulis EJ. Immunomodulatory therapies for sepsis: unexpected effects with macrolides. Int J Antimicrob Agents 2008;32:S39–43.
210. Atmatzidis S, Koutelidakis I, Chatzimavroudis G, et al. Clarithromycin modulates immune responses in experimental peritonitis. Int J Antimicrob Agents 2011;37:347–51.
211. Ichiyama T, Nishikawa M, Yoshitomi T, et al. Clarithromycin inhibits NF-kappaB activation in human peripheral blood mononuclear cells and pulmonary epithelial cells. Antimicrob Agents Chemother 2001;45:44–7.
212. Kikuchi T, Hagiwara K, Honda Y, et al. Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-kappa B transcription factors. J Antimicrob Chemother 2002;49:745–55.
213. Spyridaki A, Raftogiannis M, Antonopoulou A, et al. Effect of clarithromycin in inflammatory markers of patients with ventilator-associated pneumonia and sepsis caused by Gram-negative bacteria: results from a randomized clinical study. Antimicrob Agents Chemother 2012;56:3819–25.
214. Segel S, Miles A, Clothier B, et al. Duration of antibiotic therapy after preterm premature rupture of fetal membranes. Am J Obstet Gynecol 2003;189:799–802.
215. Alvarez J, Williams S, Ganesh V, et al. Duration of antimicrobial prophylaxis for group B streptococcus in patients with preterm premature rupture of membranes who are not in labor. Am J Obstet Gynecol 2007;197:390 e391–4.
216. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol 2010;7:653–60.
217. Dielubanza E, Schaeffer AJ. Urinary tract infections in women. Med Clin North Am 2011;95:27–41.
218. Wagenlehner F, Wullt B, Perletti G. Antimicrobials in urogenital infections. Int J Antimicrob Agents 2011;38:3–10.
219. Nicolle LE. Urinary tract infection. Crit Care Clin 2013;29: 699–715.
220. Barber A, Norton J, Spivak A, et al. Urinary tract infections: current and emerging management strategies. Clin Infect Dis 2013;57:719–24.
221. Wing DA. Pyelonephritis in pregnancy: treatment options for optimal outcomes. Drugs 2001;61:2087–96.
222. Jolley J, Wing DA. Pyelonephritis in pregnancy: an update on treatment options for optimal outcomes. Drugs 2010;70:1643–55.
223. Gupta K, Hooton T, Naber K, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52: e103–20.
224. Jolley J, Kim S, Wing DA. Acute pyelonephritis and associated complications during pregnancy in 2006 in US hospitals. J Matern Fetal Neonatal Med 2012;25:2494–8.
225. Eliakim-Raz N, Yahav D, Paul M, et al. Duration of antibiotic treatment for acute pyelonephritis and septic urinary tract infection – 7 days or less versus longer treatment: systematic review and meta-analysis of randomized controlled trials. J Antimicrob Chemother 2013;68:2183–91.
226. Wing D, Fassett M, Getahun D. Acute pyelonephritis in pregnancy: an 18-year retrospective analysis. Am J Obstet Gynecol 2014;210:219 e211–16.
227. French L, Smaill FM. Antibiotic regimens for endometritis after delivery. Cochrane Database Syst Rev 2004;4:CD001067.
228. Faro S. Postpartum endometritis. Clin Perinatol 2005;32:803–14.
229. Jaiyeoba O. Postoperative infections in obstetrics and gynecology. Clin Obstet Gynecol 2012;55:904–13.
230. Lapinsky SE. Obstetric infections. Crit Care Clin 2013;29:509–20.
231. Fihn S, Stamm WE. Interpretation and comparison of treatment studies for uncomplicated urinary tract infections in women. Rev Infect Dis 1985;7:468–78.
232. Stray-Pdersen B, Blakstad M, Bergan T. Bacteriuria in the puerperium. Risk factors, screening procedures, and treatment programs. Am J Obstet Gynecol 1990;162:792–7.
233. Duffy M. Everything you always wanted to know about graduate nursing education. Imprint. 1994;41:12–15, 17–18.
234. Iravani A, Tice A, McCarty J, et al. Short-course ciprofloxacin treatment of acute uncomplicated urinary tract infection in women. The minimum effective dose. The Urinary Tract Infection Study Group [corrected]. Arch Intern Med 1995;155: 485–94.
235. Edwards R, Clark P, Sistrom C, et al. Intrapartum antibiotic prophylaxis 1: relative effects of recommended antibiotics on gram-negative pathogens. Obstet Gynecol 2002;100: 534–9.
236. Bizzarro M, Dembry L, Baltimore R, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics 2008;121: 689–96.
237. Gregory M, Eichenwald E, Puopolo KM. Seven-year experience with a surveillance program to reduce methicillin-resistant *Staphylococcus aureus* colonization in a neonatal intensive care unit. Pediatrics 2009;123:e790–6.