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## The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor

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### Abstract

**Objective**—The amniotic cavity is normally sterile for bacteria. However, experimental evidence indicates that regular uterine contractions exert a suction-like effect whereby vaginal fluid ascends into the uterine cavity with contractions (demonstrated by sonohysterography contrast media). Consequently, this study was conducted to determine whether the presence and progress of labor are associated with an increased risk of microbial invasion of the amniotic cavity (MIAC), intraamniotic inflammation, and histologic chorioamnionitis in women with term pregnancies with intact membranes.

**Study Design**—Amniotic fluid (AF) was obtained from term singleton pregnant women with intact membranes at the time of cesarean delivery. AF was cultured for aerobic and anaerobic bacteria and genital *mycoplasma*, and white blood cell (WBC) count was determined. Patients were divided into 3 groups according to the presence or absence of labor and the progress of labor. Nonparametric statistics were used for analysis.

**Results**—Results included: (1) a total of 884 pregnant women were enrolled and divided into 3 groups: group 1, not in labor ( $n = 775$ ); group 2, in early labor (cervical dilatation less than 3 cm) ( $n = 86$ ); and group 3, in active labor (Cervical dilatation 4 cm or greater) ( $n = 23$ ); (2) the frequency of MIAC was 1% (6 of 775) in women not in labor, 3.5% (3 of 86) in patients with early labor, and 13% (3 of 23) in patients with active labor; and (3) the median AF WBC count and the frequency of histologic chorioamnionitis were also higher in the presence of labor than in the absence of labor.

**Conclusion**—We came to the following conclusions: (1) labor is associated with an increased risk of MIAC, a higher median AF WBC count, and histologic chorioamnionitis in term pregnancy with intact membranes; (2) the more advanced the cervical dilatation, the greater the risk of MIAC, a higher median AF WBC count, and histologic chorioamnionitis; and (3) in contrast, fetal inflammation (funisitis) did not increase with the presence of labor or as a function of cervical dilatation. We propose that labor predisposes to MIAC, a higher median AF WBC count, and histologic chorioamnionitis.

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## Keywords

amniotic fluid infection; amniotic fluid white blood cell count; bacteria; chorioamnionitis; labor; parturition; pregnancy; term pregnancy

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## INTRODUCTION

The amniotic cavity of women not in labor with intact membranes is considered to be free of bacteria based on studies using cultivation techniques. Microbial invasion of amniotic cavity (MIAC) was detected in approximately 19% of patients in spontaneous labor at term with intact membranes<sup>1</sup> and in 34% of patients with premature rupture of membranes (PROM) at term.<sup>2</sup>

Recent experimental evidence indicates that uterine contractions exert a suction-like effect whereby vaginal fluid can ascent into the uterine cavity with contractions (demonstrated by sonohysterography with contrast media).<sup>3</sup> Therefore, it is possible that uterine contractions during labor increase the risk of MIAC.

The purpose of this study was to determine whether the presence and the progress of labor are associated with an increased risk of intraamniotic infection, changes in the amniotic fluid (AF) white blood cell (WBC) count, and the frequency of histologic chorioamnionitis in women with term pregnancies with intact membranes.

## MATERIALS AND METHODS

### Study design

A retrospective analysis was conducted on pregnant women at term with intact membranes who delivered singleton neonates (gestational age 37 weeks or longer) at Seoul National University Hospital and who had AF retrieved at the time of cesarean delivery. This criterion (inclusion of patients whose AF was obtained at the time of cesarean delivery) was used to preserve a meaningful temporal relationship between the results of AF studies and the histological findings of the placenta and umbilical cord obtained at birth. Fetuses with major congenital anomalies or stillbirths were excluded.

Patients were divided into 3 groups according to the presence or absence of labor and the progress of labor: group 1, women not in labor; group 2, women in early labor (cervical dilatation less than 4 cm); group 3, women in active labor (Cervical dilatation 4 cm or greater). Written informed consent was obtained from all women who donated AF for research purposes. The Institutional Review Board of the Seoul National University Hospital approved the collection and the use of these samples and 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

Fluid was retrieved by puncture of the fetal membranes using 21-gauge needle under direct visualization after the careful incision of uterine wall at the time of cesarean section. AF was cultured for aerobic and anaerobic bacteria as well as genital *mycoplasmas* (*Ureaplasma urealyticum* and *Mycoplasma hominis*). MIAC was defined as a positive AF culture for microorganisms. An aliquot of AF was transported to the laboratory and examined in the hemocytometer chamber to determine WBC count.

## Histologic chorioamnionitis and funisitis

Placentas were subjected to histopathologic evaluation; sections included the chorion-amnion, the chorionic plate, and the umbilical cord. These samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin. Histopathologic examination was performed by a pathologist who was blinded to the clinical information. Acute histologic chorioamnionitis was defined as the presence of acute inflammatory change in any part of the tissue sample (e.g. amnion, chorion-decidua, umbilical cord, and chorionic plate); funisitis was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly according to the criteria previously described in detail.<sup>4</sup>

## Statistical analysis

Proportions were compared with the use of the Fisher's exact test or  $\chi^2$  test. The Kruskal-Wallis analysis of variance test was used for comparison of continuous variables among groups. Multiple comparisons between groups were performed with the Mann-Whitney U test.  $P < 0.5$  was considered significant.

## RESULTS

A total of 884 singleton pregnant women were enrolled and divided into 3 groups according to the presence or absence of labor and the progress of labor: group 1, women not in labor ( $n = 775$ ); group 2, women in early labor (cervical dilatation less than 4 cm) ( $n = 86$ ); group 3, women in active labor (cervical dilatation 4 cm or greater) ( $n = 23$ ).

The clinical indications for cesarean deliveries included previous cesarean section ( $n = 632$ ), malpresentation ( $n = 118$ ), placenta previa ( $n = 30$ ), previous uterine surgery ( $n = 25$ ), nonreassuring fetal heart testing ( $n = 17$ ), fetal macrosomia ( $n = 14$ ), failure to progress ( $n = 12$ ), and others ( $n = 36$ ).

The prevalence of a positive AF culture for microorganisms was 1% (12 of 884) in women at term pregnancy with intact membranes; the frequency of MIAC was 1% (6 of 775) in women not in labor at term. Labor at term with intact membranes was associated with MIAC in 3.5% (3 of 86) of cases if the cervical dilatation was less than 4 cm and 13% (3 of 23) if the cervical dilatation was more than 4 cm; *U. urealyticum* was the microorganism most frequently isolated from the amniotic cavity ( $n = 4$ ). Other isolates included *Staphylococcus epidermidis* ( $n = 2$ ), and 1 each of *Streptococcus agalactiae*, Group B *Streptococcus*, *S. viridans*, *Corynebacterium*, and coagulase negative *Staphylococcus*, and an unidentified Gram-positive rod. Histologic chorioamnionitis and funisitis was present in 6% (51 of 884) and 1% (10 of 884) of cases at term with intact membranes, respectively.

Table 1 compares the clinical characteristics and outcomes of the study population according to the presence or absence of labor as well as cervical dilatation. The presence of labor as well as advanced cervical dilatation were associated with a higher frequency of intraamniotic infection and histologic chorioamnionitis as well as higher median AF WBC count than the absence of labor ( $P < .01$  for each; Table 1).

Table 2 displays the characteristics of patients who have a positive AF culture for microorganisms.

## COMMENT

The principal findings of this study were: (1) MIAC and histologic chorioamnionitis is present in 1% and 6% of patients in term pregnancy with intact membranes, respectively; (2)

the presence of labor at term is associated with an increased risk of MIAC and a higher median AF WBC count; (3) patients who had entered the active phase of labor at term with intact membranes had a higher rate of MIAC and histologic chorioamnionitis than those with a cervical dilatation of less than 4 cm; and (4) histologic evidence of fetal inflammation (funisitis) was not associated with either the presence or absence of labor or the degree of cervical dilatation.

Strong evidence suggests that intrauterine infection and intraamniotic inflammation are associated with preterm delivery<sup>5–13</sup> as well as injury of preterm neonates.<sup>14–16</sup>

Similarly, epidemiologic and clinical evidence suggest an association between chorioamnionitis and cerebral palsy in patients at term.<sup>17–19</sup>

The frequency of MIAC has been reported to be 19% in spontaneous labor at term and 34% in patients with PROM at term.<sup>1,2</sup> Therefore, the presence of microorganisms in the AF is high in women at term during labor or after rupture of membranes.

The frequency of MIAC in previous studies was higher in women with spontaneous labor at term than that observed in our study (19% vs 6%).<sup>1</sup> One potential explanation for this observation is that patients in the previous study were suspected to have preterm labor but were found to have subsequently delivered a term neonate by physical examination, birthweight, the absence of complications, and a lung maturity test consistent with the presence of surfactant. These patients may represent a unique population. In contrast, all patients in the current study had intact fetal membranes and had undergone AF retrieval at the time of cesarean delivery.

However, not all patients were in spontaneous labor. Some had labor induced and this may represent a difference between the 2 studies. One limitation of this study is that we used cultivation techniques to identify MIAC. It is possible that the estimates for MIAC will be different with the use of molecular microbiologic techniques. We previously demonstrated that molecular microbiology is more sensitive than cultivation techniques in the detection of microorganisms.<sup>20,21</sup>

In keeping with other studies,<sup>22–29</sup> the most frequent microorganisms isolated from the AF were *U. urealyticum* and *Streptococcus* species, which are common constituents of normal cervicovaginal flora. This suggests that the lower genital tract is the most likely source of these microorganisms and supports the view that ascending infection occurs during the course of term labor.<sup>30–33</sup>

In conclusion, the results of this study suggest that labor is associated with MIAC and that the more advanced the cervical dilatation, the higher the risk.

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Table 1

Clinical characteristics and outcomes of the study population according to the presence or absence of labor as well as cervical dilatation

Characteristics	No labor (Group 1, n = 775)	<i>p<sup>a</sup></i>	Early labor (Group 2, n = 86)	<i>p<sup>b</sup></i>	Active labor (Group 3, n = 23)	<i>p<sup>c</sup></i>	<i>p<sup>d</sup></i>
Maternal age (y, median and range) <sup>e</sup>	32 (17–46)	NS	33 (24–44)	NS	30 (22–38)	NS	NS <sup>f</sup>
Nulliparity	149 (19.2%)	NS	23 (26.7%)	<.01	14 (60.9%)	<.001	<.001 <sup>g</sup>
Gestational age at delivery (wks, median and range) <sup>e</sup>	38.4 (37.0–41.6)	.076	38.1 (37.0–42.0)	<.001	39.9 (37.6–42.1)	<.001	<.001 <sup>f</sup>
Birthweight (g, median and range) <sup>e</sup>	3230 (1900–5410)	NS	3205 (1730–4740)	<.05	3510 (2850–4890)	<.05	<.05 <sup>f</sup>
Indication for cesarean delivery							
Previous cesarean section	579 (74.7%)		49 (57.0%)		4 (17.4%)		
Malpresentation	105 (13.5%)		11 (12.8%)		2 (8.7%)		
Placenta previa	26 (3.4%)		4 (4.7%)		0 (0%)		
Previous uterine surgery	24 (3.1%)		1 (1.2%)		0 (0%)		
Nonreassuring fetal heart testing	5 (0.6%)		8 (9.3%)		4 (17.4%)		
Fetal macrosomia	8 (1.0%)		4 (4.7%)		2 (8.7%)		
Failure to progress	0 (0%)		1 (1.2%)		11 (47.8%)		
Others	28 (3.6%)		8 (9.3%)		0 (0%)		
AF WBC count (cells/mm <sup>3</sup> ) <sup>e</sup>	2 (0 to > 1000)	NS	3 (0 to > 1000)	<.05	10 (0 to > 1000)	<.001	<.005 <sup>f</sup>
Positive AF culture	6 (0.8%)	.052	3 (3.5%)	NS	3 (13.0%)	<.01	<.001 <sup>g</sup>
Histologic chorioamnionitis	34 (4.4%)	<.01	10 (11.6%)	<.05	7 (30.4%)	<.001	<.001 <sup>g</sup>
Funisitis	8 (1.0%)	NS	2 (2.3%)	NS	0 (0%)	NS	NS <sup>g</sup>

AF, AMNIOTIC FLUID; NS, not significant; WBC, white blood cell.

<sup>a</sup> Comparison between Groups 1 and 2.

<sup>b</sup> Comparison between Groups 2 and 3.

<sup>c</sup> Comparison between Groups 1 and 3.

<sup>d</sup> Comparison between Groups 1, 2, and 3.

<sup>e</sup> Values are given as median and range.

<sup>f</sup> By Kruskal-Wallis analysis of variance test.

By  $\chi^2$  test.

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Table 2

Characteristics of 12 patients with a positive amniotic fluid culture for microorganisms

No.	Gestational age at delivery (wks)	AF WBC count (cells/mm <sup>3</sup> )	Histologic chorioamnionitis	Funisitis	Isolated microorganism	Labor
1	37.7	6	(-)	(-)	<i>Staphylococcus epidermidis</i>	(-)
2	38.4	0	(-)	(-)	<i>Ureaplasma urealyticum</i>	(-)
3	38.4	2	(-)	(-)	<i>Ureaplasma urealyticum</i>	(-)
4	38.4	8	(-)	(-)	<i>Cornibacterium</i>	(-)
5	38.6	126	(-)	(-)	<i>Cogulaise negative Staphylococcus</i>	(-)
6	39.0	3	(-)	(-)	<i>Ureaplasma urealyticum</i>	(-)
7	37.1	Greater than 1000	(-)	(-)	<i>Gram-positive rod</i>	Early
8	38.4	0	(-)	(-)	<i>Streptococcus viridans</i>	Early
9	42.0	Greater than 1000	(-)	(-)	Group B <i>Streptococcus</i>	Early
10	38.7	Greater than 1000	(+)	(-)	<i>Ureaplasma urealyticum</i>	Active
11	39.7	Greater than 1000	(+)	(-)	<i>Staphylococcus epidermidis</i>	Active
12	40.1	Greater than 1000	(-)	(-)	<i>Streptococcus agalactiae</i>	Active