

# Bacteriology of Amniotic Fluid in Women With Suspected Cervical Insufficiency

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## Abstract

**Objective:** To determine the prevalence of mid-trimester microbial invasion of the amniotic cavity (MIAC) in women with suspected cervical insufficiency.

**Methods:** A prospective observational cohort study was performed in women with suspected cervical insufficiency and visible fetal membranes who were undergoing amniocentesis to rule out MIAC between 16 and 26 weeks of gestation. Women with preterm premature rupture of membranes, regular uterine contractions, or who had a cervical cerclage were excluded. Gram staining of amniotic fluid, glucose and lactate dehydrogenase (LDH) levels in amniotic fluid, and aerobic and anaerobic amniotic fluid cultures were performed, along with polymerase chain reaction (PCR) for the detection of *Ureaplasma* and *Mycoplasma* species.

**Results:** Fifteen women with a mean gestational age of 22.6 ± 2.3 weeks were included in the study. The diagnosis of MIAC was confirmed in 47% (7/15), of whom 20% (3/15) were infected with more than one bacterial strain and 33% (5/15) with *Ureaplasma* species. According to receiver-operator curve analyses, amniotic fluid levels of glucose were associated with MIAC ( $P = 0.02$ ), but not amniotic fluid LDH ( $P = 0.25$ ).

**Conclusion:** MIAC is present in approximately one half of women with suspected cervical insufficiency and visible fetal membranes at speculum examination.

la présence d'une IMCA entre la 16<sup>e</sup> et la 26<sup>e</sup> semaine de gestation. Les femmes présentant une rupture prématurée des membranes préterme, des contractions utérines régulières ou ayant subi un cerclage cervical ont été exclues. La coloration de Gram du liquide amniotique, la mesure des taux de glucose et de lactate-déshydrogénase (LDH) dans ce dernier, et des cultures aérobiques et anaérobiques du liquide amniotique ont été effectuées, en plus d'une amplification en chaîne par polymérase (PCR) menée en vue de dépister les espèces *Ureaplasma* et *Mycoplasma*.

**Résultats :** Quinze femmes dont l'âge gestationnel moyen était de 22,6 ± 2,3 semaines ont été invitées à participer à l'étude. Le diagnostic d'IMCA a été confirmé chez 47 % (7/15) des participantes, 20 % (3/15) desquelles étaient infectées par plus d'une souche bactérienne et 33 % (5/15), par l'espèce *Ureaplasma*. En fonction des analyses de courbe récepteur-opérateur, l'IMCA était associée aux taux de glucose du liquide amniotique ( $P = 0,02$ ), mais non aux taux de LDH de ce dernier ( $P = 0,25$ ).

**Conclusion :** L'IMCA est présente chez environ la moitié des femmes pour lesquelles une insuffisance cervicale est soupçonnée et dont les membranes fœtales sont visibles à l'examen au spéculum.

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## Résumé

**Objectif :** Déterminer la prévalence de l'invasion microbienne de la cavité amniotique (IMCA) au cours du deuxième trimestre chez les femmes pour lesquelles une insuffisance cervicale est soupçonnée.

**Méthodes :** Une étude de cohorte observationnelle prospective a été menée auprès de femmes, pour lesquelles une insuffisance cervicale était soupçonnée et qui présentaient des membranes fœtales visibles, qui subissaient une amniocentèse afin d'écartier

**Key Words:** Pregnancy, prematurity, cervical insufficiency, cerclage, amniotic fluid, microbiology, microbial invasion of the amniotic cavity (MIAC)

Competing Interests: None declared.

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## INTRODUCTION

Although “cervical insufficiency” has been recognized for many years as a specific condition of pregnancy and has been associated with a specific treatment (cerclage), diagnostic criteria for this condition remain poorly defined. Proposed criteria include a wide variety of clinical conditions: prior second trimester fetal loss, cervical dilatation without contractions, bulging fetal membranes, hourglass membranes, or short cervical length on ultrasound.<sup>1,2</sup> Although randomized trials have failed to demonstrate benefit from prophylactic cervical cerclage for most of these women, such as those with prior single second-trimester loss or short cervical length,<sup>3-5</sup> some retrospective studies

**Table 1. Bacteriology of amniotic fluid and intra-amniotic biological markers (glucose, LDH) in women with suspected cervical insufficiency diagnosed with microbial invasion of the amniotic cavity (MIAC)**

Patient	Cultures Results	PCR ( <i>M. hominis</i> , <i>U. parvum</i> , <i>U. urealyticum</i> )	Glucose mmol/L (mg/dL)	LDH U/L
1	<i>Ureaplasma</i> spp.	Negative	2.2 (39.6)	65
2	<i>Streptococcus mutans</i>	Negative	0.5 (9.0)	258
3	<i>Fusobacterium</i> spp. <i>Bacteroides</i> spp.	Negative	0.9 (16.2)	289
4	<i>Fusobacterium</i> spp. <i>Ureaplasma</i> spp.	<i>U. parvum</i>	0.2 (3.6)	2,055
5	Group B <i>Streptococcus</i> <i>Ureaplasma</i> spp.	<i>U. urealyticum</i> & <i>U. parvum</i>	0.9 (16.2)	141
6	Negative	<i>U. urealyticum</i>	1.2 (21.6)	201
7	Negative	<i>Ureaplasma</i> spp.	0.7 (12.6)	398

have indicated that emergency or “rescue” cervical cerclage could prolong pregnancy in women with early asymptomatic cervical dilatation.<sup>6,7</sup> While prolongation of pregnancy could be associated with greater gestational age at delivery, it has been linked with higher rates of chorioamnionitis and PPRM.<sup>6,8</sup> Indeed, women with short cervical length are at higher risk for intra-amniotic infection and chorioamnionitis.<sup>9,10</sup>

Performing amniocentesis to rule out MIAC prior to the placement of rescue cerclage has been recommended.<sup>11,12</sup> Mays et al. showed that women who had MIAC excluded prior to rescue cerclage had a greater chance of delivering after 37 weeks (82%) than women with MIAC who did not have rescue cerclage (0%) or those who did not have amniocentesis to rule out MIAC prior to rescue cerclage (0%).<sup>11</sup> However, this practice has not been standardized.

The purpose of our study was to determine the prevalence of MIAC in our population of women with suspected cervical insufficiency and visible fetal membranes.

## METHODS

We performed a prospective observational cohort study of women with suspected cervical insufficiency who were undergoing amniocentesis to rule out MIAC between 16 and 26 weeks of gestation in our two tertiary care

institutions between June 2004 and October 2007. Only women with painless cervical dilatation and visible fetal membranes at the external cervical os at speculum examination were included. Patients with cervical cerclage placed earlier in pregnancy or before amniocentesis, rupture of membranes before amniocentesis, or the presence of regular uterine contractions were excluded.

Clinical protocols for amniocentesis and amniotic fluid processing were similar in both centres. All amniocenteses were performed under ultrasonographic guidance by a maternal-fetal medicine specialist. Twelve to 20 mL of amniotic fluid were withdrawn from the amniotic sac (or from both sacs in the case of twins). All samples were adequate for microbiological analyses, with no need for repeat sampling. Amniotic fluid analyses included glucose and LDH levels, and aerobic and anaerobic cultures. Polymerase chain reaction was performed for detection of *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Mycoplasma hominis*. MIAC was confirmed by a positive result in Gram staining, aerobic or anaerobic bacterial culture, *Mycoplasma* culture, or PCR.

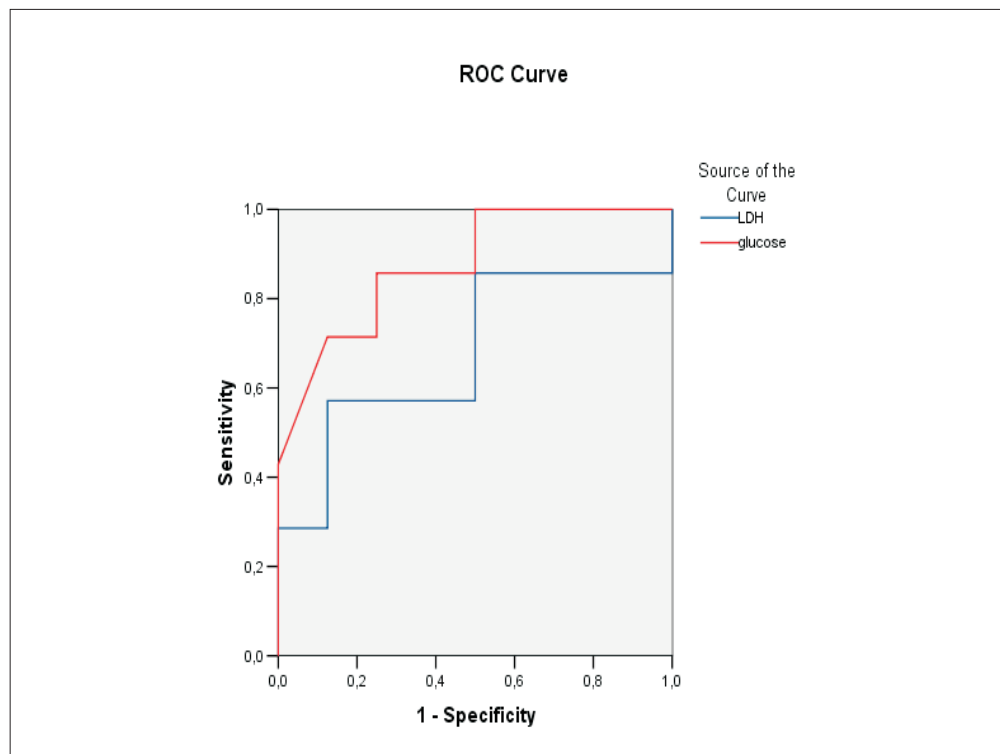
Clinical management followed the direction of the maternal-fetal specialist in charge of the patient. Most women with MIAC received intravenous antibiotic therapy, while those without MIAC had emergency cerclage if there was no contraindication.

The primary outcome was the rate of MIAC in the study cohort. Secondary outcomes were the predictive values of amniotic fluid LDH and glucose levels to detect MIAC. Predictive values were calculated by ROC analyses. Non-parametric tests were performed when appropriate, using SPSS for Windows (Version 13.0, Chicago IL). *P* values < 0.05 were considered significant. Approval was obtained from Sainte-Justine Hospital and Centre

## ABBREVIATIONS

LDH	lactate dehydrogenase
MIAC	microbial invasion of the amniotic cavity
PCR	polymerase chain reaction
PPROM	preterm premature rupture of the membranes
ROC	receiver operator curve

**Receiver-operator curve analyses of intra-amniotic fluid glucose and LDH levels as predictors of MIAC. Area under the curve: glucose = 0.875,  $P = 0.015$ ; LDH = 0.679,  $P = 0.247$**



Hospitalier Universitaire de Québec scientific and ethics research committees.

## RESULTS

Twenty-one women with suspected cervical insufficiency between 16 and 26 weeks of gestation who underwent amniocentesis were originally included. Six women were subsequently excluded: four had cerclage placed early in the second trimester, and fetal membranes were not visible on speculum examination in two others despite short cervical length (12 mm in one case and 5 mm in the other). Of the six women excluded, four (67%) had MIAC confirmed, and a fifth had suspected MIAC although not confirmed (negative culture with very low glucose and high LDH level). Thus, 15 women were included in analyses. The mean gestational age at the time of amniocentesis was  $22.6 \pm 2.3$  weeks. Three patients (20%) had a twin pregnancy, and both amniotic cavities were punctured in all three patients. MIAC was found on one (33%) amniocentesis in the amniotic cavity of the first twin. In all cases, the amniotic cavity of the second twin was sterile, with normal glucose and LDH values. The level of LDH and glucose from the amniotic cavity of the first fetus were used for the ROC analyses.

The diagnosis of MIAC was confirmed in seven women (47%) (95% CI 21–72%) with visible membranes and no cerclage, including five women who were infected by *Ureaplasma* species. Three women (20%) were infected by more than one bacterial strain. MIAC was confirmed by PCR exclusively in two patients (13%). Details concerning results of amniotic fluid bacterial cultures are presented in Table 1, together with information on the other indicators of infection. ROC analyses for the predictive values of amniotic fluid LDH and glucose levels for MIAC are presented in the Figure. Amniotic fluid LDH > 250 U/L was associated with 57% sensitivity and 87% specificity, while amniotic fluid glucose < 1.0 mmol/L had 71% sensitivity and 87% specificity. The area under the curve was statistically different from 0.05 for the ROC of glucose only.

## DISCUSSION

MIAC was found in seven (47%) of the 15 women with suspected cervical insufficiency and visible fetal membranes at between 16 and 26 weeks of gestation. This finding is different from the rate of MIAC in asymptomatic women, which has been reported as between 0 and 11% at mid-trimester genetic amniocentesis, with the vast majority of women with MIAC having a subsequent preterm birth, sometimes several weeks after the amniocentesis.<sup>13–16</sup> Therefore, in

**Table 2. Studies reporting the incidence of MIAC in women with second trimester cervical insufficiency**

Author (year)	Country	N	Inclusion criteria	Definition of MIAC	Infection rate
MacDougall J & Siddle N, 1991 <sup>19</sup>	UK	16	Women who underwent emergency cerclage between 16 and 28 weeks' gestation with cervical dilatation between 3 and 10 cm	Positive culture (aerobic and anaerobic)	13% (2/16)
Romero R et al., 1992 <sup>12</sup>	USA	33	Women with cervical dilatation $\geq$ 2 cm, intact membranes, and without active labour between 14 and 24 weeks' gestation	Positive Gram stain or culture (aerobic, anaerobic and for <i>Mycoplasma</i> )	52% (17/33)
Mays JK et al., 2000 <sup>11</sup>	USA	18	Candidates for rescue cerclage (internal os dilated at least 2 cm, 50% effaced, with membranes visible at the external os by speculum examination)	AF glucose level $\leq$ 14 mg/dL, AF LDH level $>$ 400 U/L, positive Gram stain and/or culture (aerobic and anaerobic)	39% (7/18)
Ishikawa K et al., 2003 <sup>20</sup>	Japan	61	Women with singleton pregnancies between 21 and 31 weeks' gestation who had visible or protruding intact chorioamniotic membranes on admission	WBC counts $>$ 13,000 / $\mu$ L and/or CRP values $>$ 1.0 mg/dL	23% (14/61)
Current study 2008	Canada	15	Women between 16 and 26 weeks' gestation with suspected cervical insufficiency and visible fetal membranes.	Positive culture (aerobic, anaerobic and mycoplasma), Gram stain, or PCR for <i>Ureaplasma/Mycoplasma</i>	47% (7/15)
<b>TOTAL</b>		<b>143</b>			<b>33% (47/143)</b>

AF: amniotic fluid; WBC: white blood cells; CRP: C-reactive protein.

response to these data and the findings of the current study, one could suggest that preterm birth and possibly cervical insufficiency may be a consequence of MIAC that has been present for several days or weeks.

Although performing amniocentesis to rule out MIAC for PPRM is a common practice in several countries, performing amniocentesis to exclude MIAC in women with suspected cervical insufficiency and visible fetal membranes is not a current practice throughout the world, and published data are scattered or rare.<sup>11,12,17-21</sup> To compare our results and to counterbalance the small sample size, we conducted a systematic review of the literature (randomized trials, cohort and case-control studies with at least 4 patients in each of them) concerning MIAC in women with suspected cervical insufficiency (Table 2). An overall MIAC rate of 33% was found in women with suspected cervical insufficiency and dilated cervix. Although this result is in agreement with our findings, it is likely that a MIAC rate could have been higher if more sensitive detection methods, such as PCR, had been used in all studies. Moreover, the extent of membrane protrusion might also have influenced MIAC rate. Ishikawa et al.<sup>20</sup> found a significant difference between the infection rate in women with membranes visible through the cervix but not protruding beyond the external cervical os (9.8%) and the rate in women who had membranes protruding into the vagina (50%) ( $P < 0.05$ ).

Since the MIAC rate in women with a short cervix is between 2% and 9%,<sup>9,22</sup> it seems that there is a positive relationship between the degree of cervical effacement/dilatation and the MIAC rate.

In response to our study, one could question whether amniocentesis should be a part of the standard investigation of all women with suspected cervical insufficiency and/or short cervical length. Our study cannot answer this specific question, but indicates future research possibilities. First, we confirmed that MIAC is very prevalent in women with suspected cervical insufficiency and visible fetal membranes. Since antibiotics have been shown to be effective in eradicating MIAC in specific situations, antibiotic therapy could be considered as an alternative form of management or added to cervical cerclage.<sup>21,23,24</sup> This suggestion is supported by recent publications demonstrating the benefits of antibiotic therapy in preventing preterm births in high-risk populations.<sup>25-27</sup> Second, since the variable sensitivity of the usual microbiology cultures and the time required to obtain laboratory results could affect the clinical management of women with suspected cervical insufficiency, we reported the predictive values of commonly used markers (LDH, glucose). The predictive values of these markers for the detection of MIAC have been previously reported in women with preterm labour.<sup>28,29</sup> Our findings of a low glucose value being a predictor of intra-amniotic infection are

consistent with those of earlier studies, but a larger sample size would be needed to predict the cut-off value in this specific clinical condition where MIAC is highly prevalent (50% compared with 13.6% and 12.0%). Inflammatory markers such as interleukin-6 or matrix metalloproteinase-8 seem to have better predictive value, and further investigations should be undertaken.<sup>30–32</sup> Those parameters were not part of the usual amniotic fluid analyses during our study. Finally, details concerning the presence or absence of amniotic sludge were not disclosed in the current study but should be considered as a potential variable in future.<sup>10,33–35</sup>

Our observational study was not designed to evaluate the benefit of MIAC diagnosis on pregnancy outcomes. The main limitation is the fact that these women's follow-up and treatments were based on amniocentesis results. Some women received antibiotics or had labour induced after a diagnosis of MIAC was obtained. Therefore, few conclusions could be drawn from pregnancy outcomes in regard to MIAC in the current study.

## CONCLUSION

MIAC can be found in one third to one half of women with suspected cervical insufficiency and visible fetal membranes. Therefore, amniocentesis should be considered prior to expectant management or emergency cerclage in this situation. Further studies investigating sensitive and specific markers of intra-amniotic infection/inflammation are warranted.

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## REFERENCES

- Romero R, Espinoza J, Erez O, Hassan S. The role of cervical cerclage in obstetric practice: can the patient who could benefit from this procedure be identified? *Am J Obstet Gynecol* 2006; 194:1–9.
- Abortions. In: Cunningham FG, Gant NF, Leveno KJ, Gilstrap LC III, Hauth JC, Wenstrom KD, eds. *William's Obstetrics*. Chicago: McGraw-Hill;2005:855–80.
- Rush RW, Isaacs S, McPherson K, Jones L, Chalmers I, Grant A. A randomized controlled trial of cervical cerclage in women at high risk of spontaneous preterm delivery. *Br J Obstet Gynaecol* 1984;91:724–30.
- To MS, Alfirevic Z, Heath VC, Cicero S, Cacho AM, Williamson PR, et al. Cervical cerclage for prevention of preterm delivery in women with short cervix: randomised controlled trial. *Lancet* 2004;363:1849–53.

- Rust OA, Atlas RO, Jones KJ, Benham BN, Balducci J. A randomized trial of cerclage versus no cerclage among patients with ultrasonographically detected second-trimester preterm dilatation of the internal os. *Am J Obstet Gynecol* 2000;183:830–5.
- Cockwell HA, Smith GN. Cervical incompetence and the role of emergency cerclage. *J Obstet Gynaecol Can* 2005;27:123–9.
- Daskalakis G, Papantoniou N, Mesogitis S, Antsaklis A. Management of cervical insufficiency and bulging fetal membranes. *Obstet Gynecol* 2006;107:221–6.
- Hassan SS, Romero R, Maymon E, Berry SM, Blackwell SC, Treadwell MC, et al. Does cervical cerclage prevent preterm delivery in patients with a short cervix? *Am J Obstet Gynecol* 2001;184:1325–9.
- Hassan S, Romero R, Hendler I, Gomez R, Khalek N, Espinoza J, et al. A sonographic short cervix as the only clinical manifestation of intra-amniotic infection. *J Perinat Med* 2006;34:13–9.
- Bujold E, Pasquier JC, Simoneau J, Arpin MH, Duperron L, Morency AM, et al. Intra-amniotic sludge, short cervix and risk of preterm delivery. *J Obstet Gynaecol Can* 2006;28:198–202.
- Mays JK, Figueroa R, Shah J, Khakoo H, Kaminsky S, Tejani N. Amniocentesis for selection before rescue cerclage. *Obstet Gynecol* 2000;95:652–5.
- Romero R, Gonzalez R, Sepulveda W, Brandt F, Ramirez M, Sorokin Y, et al. Infection and labor. VIII. Microbial invasion of the amniotic cavity in patients with suspected cervical incompetence: prevalence and clinical significance. *Am J Obstet Gynecol* 1992;167:1086–91.
- Gray DJ, Robinson HB, Malone J, Thomson RB Jr. Adverse outcome in pregnancy following amniotic fluid isolation of *Ureaplasma urealyticum*. *Prenat Diagn* 1992;12:111–7.
- Horowitz S, Mazor M, Romero R, Horowitz J, Glezerman M. Infection of the amniotic cavity with *Ureaplasma urealyticum* in the midtrimester of pregnancy. *J Reprod Med* 1995;40:375–9.
- Yoon BH, Oh SY, Romero R, Shim SS, Han SY, Park JS, 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.
- Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of *Ureaplasma urealyticum* in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. *J Infect Dis* 2003;187:518–21.
- Ramsey PS, Nuthalapaty FS, Lu G, Ramin S, Nuthalapaty ES, Ramin KD. Contemporary management of preterm premature rupture of membranes (PPROM): a survey of maternal-fetal medicine providers. *Am J Obstet Gynecol* 2004;191(4):1497–502.
- Lacerte M, Bujold E, Audibert F, Mayrand MH. Amniocentesis for PPRM management: a feasibility study. *J Obstet Gynaecol Can* 2008;30(8):659–664.
- MacDougall J, Siddle N. Emergency cervical cerclage. *Br J Obstet Gynaecol* 1991;98:1234–8.
- Ishikawa K, Watanabe H, Tadokoro N, Oshima K, Nishikawa M, Inaba N. Outcome of prolapsed chorioamniotic membrane: relationship between the degree of herniation, infection, and pregnancy prolongation. *Am J Perinat* 2003;20:381–9.
- Morency AM, Rallu F, Laferrière C, Bujold E. Eradication of intra-amniotic *Streptococcus mutans* in a woman with a short cervix. *J Obstet Gynaecol Can* 2006;28:898–902.
- Rust OA, Atlas RO, Reed J, Van Gaalen J, Balducci J. Revisiting the short cervix detected by transvaginal ultrasound in the second trimester: why cerclage therapy may not help. *Am J Obstet Gynecol* 2001;185:1098–105.
- Romero R, Hagay Z, Nores J, Sepulveda W, Mazor M. Eradication of *Ureaplasma urealyticum* from the amniotic fluid with transplacental antibiotic treatment. *Am J Obstet Gynecol* 1992;166:618–20.

24. Mazor M, Chaim W, Horowitz S, Leiberman JR, Glezerman M. Successful treatment of preterm labour by eradication of *Ureaplasma urealyticum* with erythromycin. *Arch Gynecol Obstet* 1993;253:215–8.
25. Morency AM, Bujold E. The impact of second-trimester antibiotics on the rate of preterm birth. *J Obstet Gynaecol Can* 2007;29:35–44.
26. Morency AM, Bujold E. Treatment of bacterial vaginosis in pregnancy: a new perspective. *J Obstet Gynaecol Can* 2007;29:115–6.
27. Ugwumadu A, Manyonda I, Reid F, Hay P. Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. *Lancet* 2003;361:983–8.
28. Garry D, Figueroa R, Aguero-Rosenfeld M, Martinez E, Visintainer P, Tejani N. A comparison of rapid amniotic fluid markers in the prediction of microbial invasion of the uterine cavity and preterm delivery. *Am J Obstet Gynecol* 1996;175(5):1336–41.
29. Romero R, Jimenez C, Lohda AK, Nores J, Hanaoka S, Avila C, et al. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. *Am J Obstet Gynecol* 1990;175(5):1336–41.
30. Lee KY, Jun HA, Kim HB, Kang SW. Interleukin-6, but not relaxin, predicts outcome of rescue cerclage in women with cervical incompetence. *Am J Obstet Gynecol* 2004;191:784–9.
31. Bashiri A, Horowitz S, Huleihel M, Hackmon R, Dukler D, Mazor M. Elevated concentrations of interleukin-6 in intra-amniotic infection with *Ureaplasma urealyticum* in asymptomatic women during genetic amniocentesis. *Acta Obstet Gynecol Scand* 1999;78:379–82.
32. Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, et al. A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. *Am J Obstet Gynecol* 2007;197:292.e1–5.
33. Espinoza J, Goncalves LF, Romero R, Nien JK, Stites S, Kim YM, et al. The prevalence and clinical significance of amniotic fluid ‘sludge’ in patients with preterm labor and intact membranes. *Ultrasound Obstet Gynecol* 2005;25(4):346–52.
34. Romero R, Kusanovic JP, Espinoza J, Gotsch F, Nhan-Chang CL, Erez O, et al. What is amniotic fluid ‘sludge’? *Ultrasound Obstet Gynecol* 2007;30(5):793–8.
35. Kusanovic JP, Espinoza J, Romero R, Goncalves LF, Nien JK, Soto E, et al. Clinical significance of the presence of amniotic fluid ‘sludge’ in asymptomatic patients at high risk for spontaneous preterm delivery. *Ultrasound Obstet Gynecol* 2007;30(5):706–14.