OBSTETRICS

Evidence that antibiotic administration is effective in the treatment of a subset of patients with intra-amniotic infection/inflammation presenting with cervical insufficiency

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BACKGROUND: Cervical insufficiency is a risk factor for spontaneous midtrimester abortion or early preterm birth. Intra-amniotic infection has been reported in 8-52% of such patients and intra-amniotic inflammation in 81%. Some professional organizations have recommended perioperative antibiotic treatment when emergency cervical cerclage is performed. The use of prophylactic antibiotics is predicated largely on the basis that they reduce the rate of complications during the course of vaginal surgery. However, it is possible that antibiotic administration can also eradicate intra-amniotic infection/inflammation and improve pregnancy outcome.

OBJECTIVE: To describe the outcome of antibiotic treatment in patients with cervical insufficiency and intra-amniotic infection/inflammation.

STUDY DESIGN: The study population consisted of 22 women who met the following criteria: (1) singleton pregnancy; (2) painless cervical dilatation of >1 cm between 16.0 and 27.9 weeks of gestation; (3) intact membranes and absence of uterine contractions; (4) transabdominal amniocentesis performed for the evaluation of the microbiologic and inflammatory status of the amniotic cavity; (5) presence of intra-amniotic infection/inflammation; and (6) antibiotic treatment (regimen consisted of ceftriaxone, clarithromycin, and metronidazole). Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas, and polymerase chain reaction for *Ureaplasma* spp. was performed. Intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms or a positive polymerase chain reaction for *Ureaplasma* spp., and intra-amniotic inflammation was suspected when there was an elevated amniotic fluid white blood cell count ($\geq$19 cells/mm^3) or a positive rapid test for metalloproteinase-8 (sensitivity 10 ng/mL). For the purpose of this study, the "gold standard" for diagnosis of intra-amniotic inflammation was an elevated interleukin-6 concentration (>2.6 ng/mL)

using an enzyme-linked immunosorbent assay. The results of amniotic fluid interleukin-6 were not available to managing clinicians. Follow-up amniocentesis was routinely offered to monitor the microbiologic and inflammatory status of the amniotic cavity and fetal lung maturity. Treatment success was defined as resolution of intra-amniotic infection/inflammation or delivery $\geq$34 weeks of gestation.

RESULTS: Of 22 patients with cervical insufficiency and intra-amniotic infection/inflammation, 3 (14%) had microorganisms in the amniotic fluid. Of the 22 patients, 6 (27%) delivered within 1 week of amniocentesis and the remaining 16 (73%) delivered more than 1 week after the diagnostic procedure. Among these, 12 had a repeat amniocentesis to assess the microbial and inflammatory status of the amniotic cavity; in 75% (9/12), there was objective evidence of resolution of intra-amniotic inflammation or intra-amniotic infection demonstrated by analysis of amniotic fluid at the time of the repeat amniocentesis. Of the 4 patients who did not have a follow-up amniocentesis, all delivered $\geq$34 weeks, 2 of them at term; thus, treatment success occurred in 59% (13/22) of cases.

CONCLUSION: In patients with cervical insufficiency and intra-amniotic infection/inflammation, administration of antibiotics (ceftriaxone, clarithromycin, and metronidazole) was followed by resolution of the intra-amniotic inflammatory process or intra-amniotic infection in 75% of patients and was associated with treatment success in about 60% of cases.

Key words: Antimicrobial agents, interleukin-6, ceftriaxone, cephalosporins, cerclage, clarithromycin, metronidazole, MMP-8, amniotic fluid, prematurity, pregnancy, *Ureaplasma urealyticum,* chorioamnionitis, biomarker

T he term "cervical insufficiency" refers to a condition in which patients present with a dilated cervix in the

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EDITORS' CHOICE

midtrimester of pregnancy, protruding membranes in the absence of uterine contractions, or vaginal bleeding.[1] The standard treatment is placement of an emergency cerclage.[1–19] The best scientific evidence in support of this intervention derives from a randomized clinical trial that included patients with cervical insufficiency (ie, membranes at or below a dilated external cervical os <27 weeks) who were treated with bed rest and antibiotics and randomly

assigned to emergency cerclage and indomethacin vs bed rest only.[2] Patients who received emergency cerclage, indomethacin, and antibiotics had a significantly lower rate of preterm delivery (<34 weeks) than those allocated to bed rest and antibiotics (53.8% [7/13] vs 100% [10/10], $P = .02$).[2]

Intra-amniotic infection ascertained by amniocentesis has been reported in 8-52% of patients presenting with cervical insufficiency.[20–27] The use of a cervical cerclage in such patients is associated with poor pregnancy outcome, given

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AJOG at a Glance

Why was this study conducted?
This study explored whether intra-amniotic infection/inflammation in patients with cervical insufficiency can be treated with antibiotics.

Key findings
Seventy-five percent of patients with intra-amniotic infection/inflammation had objective evidence of resolution of the intra-amniotic infectious/inflammatory process after treatment with antibiotics.

What does this add to what is known?
The conventional view is that cervical insufficiency complicated by intra-amniotic infection/inflammation leads to inevitable delivery. Herein we present the first objective evidence that intra-amniotic infection/inflammation can be eradicated with antibiotic treatment, as proven by serial amniotic fluid analysis before and after treatment. This has implications for optimizing patient care.

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that pregnant women have developed clinical chorioamnionitis, rupture of the chorioamniotic membranes, or vaginal bleeding, requiring delivery.[21] In contrast, patients with cervical insufficiency but without intra-amniotic infection who undergo cerclage placement have a better pregnancy outcome.[21,22,25]

Microorganisms and their products (ie, endotoxin,[28,29] peptidoglycans, or glucans[30]) can elicit an intra-amniotic inflammatory response,[31–45] which can be ascertained by measuring amniotic fluid proinflammatory cytokines such as interleukin (IL)-6 or matrix metalloproteinase-8 (MMP-8).[46–93] Intra-amniotic inflammation has been identified in patients with cervical insufficiency[23,94,95] and is associated with preterm delivery as well as adverse pregnancy and neonatal outcomes.[23,94] A subset of patients with intra-amniotic inflammation had no demonstrable microorganisms and, therefore, represented cases of sterile intra-amniotic inflammation, caused by danger signals or alarmins,[96–102] which activate the inflammasome. Inflammasome activation can lead to preterm delivery.[90,103–108]

The traditional view has been that intra-amniotic infection in patients with cervical insufficiency cannot be successfully treated, and the same has been so for intra-amniotic inflammation. The objective of this report is to communicate a case series in which women with cervical insufficiency and intra-amniotic infection/inflammation were treated with antimicrobial agents, which resulted in eradication of intra-amniotic infection/inflammation in a subset of patients.

Materials and Methods
Study design
This was a retrospective case series study of patients admitted to Seoul National University Hospital between January 2004 and April 2014 who met the following criteria: (1) singleton gestation; (2) midtrimester painless cervical dilatation (>1 cm) with membranes visible through the cervical os and intact membranes confirmed by sterile speculum examination; (3) gestational age between 16 and 27.9 weeks; (4) absence of uterine contractility based on patient's self-report and a uterine tocodynamometer assessment; (5) transabdominal amniocentesis to evaluate the microbiologic and inflammatory status of the amniotic cavity; (6) presence of intra-amniotic infection/inflammation; and (7) administration of antibiotics (regimen consisted of ceftriaxone, clarithromycin, and metronidazole).

Intra-amniotic infection was defined as a positive amniotic fluid culture or positive polymerase chain reaction (PCR) assay for *Ureaplasma* spp., while intra-amniotic inflammation was defined as an elevated amniotic fluid IL-6 concentration (>2.6 ng/mL), as previously reported.[72,92,109–119] Cervical

dilatation was determined by visual examination at the time of sterile speculum examination.[23,26] Previous studies have shown a good correlation between cervical dilatation observed visually and through digital examination.[120] Every effort was made to avoid digital examination to decrease the risk of ascending intra-amniotic infection.

Amniocentesis was routinely offered to all patients admitted with the diagnosis of cervical insufficiency to assess the microbiologic status of the amniotic cavity and the presence of intra-amniotic inflammation. This is based on previous studies that reported a 52% prevalence of microbial invasion of the amniotic cavity[21] and an 81% prevalence of intra-amniotic inflammation[23] in patients with cervical insufficiency. The prognosis under these circumstances is poor.[21–23,25,26]

All patients provided written informed consent. The Institutional Review Board of the Seoul National University Hospital approved the collection and use of these samples and clinical information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protections of the Department of Health and Human Services of the United States.

Amniotic fluid analysis
Amniotic fluid was cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas (*Ureaplasma* spp. and *Mycoplasma hominis*). Samples were also assayed for *Ureaplasma* spp. using PCR with specific primers and examined in our clinical microbiology laboratory using methods previously described.[26] This test has been available in the clinical microbiology laboratory of the hospital since 2007. A PCR assay for *Ureaplasma* spp. was performed using stored amniotic fluid, which was obtained before 2007. An aliquot of amniotic fluid was examined in a hemocytometer chamber to determine the white blood cell (WBC) count.[57] Between March 2005 and December 2010, a rapid MMP-8 bedside test (MMP-8 PTD Check Test, SK Pharma Co, Ltd, Kyunggi-do, Republic of Korea), was performed

and used in patient management. Details of the MMP-8 rapid test have been previously described.[109,121−123] In a subset of patients, MMP-8 concentration in amniotic fluid was measured in our laboratory using a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc, Bucks, UK) and the results were available to clinicians. Intraamniotic inflammation was suspected when there was an elevated amniotic fluid WBC count ($\geq$19 cells/mm^3),[69,124] a positive rapid test for MMP-8,[109,121−123] or an MMP-8 concentration >23 ng/mL.[23,81,124,125]

Amniotic fluid not used for clinical diagnostic tests was centrifuged and stored in polypropylene tubes at −70°C. The stored amniotic fluid was analyzed for IL-6, which was measured using a commercially available enzyme-linked immunoassay (R&D Systems, Minneapolis, MN) in 2017 and 2018. The amniotic fluid IL-6 concentrations were measured for research purposes, and the results were not used in clinical decision management. The sensitivity of the assay was 0.7 pg/mL. The intra- and interassay coefficients of variation were <10%. For the purpose of this study, the final diagnosis of intra-amniotic inflammation was made when IL-6 concentration was >2.6 ng/mL[72].

Clinical management

Intra-amniotic inflammation, isolation of microorganisms by amniotic fluid culture, or the detection of *Ureaplasma* nucleic acids was an indication for antibiotic administration. We used a combination of antimicrobial agents previously described by our group in the management of patients with preterm premature rupture of membranes (PROM),[126,127] including ceftriaxone 1 g (intravenous) every 24 hours, clarithromycin 500 mg (oral) every 12 hours, and metronidazole (intravenous) 500 mg every 8 hours. Metronidazole was administered for a maximum of 4 weeks. A follow-up amniocentesis was offered to monitor the microbiologic and inflammatory status of the amniotic cavity and fetal lung maturity. The use and discontinuation of antibiotics, tocolytics,

and progesterone; interval of follow-up amniocentesis; and the placement of a cervical cerclage were left to the discretion of treating clinicians because of the lack of uniformity among attending physicians. Tocolytics used were ritodrine, magnesium sulfate, or atosiban. Nonsteroidal anti-inflammatory agents were not used as tocolytic agents in this study.

Treatment success

Treatment success was defined as the resolution of intra-amniotic infection/inflammation (defined as a negative amniotic fluid culture or negative PCR assay for *Ureaplasma* spp., and as an IL-6 <2.6 ng/mL, respectively) or delivery at or after 34 weeks of gestation.

Diagnosis of chorioamnionitis and neonatal morbidity

Acute histologic chorioamnionitis was defined as the presence of acute inflammatory changes in the choriodecidua and amnion, respectively; acute funisitis was diagnosed by the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly, using previously published criteria.[128−131] Clinical chorioamnionitis was diagnosed in the presence of a maternal temperature of $\geq$37.8°C and $\geq$2 of the following criteria: (1) uterine tenderness; (2) malodorous vaginal discharge; (3) maternal leukocytosis (WBC count of >15,000 cells/mm^3); (4) maternal tachycardia (>100 beats/min); and (5) fetal tachycardia (>160 beats/min).[132,133]

Significant neonatal morbidity was defined as the presence of any of the following conditions: respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage (grade $\geq$II), proven congenital neonatal sepsis, and necrotizing enterocolitis. These conditions were diagnosed according to definitions previously described in detail.[128,134]

Statistical analysis

Continuous variables were compared between 2 groups using the Mann-Whitney U test. Proportions were compared with a Fisher's exact test. A P value < .05 was considered statistically

significant. Analysis was performed by SPSS, version 22 (SPSS Inc, Chicago, IL).

Results

Characteristics of the study population

During the study period, 44 patients with cervical insufficiency had 1 or more amniocentesis. Among these patients, stored amniotic fluid was available for IL-6 determination in 41 patients. Intra-amniotic infection/inflammation was identified in 68% (28/41). Among the 28 patients with intra-amniotic infection/inflammation, the antibiotic regimen (clarithromycin, ceftriaxone, and metronidazole) was administered in 22 cases. The antibiotic regimen was not given to the other 6 patients for the following reasons: 1) intra-amniotic infection/inflammation was not suspected because the patients had a low amniotic fluid WBC count when the MMP-8 rapid test was not available (before March 2005 and after January 2011) (n = 5); however, intra-amniotic inflammation was diagnosed by an elevated IL-6 concentration measured afterward; (2) in 1 patient who had a fever, the managing clinician preferred to use another antibiotic regimen.

Twenty-two patients with cervical insufficiency had intra-amniotic infection and/or intra-amniotic inflammation and were treated with the antimicrobial agent combination (ceftriaxone, clarithromycin, and metronidazole). Amniotic fluid culture was positive in 2 patients. Microorganisms identified by culture *Candida albicans* (n = 1) and *Streptococcus anginosus* (n = 1). Intra-amniotic inflammation was identified in 20 patients with a negative amniotic fluid culture for microorganisms. In one instance, nucleic acids for *Ureaplasma* spp. were detected using a specific PCR assay.

The Figure shows the distribution of patients. Among the 22 patients, 6 (27%) delivered within 1 week after amniocentesis and 16 (73%) remained undelivered for at least 1 week after amniocentesis. A repeat amniocentesis to evaluate the therapeutic response to antimicrobial agents was offered to

FIGURE
Flow diagram of the study population
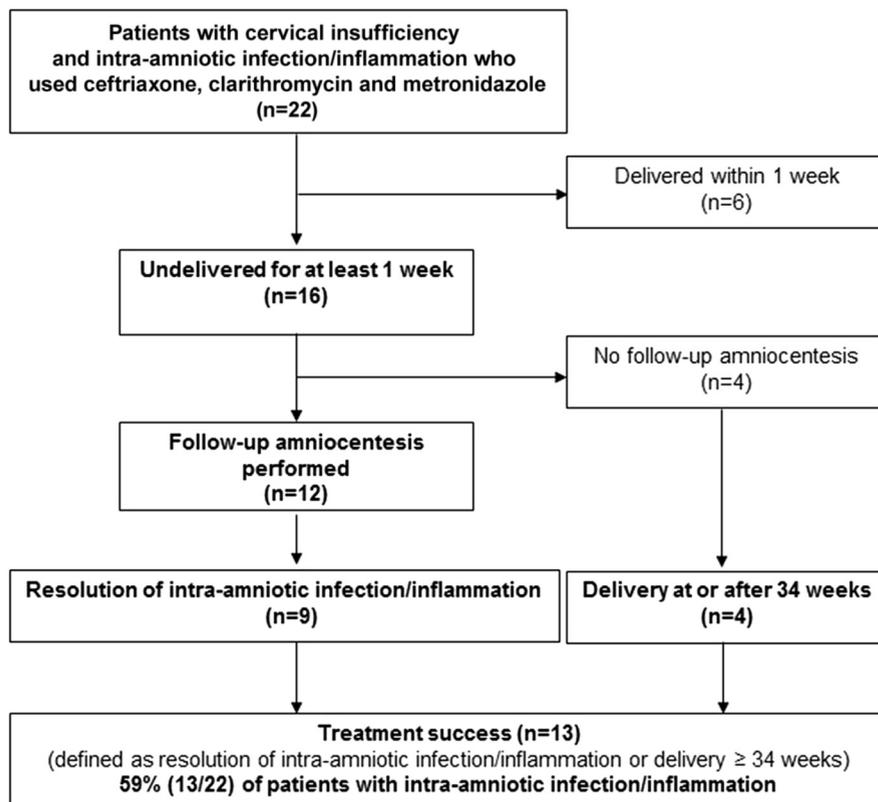
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patients and performed in 75% (12/16) of cases; in 75% (9/12) of those cases, there was objective evidence of resolution of intra-amniotic infection or intra-amniotic inflammation. Of the 4 patients who did not have a follow-up amniocentesis, all delivered ≥34 weeks, 2 of them at term (≥37 weeks). Thus, treatment success occurred in 59% (13/22) of patients with cervical insufficiency and intra-amniotic infection/inflammation who received the antibiotic regimen (Figure).

Table 1 compares the clinical characteristics and outcomes of the patients who delivered within 1 week of amniocentesis and those who remained undelivered for at least 1 week after administration of the antibiotic regimen. Patients who delivered within 1 week of amniocentesis had a significantly higher median amniotic fluid IL-6 concentration and a higher rate of history of preterm delivery than those who remained

undelivered for at least 1 week after amniocentesis ($P = .005$ and $P = .025$, respectively). The median amniotic fluid WBC count and the rate of clinical chorioamnionitis were higher in patients who delivered within 7 days of amniocentesis than in those who were undelivered for at least 1 week after amniocentesis; however, the differences did not reach statistical significance ($P > .05$ for each, Table 1). Thus, patients with cervical insufficiency who delivered within 1 week of amniocentesis had a more intense intra-amniotic inflammatory response, as determined by the concentrations of a soluble component of the inflammatory response (ie, IL-6). Vaginal progesterone was administered to 2 patients who remained undelivered for at least 1 week.

Among the 16 patients who remained undelivered for 1 week after amniocentesis, 12 underwent a follow-up amniocentesis. The median interval

between amniocenteses was 8 days (interquartile range, 7-14 days). There were no significant differences in baseline characteristics, results of amniotic fluid analysis at the time of the initial amniocentesis, and median gestational age at delivery between patients who were undelivered for at least 1 week and underwent a follow-up amniocentesis and those who did not undergo another prenatal test.

Table 2 displays the clinical characteristics according to the results of amniotic fluid analysis obtained at the time of a repeat amniocentesis. Resolution of intra-amniotic infection/inflammation was observed in 75% (9/12) of patients. None of the neonates born to the 9 mothers with resolution of the intra-amniotic inflammatory response had significant complications. The median interval between the initial and follow-up amniocenteses that confirmed resolution of intra-amniotic infection/inflammation was 24 days (interquartile range, 14—30 days). Among the 9 patients, 8 delivered at or beyond 34 weeks of gestation, and 6 delivered at term. The median gestational age at amniocentesis and delivery of patients who delivered at or beyond 34 weeks was 23.4 weeks and 37.8 weeks, respectively. There were no significant differences in the median amniotic fluid WBC count and IL-6 concentration among the 3 groups of patients ($P > .1$).

Changes in amniotic fluid culture, intra-amniotic inflammatory markers, and pregnancy and neonatal outcomes in patients with a follow-up amniocentesis

Table 3 shows the characteristics and outcomes of 12 patients who received antibiotics and underwent a follow-up amniocentesis. Nine patients (cases 1—9) had biochemical evidence of successful treatment of intra-amniotic inflammation. Bulging membranes (defined as the prolapse of membranes beyond the external os) were present in 10 of 12 patients (except cases 3 and 6). Three of 4 patients (cases 1, 2, and 4) who did not have cerclage placement had spontaneous reduction of membranes back into the uterine cavity while

receiving antibiotic treatment. This was an unexpected observation made during the course of the study.

Of 9 patients in whom the resolution of intra-amniotic inflammation was confirmed by IL-6 determinations retrospectively, 7 underwent an additional amniocentesis after resolution. The number of amniocenteses after resolution was 1 for 6 patients and 2 for 1 patient. The procedures were performed due to the occurrence of labor or rupture of membranes (n = 3), a positive result of rapid MMP-8 test at the last amniocentesis (n = 1), acute elevation of maternal serum C-reactive protein and mild fever (n = 1), or reevaluation of intra-amniotic infection/inflammation at the discretion of the clinician caring for the patient (n = 2).

Intra-amniotic infection was eradicated in case 1: *Ureaplasma* spp. had been detected by PCR. This patient had an amniotic fluid IL-6 concentration of 4.8 ng/mL, a WBC count of 35 cells/mm^3, and a positive rapid test for MMP-8 at initial amniocentesis (24.6 weeks). The antibiotics were administered after the first amniocentesis. One week later (25.6 weeks), membrane rupture occurred but follow-up amniocentesis revealed improvement of inflammatory markers (IL-6 concentration of 0.19 ng/mL, WBC count of 6 cells/mm^3, and a weakly positive MMP-8 rapid kit test). The PCR test for *Ureaplasma* spp. was still positive. The fourth amniocentesis at 27.6 weeks showed no evidence of *Ureaplasma* spp. by cultivation or specific PCR assay, and all parameters of intra-amniotic inflammation improved (ie, IL-6 concentration of 0.17 ng/mL, WBC count of 6 cells/mm^3, and a negative MMP-8 rapid kit test). At 34 weeks of gestation, the patient underwent labor induction secondary to preterm PROM. A neonate weighing 2000 g was delivered and did not develop any complications. There was no evidence of acute histologic chorioamnionitis or funisitis in placental pathologic examination.

Intra-amniotic infection with *Candida albicans* was identified in 1 patient (case 12). There was evidence of an intense amniotic fluid inflammatory response (ie, IL-6 concentration of 35.4 ng/mL and WBC count of 2556 cells/mm^3), and the amniotic fluid culture was eventually positive for *Candida albicans*. A repeat amniocentesis showed a very high WBC count (1305 cells/mm^3) and the managing physician and patient elected to proceed with labor induction given the concern for congenital candidiasis. The repeat amniotic fluid culture showed that *Candida*

albicans was still present. After completion of corticosteroid administration for fetal lung maturation, the patient delivered at 28.7 weeks of gestation (10 days from initial amniocentesis) by oxytocin administration. The birthweight was 1100 g, and Apgar scores at 1 and 5 minutes were 7 and 8, respectively. The neonate survived without any significant morbidity, and placental examination

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TABLE 1

Clinical characteristics and outcomes of patients with cervical insufficiency who received antimicrobial agents

	Delivery before 1 week (N = 6)	Delivery after 1 week (N = 16)	*P* value
Maternal age (years)	35 (28—38)	32 (24—39)	.97
Nulliparity	16.7% (1/6)	43.8% (7/16)	.35
History of preterm delivery	66.7% (4/6)	12.5% (2/16)	.025
GA at amniocentesis (weeks)	22.2 (19.7—25.0)	23.2 (21.3—27.4)	.29
Positive amniotic fluid culture	16.7% (1/6)	6.3% (1/16)	.48
Positive amniotic fluid polymerase chain reaction for *Ureaplasma* spp.	0% (0/4)	7.1% (1/14)	1.00
Amniotic fluid white blood cell count (cells/mm^3)	143 (0—430)	7 (0—2556)	.070
Amniotic fluid white blood cell count ≥19 cells/mm^3	83.3% (5/6)	31.3% (5/16)	.056
Amniotic fluid interleukin-6 (ng/mL)	47.7 (5.7—49.3)	9.4 (2.8—35.4)	.005
Amniotic fluid interleukin-6 >2.6 ng/mL	100% (6/6)	100% (16/16)	1.00
Cervical dilatation >3 cm	83.3% (5/6)	68.8% (11/16)	.63
Cervical cerclage for cervical insufficiency	33.3% (2/6)	75.0 % (12/16)	.14
Use of tocolytics	66.7% (4/6)	37.5% (6/16)	.34
Use of natural vaginal progesterone	0% (0/6)	12.5% (2/16)	1.00
Use of 17α-hydroxyprogesterone caproate	0% (0/6)	0% (0/16)	1.00
Antenatal corticosteroids administration	33.3% (2/6)	43.8% (7/16)	1.00
Gestational age at delivery (weeks)	23.0 (20.0—25.7)	37.0 (27.0—41.3)	<.001
Preterm labor[a]	33.3% (2/6)	18.8% (3/16)	.59
Preterm premature rupture of membranes[a]	33.3% (2/6)	6.3% (1/16)	.17
Clinical chorioamnionitis	33.3% (2/6)	0% (0/16)	.065
Acute histologic chorioamnionitis	75% (3/4)	57.1% (4/7)	1.00
Funisitis	25% (1/4)	42.9% (3/7)	1.00

Patients are classified into those who delivered within 1 week of amniocentesis and those who remained undelivered for at least 1 week.

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

[a] Cases in which preterm delivery before 37 weeks occurred were included.

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TABLE 2
Antenatal and postnatal characteristics of patients who were treated with antimicrobial agents and underwent follow-up amniocentesis after 1 week from initial amniocentesis

	Resolution of intra-amniotic inflammation		
	Confirmed (n = 9)		Not confirmed (n = 3)
	Delivery ≥34 weeks (n = 8)	Delivery <34 weeks (n = 1)	Delivery <34 weeks (n = 3)
Nulliparity	50% (4/8)	100% (1/1)	33.3% (1/3)
History of preterm delivery	12.5% (1/8)	0% (0/1)	0% (0/3)
Initial amniocentesis			
Gestational age at amniocentesis	23.4 (21.0—26.7)	23.7	21.4 (21.3—27.4)
Positive amniotic fluid culture	0% (0/8)	0% (0/1)	33.3% (1/3)
Positive amniotic fluid polymerase chain reaction for *Ureaplasma* spp.	14.3% (1/7)	0% (0/1)	0% (0/3)
Amniotic fluid white blood cell count (cells/mm^3)	16 (0—282)	4	24 (1—2556)
Amniotic fluid interleukin-6 (ng/mL)	8.9 (2.8—26.2)	3.8	24.8 (10.6—35.4)
Days from initial amniocentesis to resolution[a]	23 (6—30)	30	—
Number of amniocenteses	3.5 (3—7)	3	4 (3—5)
Duration of new antibiotic regimen use (days)	27 (7—66)	31	39 (9—43)
Cervical dilatation > 3cm (%)	75% (6/8)	100% (1/1)	100% (3/3)
Cervical cerclage for cervical insufficiency	50% (4/8)	100% (1/1)	100% (3/3)
Gestational age at delivery (weeks)	37.8 (34.0—41.3)[b]	29.9	27.4 (27.0—28.7)[b]
Days from initial amniocentesis to delivery	101 (66—131)[b]	43	39 (9—43)[b]
Clinical chorioamnionitis	0% (0/8)	0% (0/1)	0% (0/3)
Acute histologic chorioamnionitis	0% (0/3)	100% (1/1)	100% (3/3)
Funisitis	0% (0/3)	100% (1/1)	66.7% (2/3)
Neonatal mortality	0% (0/8)	0% (0/1)	0% (0/3)
Significant neonatal morbidity	0% (0/8)	0% (0/1)	66.7% (2/3)

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

[a] The first amniocentesis without evidence of intra-amniotic infection/inflammation; [b] *P* value < .05.

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showed acute histologic chorioamnionitis and funisitis.

Resolution of intra-amniotic inflammation was noted in 8 patients with a negative amniotic fluid culture and PCR (cases 2-9). Among these, 7 delivered at term (cases 3-8) or near term (case 2, delivered at 36.9 weeks by elective cesarean delivery). One patient (case 9) delivered at 29.9 weeks despite the resolution of intra-amniotic inflammation. The initial amniotic fluid inflammatory markers were as follows: IL-6 concentration, 3.8 ng/mL and WBC count, 4 cells/mm^3. After 4 weeks of treatment with the antibiotic regimen, the amniotic fluid IL-6 concentration decreased to 1.0 ng/mL and the amniotic fluid WBC count was 2 cells/mm^3. Antibiotics were discontinued, and the patient was discharged from the hospital. Ten days later, the patient was readmitted with spontaneous preterm labor, and the amniotic fluid IL-6 concentration had increased to 2.4 ng/mL. The patient progressed to spontaneous delivery at 29.9 weeks. The neonate weighed 1610 grams and survived without any significant morbidity. Acute histologic chorioamnionitis and funisitis were diagnosed upon placental examination.

Antibiotic treatment was not successful in 2 patients with intra-amniotic inflammation but without intra-amniotic infection (cases 10 and 11). Of these 2, microbial invasion of the amniotic cavity (by *Candida tropicalis* after 6 weeks from the initial amniocentesis in case 10, and by *Enterococcus faecalis* after 8 days from the

TABLE 3
Characteristics and outcomes of 12 patients with administration of antimicrobial agents who underwent follow-up amniocentesis

Case no.	Gestational age (weeks) Amniocentesis	Delivery	Cervix dilatation (cm)	Bag bulging	Cerclage for cervical insufficiency	Analysis of amniotic fluid (initial amniocentesis) Culture	Polymerase chain reaction for *Ureaplasma* spp.	Interleukin-6 (ng/mL)	White blood cell count (cells/mm³)	Matrix metalloproteinase-8 rapid test	Corticosteroid for fetal lung maturity	Interval between initial amniocentesis and resolution (days)[b]	Acute histologic chorioamnionitis/funisitis	Outcome (mother/newborn)
Group A. Resolution confirmed and delivery at or beyond 34 weeks														
1	24.6	34	3	Yes	No	Neg	Pos	4.8	35	Pos	Yes	21	-/-	Rupture of membranes at 25.7 weeks Induction of labor at 34 weeks Survival without morbidity
2	25	36.9	3	Yes	No[a]	Neg	Neg	11.4	1	N/A	No	21	-/-	Survival without morbidity
3	23.9	38.3	1.5	No	No	Neg	N/A	18.1	14	Neg	No	14	N/A	Survival without morbidity
4	26.7	37	4	Yes	No	Neg	Neg	2.9	0	Pos	Yes	30	N/A	Survival without morbidity
5	22.6	41.3	3	Yes	Yes	Neg	Neg	10.4	18	N/A	No	27	N/A	Survival without morbidity
6	22.9	41.1	1.5	No	Yes	Neg	Neg	2.8	0	N/A	No	6	N/A	Survival without morbidity
7	22.7	37.3	4	Yes	Yes	Neg	Neg	26.2	282	N/A	Yes	30	-/-	Survival without morbidity
8	21.1	39.9	3	Yes	Yes	Neg	Neg	N/A (7.4 on 22.3 week)	144	N/A	No	24	N/A	Survival without morbidity
Group B. Resolution confirmed but delivery before 34 weeks														
9	23.7	29.9	5.5	Yes	Yes	Neg	Neg	3.8	4	N/A	Yes	30	+/+	Spontaneous labor Survival without morbidity

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(continued)

TABLE 3
Characteristics and outcomes of 12 patients with administration of antimicrobial agents who underwent follow-up amniocentesis (continued)

Case no.	Gestational age (weeks)		Cervix dilatation (cm)	Bag bulging	Cerclage for cervical insufficiency	Analysis of amniotic fluid (initial amniocentesis)					Corticosteroid for fetal lung maturity	Interval between initial amniocentesis and resolution (days)[b]	Acute histologic chorioamnionitis/ funisitis	Outcome (mother/ newborn)
	Amniocentesis	Deli-very				Culture	Polymerase chain reaction for *Ureaplasma* spp.	Interleukin-6 (ng/mL)	White blood cell count (cells/ mm^3)	Matrix metalloproteinase-8 rapid test				
Group C. Resolution not confirmed and delivery before 34 weeks														
10	21.3	27.4	3	Yes	Yes	Neg	Neg	24.8	1	Pos	Yes	-	+/+	Spontaneous labor Respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage
11	21.4	27	3	Yes	Yes	Neg	Neg	10.6	24	Pos	Yes	-	+/-	Spontaneous labor Bronchopulmonary dysplasia
12	27.4	28.7	9	Yes	Yes	*Candida albicans*	Neg	35.4	2556	N/A	Yes	-	+/+	Persistent positive culture for candida and frequent contractions Augmentation of labor Survival without morbidity

N/A, not assessed; *Neg*, negative; *Pos*, positive.

[a] Prophylactic cerclage was performed at 14.4 weeks of gestation; [b] The first amniocentesis without evidence of intra-amniotic infection/inflammation.

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TABLE 4
Characteristics and outcomes of 4 patients with administration of antimicrobial agents who delivered after 1 week of amniocentesis without follow-up amniocentesis

Case no.	Gestational age (weeks)		Cervix dilatation (cm)	Bag bulging	Cerclage for cervical insufficiency	Analysis of amniotic fluid					Corticosteroid for fetal lung maturity	Acute histologic chorioamnionitis/ funisitis	Neonatal outcome
	Amniocentesis	Delivery				Culture *Ureaplasma* spp.	Polymerase chain reaction for *Ureaplasma* spp.	Interleukin-6 (ng/mL)	White blood cell count (cells/mm³)	Matrix metallo proteinase-8 rapid kit			
13	21.6	39	3	Yes	Yes	Neg	Neg	8.3	1	Pos	No	N/A	Survival without morbidity
14	23	36.1	2	No	Yes	Neg	Neg	2.8	2	N/A	No	N/A	Survival without morbidity
15	23.4	39.4	2	Yes	Yes	Neg	N/A	3.0	1	Pos	No	N/A	Survival without morbidity
16	24.6	35.7	2	Yes	Yes	Neg	Neg	16.2	9	N/A	No	N/A	Survival without morbidity

N/A, not assessed; *Neg*, negative; *Pos*, positive.

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initial amniocentesis in case 11) was identified in the follow-up amniocentesis. These 2 patients delivered at 27.4 weeks (1220 g) and 27 weeks (1220 g), respectively, because of spontaneous labor. The neonates survived but had respiratory distress syndrome (case 10), broncho-pulmonary dysplasia (cases 10 and 11), and intraventricular hemorrhage (case 10).

Pregnancy and neonatal outcomes in patients who did not have a follow-up amniocentesis

Table 4 shows the clinical characteristics and outcomes of 4 patients who received antibiotics and remained undelivered for at least 1 week after amniocentesis, but did not have a follow-up amniocentesis. All 4 patients underwent an emergency cervical cerclage, and antibiotics were postoperatively administered for 4-5 days. Follow-up amniocentesis was not performed because there was no evidence of intra-amniotic infection/inflammation at initial amniocentesis (low amniotic fluid WBC count and negative culture; rapid MMP-8 test was not available at this time) in cases 14 and 16, or because of the decision made by the attending physician (cases 13 and 15). All patients in this group delivered after 34 weeks of gestation.

Table 5 summarizes the characteristics and outcomes of the 6 patients who delivered within 1 week of amniocentesis and received antibiotics. These patients had a significantly higher amniotic fluid IL-6 concentration than those who remained undelivered for at least 1 week after amniocentesis ($P = .005$, Table 1). The indication of delivery was clinical chorioamnionitis (cases 21 and 22), progression of spontaneous labor after preterm PROM (case 19) or with intact membranes (case 20), abruptio placentae (case 17), or the patient's refusal to maintain pregnancy (case 18). Only 1 neonate survived with broncho-pulmonary dysplasia (case 17) and the other 5 neonates died shortly after birth.

Comment
Principal findings of the study
Principal findings were as follows: (1) eradication of intra-amniotic infection/

TABLE 5
Characteristics and outcomes of 6 patients delivered within 1 week of amniocentesis who received antibiotics

| | Gestational age (weeks) | | | | | Analysis of amniotic fluid | | | | | | | |
Case no.	Amniocentesis	Delivery	Cervix dilatation (cm)	Bag bulging	Cerclage for cervical insufficiency	Culture	Polymerase chain reaction for *Ureaplasma* spp.	Interleukin-6 (ng/mL)	White blood cell count (cells/mm^3)	Matrix metalloproteinase-8 rapid kit	Corticosteroid for fetal lung maturity	Acute histologic chorioamnionitis/funisitis	Outcome (mother/newborn)
17	24.1	24.9	2	Yes	Yes	Neg	N/A	47.7	430	Pos	Yes	+/-	Preterm labor and delivery due to placenta abruptio / Survival with bronchopulmonary dysplasia
18	23.6	24.4	3	Yes	No	Neg	Neg	5.7	0	Pos	No	N/A	Patient elected termination of pregnancy at other hospital / Neonatal death
19	20.7	21.6	4	Yes	Yes	Neg	N/A	26.9	123	Pos	No	+/+	Progress to spontaneous labor after rupture of membranes / Neonatal death
20	19.7	20	Full dilatation	Yes	No	Neg	Neg	48.7	360	N/A	No	+/-	Spontaneous preterm labor / Neonatal death
21	19.7	20.3	4	Yes	No	*Streptococcus anginosus*	Neg	47.7	81	N/A	No	N/A	Clinical chorioamnionitis / Patient elected termination of pregnancy / Neonatal death
22	25	25.7	3	Yes	No	Neg	Neg	49.3	162	Pos	Yes	-/-	Clinical chorioamnionitis after rupture of membranes / Neonatal death

N/A, not assessed; *Neg*, negative; *Pos*, positive.

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inflammation was achieved in 75% (9/12) of patients with cervical insufficiency who were treated with antimicrobial agents; and (2) the overall treatment success (defined as resolution of intra-amniotic infection/inflammation or delivery ≥34 weeks) was 59% (13/22). These observations provide strong evidence that antibiotic administration can be effective in treating intra-amniotic infection/inflammation in patients with cervical insufficiency.

Recognition of cervical insufficiency as a clinical entity

The initial recognition of cervical insufficiency is attributed to Culpeper, Cole, and Rowland in their 1658 publication "Practice of Physick,"[135] and the term "cervical incompetence" was first mentioned in 1865 by Gream.[136] In 1950, Lash and Lash described a condition called "incompetent internal os of the cervix" as a cause of habitual abortion.[137] Currently, the term "cervical insufficiency" has replaced "cervical incompetence" to avoid the negative connotation of "incompetence."[138] The standard treatment for this condition, cervical cerclage, was introduced in 1955 by Shirodkar[139] and in 1957 by McDonald.[140] The role of cerclage in obstetrics has remained controversial for more than 60 years.[15,141–155]

Intra-amniotic infection in cervical insufficiency

Goodlin[20] proposed a role for intra-amniotic infection and amniocentesis to study the microbial status of the amniotic cavity and decompression of the uterus by removing amniotic fluid prior to placement of a cerclage. Goodlin reported that 22.2% (2/9) of patients with cervical insufficiency had microorganisms in the amniotic cavity (1 had *Escherichia coli* and the other had group B *Streptococcus*).[20] He proposed treatment of intra-amniotic infection with the administration of ampicillin into the amniotic cavity at the time of amniocentesis. The patient with group B *Streptococcus* carried the pregnancy to term, suggesting a therapeutic value for antibiotic administration.[20]

Subsequent studies have shown that microorganisms are present in the amniotic cavity in 8−52% of patients with cervical insufficiency.[20−27] For example, in a study including 33 patients with cervical insufficiency, 51.5% (17/33) had a positive amniotic fluid culture for microorganisms.[21] Four of these patients had a cervical cerclage because the amniotic fluid Gram stain results were negative. All had complications, which consisted of rupture of membranes, clinical chorioamnionitis, or bleeding. When a cervical cerclage was not placed in patients with intra-amniotic infection, all (13/13) had a preterm delivery. On the other hand, patients without intra-amniotic infection had a favorable outcome, suggesting that the microbial status of the amniotic cavity is a major determinant of pregnancy outcome in patients with cervical insufficiency.

Intra-amniotic inflammation in cervical insufficiency

Microbial products (endotoxin or lipopolysaccharide, peptidoglycans, and glucans) and microorganisms can elicit an inflammatory response.[31−45] In practice, it is easier and faster to diagnose intra-amniotic inflammation than intra-amniotic infection because an elevated amniotic fluid WBC count or inflammatory molecules (such as IL-6 or MMP-8) can be rapidly detected in clinical practice, whereas the results of amniotic fluid culture or molecular microbiologic techniques will take longer than a bedside test.[109,117−119,121−123,156,157] It is now recognized that some cases of intra-amniotic inflammation occur in the absence of demonstrable microorganisms detected with cultivation or molecular methods ("sterile intra-amniotic inflammation"),[72,98−100,113,115,125] and this condition is more common than intra-amniotic infection in patients with a sonographic short cervix.[78,113] The same has been reported in patients with cervical insufficiency with the use of cultivation techniques.[23]

Specifically, Lee et al[23] reported that 81% (42/52) of patients with cervical insufficiency had evidence of intra-amniotic inflammation, defined as an amniotic fluid concentration of MMP-8

>23 ng/mL. Only 4 patients in that study had evidence of intra-amniotic infection using cultivation methods. Therefore, most cases of intra-amniotic inflammation were not attributed to bacteria. The prevalence of intra-amniotic inflammation in other studies has ranged from 22.2% (16/72) to 64.5% (20/31) using different cutoffs of IL-6 concentrations for the definition of intra-amniotic inflammation.[94,95]

The prognosis of patients with cervical insufficiency and intra-amniotic inflammation is poor, with 50% delivering within 7 days; in 84% of cases, patients had a preterm delivery <34 weeks.[23] Importantly, Lee et al reported that there were no differences in the amniocentesis-to-delivery interval or the rate of adverse pregnancy outcome between patients with intra-amniotic inflammation and a negative amniotic fluid culture and patients with proven intra-amniotic infection.[23] Collectively, these data suggest that intra-amniotic inflammation is a poor prognostic factor in cervical insufficiency.

Antimicrobial agents to treat cervical insufficiency

In this study, clinicians elected to use a combination of ceftriaxone, clarithromycin, and metronidazole based on previous studies of the pharmacokinetics of antibiotics during pregnancy.[158,159] The rationale has been described in previous reports.[126,127] In brief, erythromycin or azithromycin were frequently inadequate in eradicating intra-amniotic infection with *Ureaplasma* spp., the most common microorganism identified in the amniotic fluid of patients at risk for preterm delivery, perhaps due to limited transplacental passage, which results in inadequate antimicrobial activity in amniotic fluid (only 3% of erythromycin and 2.6% of azithromycin cross the placenta).[158] Clarithromycin, on the other hand, has a much higher rate of transplacental passage, and is effective for the treatment of intra-amniotic infection with *Ureaplasma* spp.[158] Metronidazole was used because of its powerful effect against anaerobic

bacteria, which are frequently implicated in intra-amniotic infection.[160–163] These organisms are difficult to identify using cultivation techniques.[164] Ceftriaxone was included because of its enhanced coverage of aerobic bacteria and high rate of transplacental passage.[159,165] In a previous study, we demonstrated successful eradication of intra-amniotic infection and/or inflammation in at least 33% of patients with preterm PROM after the administration of this antibiotic regimen.[127]

Can intra-amniotic infection be treated in patients with cervical insufficiency?

Goodlin[20] reported the successful treatment of intra-amniotic infection in 1 case after the administration of a single dose of 1 gram of ampicillin into the amniotic cavity. However, since that report in 1979, there is a paucity of investigation about the treatment of intra-amniotic infection and inflammation. Antibiotics have been successful in eradicating amniotic fluid infection in animal models.[166,167] Fidel et al[166] reported that antibiotic administration within 12 hours of transcervical inoculations of *Escherichia coli*, but not after 18 hours, increased the duration of pregnancy (by reducing the rate of preterm delivery) and neonatal survival in rabbits. More recently, Grigsby et al[167] showed that maternal azithromycin can eradicate *Ureaplasma* spp. from the amniotic fluid and key fetal organs in a nonhuman primate model. Gravett et al[168] reported that the combination of immunomodulators (dexamethasone and indomethacin) and antibiotics was able to eradicate intra-amniotic infection, suppress intra-amniotic and placental inflammation, and prolong gestation in a nonhuman primate model.

The results reported herein show that antibiotic administration can eradicate intra-amniotic infection, as demonstrated by repeat amniotic fluid analyses of 1 patient who had *Ureaplasma* spp. detected by PCR at 24.6 weeks of gestation. This patient delivered at 34 weeks and the neonate had no significant morbidity. In contrast, 1 patient was

admitted at 27.4 weeks and received antibiotics, and the amniotic fluid culture eventually became positive for *Candida albicans*. A repeat amniocentesis showed a very high WBC count (1305 cells/mm³) and the managing physician and patient elected to proceed with labor induction given the concern of congenital candidiasis. The repeat amniotic fluid culture showed that *Candida albicans* was still present. Such observation underscores the importance of identifying the specific microorganism involved and selecting antibiotics that are effective against the pathogen. Previous reports have shown that fluconazole may be effective in cases of intra-amniotic infection with *Candida albicans*.[169]

Antibiotics can eradicate intra-amniotic inflammation in cervical insufficiency

An observation made during the course of this case series is that antibiotic administration in patients with intra-amniotic inflammation was effective in treating intra-amniotic inflammation, even when microorganisms were not detectable. We have reported similar results in patients with preterm PROM.[126,127]

The mechanisms whereby antibiotics can treat intra-amniotic inflammation are not clear. One possibility is that these agents are effective against microorganisms that were not detected using cultivation techniques[99,113,114,164,170–177] or PCR-specific assays for *Ureaplasma* spp. Future studies using broad-range PCR assays or cell-free microbial DNA[178–181] could help to answer this important question.

It is also possible that macrolides (ie, clarithromycin) suppress intra-amniotic inflammation caused by danger signals by down-regulating the expression of proinflammatory transcription factors, such as nuclear factor κB and activator protein-1, which can induce the production of proinflammatory cytokines. Such observations have been made in other organ systems.[182–184] It is unlikely that antibiotics were effective in reducing decidual infection because careful studies to locate microorganisms in the chorioamniotic membranes and decidua

have shown that organisms first invade the amniotic cavity and are only a secondary presence in the decidua. Thus, the initial view that organisms would be present in the decidua and, from there, would invade the amniotic cavity is contradicted by empirical evidence using morphologic and molecular microbiologic techniques.[185]

A relevant question is the relative contribution of antibiotics and cervical cerclage to the outcomes in this study. There are no data to support that cervical cerclage alone can eradicate intra-amniotic inflammation. A cervical cerclage was placed in 64% of patients, and it may have had a beneficial effect in improving pregnancy outcome. However, it can be postulated that some patients with intra-amniotic infection/inflammation benefited from the administration of the combination of antimicrobial agents, which eradicated bacteria from the amniotic cavity or down-regulated the inflammatory response.

For those patients in whom infection or inflammation was successfully treated, a cervical cerclage may have contributed to the prevention of preterm labor or pregnancy loss by closing the cervix and preventing further exposure of the chorioamniotic membranes to microorganisms present in the vagina. Therefore, the improved clinical outcomes observed in this study may have been attributable to the combination of antibiotic administration, with a direct effect on microbial invasion of the amniotic cavity and intra-amniotic inflammation, and cerclage, which prevented reinfection or recurrence of the inflammatory process. Cerclage placement may also have had a protective role by supporting the cervix, as cervical disorders may be a cause of preterm labor and midtrimester pregnancy loss.

Strengths and limitations

This is an observational study in which women with intra-amniotic infection/inflammation were treated with antibiotics and monitored with follow-up amniocenteses. It represents an objective demonstration that a case of intra-amniotic infection was successfully

treated, and that intra-amniotic inflammation could also be down-regulated and followed by a favorable pregnancy outcome.

Although Goodlin[20] reported the first case of eradication of intra-amniotic infection in cervical insufficiency, the current study reports the successful treatment of intra-amniotic inflammation in the absence of detectable microorganisms. This was possible because of the availability of rapid tests for detection of intra-amniotic inflammation, such as amniotic fluid WBC count and a rapid amniotic fluid test for MMP-8. It is unlikely that the eradication of intra-amniotic inflammation can be attributed to the administration of corticosteroids, because these agents were not administered in 69.2% (9/13) of patients in whom treatment was successful.

A limitation of this study is that it is not a randomized clinical trial, but rather a case series, and therefore, further studies to confirm these observations are desirable. Another limitation is that we combined patients with intra-amniotic infection and those with intra-amniotic inflammation but without proven intra-amniotic infection. Lee et al[23] previously demonstrated that these 2 conditions have similar pregnancy outcomes. Additional research is required to examine whether there is a differential effect of antibiotics in patients with intra-amniotic infection vs those with "sterile" intra-amniotic inflammation. The main claim of this paper is that antimicrobial agents can be useful in eradicating intra-amniotic inflammation and, in some cases, intra-amniotic infection. While our data suggest that the frequency of complications in the neonatal period is lower, other studies will need to examine long-term follow-up.

This study included patients with cervical insufficiency diagnosed by physical examination, and therefore it does not address the entity of an asymptomatic short cervix diagnosed by ultrasound. We previously demonstrated that microbial invasion of the amniotic cavity in asymptomatic patients with a short cervix can be treated with antibiotics, as demonstrated by repeat amniocentesis in which amniotic fluid cultures became negative, and patients delivered close to term.[186]

The choice of antimicrobial agents used in this study, route of administration, and duration of treatment represent the choice of clinicians practicing in a university hospital that has studied intra-amniotic infection/inflammation, the microbiology of infection, and the short- and long-term consequences of these conditions. It is possible that similar success could be achieved with other antibiotic regimens that provide adequate coverage for the most frequent microorganisms found in the amniotic cavity (genital mycoplasmas) and administered in a different manner.

Medicine is evolving toward the individualized treatment of patients, and we believe that, with the application of modern molecular microbiologic techniques, it may be possible to tailor treatment for a specific patient based on the results of amniotic fluid analysis.

Conclusion

Antibiotic administration to women with cervical insufficiency and intra-amniotic infection/inflammation can eradicate these pathologic processes in a subset of patients. ■

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