Cervical ureaplasma colonization affects intraamniotic inflammation in preterm labor with intact membrane: a cohort study

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Research Article

Keywords: Amniotic fluid, Chorioamnionitis, Preterm birth, Ureaplasma

Posted Date: May 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-453082/v1

Abstract

Background: A causative role between cervical *ureaplasma* colonization and adverse outcomes during pregnancy has remained controversial. We investigated whether cervical *ureaplasma* colonization affects the biochemically or histologically intraamniotic inflammation in preterm birth.

Methods: Amniotic fluid was retrieved during delivery. Various chorioamnionitis-related cytokines (interleukin (IL)-1β, -6, -8, -10, and tumor necrosis factor-α) and regulators (matrix metalloproteins (MMP)-8 and MMP-9) were measured with Human Magnetic Luminex screening assay. We tested cervical swab specimens using real-time polymerase chain reaction assays for the detection of *ureaplasma* spp. colonization. Considering the clinical situation that causes intraamniotic infection, we arbitrarily divided into three categories of preterm labor with intact membrane, preterm premature rupture of membrane (PPROM), and control group with no exposure to preterm labor or preterm premature rupture of membrane.

Results: The incidence of cervical *ureaplasma* colonization was 49.3% (136/276). The incidence of histologic chorioamnionitis was 27.5% (76/200). All differences in cytokines and regulators according to histologic chorioamnionitis were significant. Of the 153 cases that experienced preterm labor with intact membrane, IL-10, MMP-8, and MMP-9 levels in the *ureaplasma* positive group were significantly higher than those of the *ureaplasma* negative group. According to logistic regression analysis adjusted to preterm labor with intact membrane, PPROM, and gestational age at delivery, cervical *ureaplasma* colonization was an independent risk factor of histologic chorioamnionitis (odd ratio: 2.622, 95% confidence interval: 1.443-4.766).

Conclusions: Cervical *ureaplasma* colonization augments biochemically intraamniotic inflammation in preterm labor with intact membrane, and was an independent risk factor of histologic chorioamnionitis.

Introduction

Preterm birth (defined by a birth occurring at less than 37 weeks' gestation) is a major determinants of maternal and neonatal morbidities [1]. Though improvements of perinatal and neonatal outcomes, preterm birth has consistently increased in the world [2]. Among the causes of preterm birth, intraamniotic infection accounts for 25 to 40 percent of preterm births [3, 4]. However, intraamniotic infection is not clinically apparent in most case of preterm labor or preterm birth [4]. Accumulating evidences suggests that intraamniotic inflammation is associated with a variety of dysregulated cytokines such as interleukin (IL)-1ß, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α) and inflammatory regulators such as matrix metalloproteinase (MMP)-8 and MMP-9 [5-11]. Inflammatory cytokines of amniotic fluid have been shown associated with delivery at lower gestational ages in women with preterm labor [9]. Although bacterial infections within the uterus of the pregnant women can occur between the maternal tissues and the fetal membrane, increased inflammatory cytokines cause fetal inflammatory response syndrome and are known to affect not only preterm birth but also lung and brain of preterm infant [4, 12-14].

Of all pathogenic bacteria associated with intraamniotic infection, *ureaplasma* is the most common organism that is isolated from infected amniotic fluid and the placenta and is associated with an increased risk for preterm labor and perinatal morbidity [6, 15-18]. *Ureaplasma*, which colonizes the lower genitalia, penetrates into the choriodecidual space and can cause preterm labor [14]. On the contrary, *ureaplasma* is considered to be a commensal organism within the female lower genital tract according to recent reports demonstrating no differences in the rates of *ureaplasma* colonization between women with and without symptomatic genital infection [19, 20]. In particular, *ureaplasma*-associated inflammation within the chorioamnion may vary between 0-100% [21]. A causative role between *ureaplasma* colonization in the lower genital tract and adverse outcomes during pregnancy has remained controversial. Therefore, in the present study, we investigated whether cervical *ureaplasma* colonization affects the intraamniotic inflammation in preterm birth.

Methods

1. Collection of amniotic fluids

Before the collection of amniotic fluid, an agreement with Keimyung Human Bio-Resource Bank was made, an approval from the National Biobank of Korea was received [22, 23]. For the collection of amniotic fluid during their cesarean section, we acquired informed consent from pregnant women with threatened preterm birth between 24-34 weeks of gestation. After administration of spinal anesthesia to the pregnant women, low transverse cesarean incision and hysterotomy was conducted. The myometrium was incised with shallow strokes to avoid amniotic sac rupture. When the amniotic sac was exposed (Fig. 1), amniocentesis was performed using a 21-gauge needle. In vaginal delivery, it was performed only when the intact membranes were exposed. To reduce any further risk of pregnant women and baby during delivery, amniocentesis was not performed in patients with general anesthesia, severe oligohydramnios (amniotic fluid index < 1.0), suspected placental abruption with fetal distress, or unexpected amniotic sac rupture. The collected amniotic fluid was centrifuged, and the supernatant was aliquoted and stored at -80°C at the Keimyung Human Bio-Resource Bank. Samples were not subjected to freeze-thaw cycles before being assayed. This study was approved by the Dongsan Medical Center Institutional Review Board (approval No. DSMC 2020-01-001-001), and conducted in accordance with the principles of the Declaration of Helsinki. Amniotic fluids between January 2017 and December 2019 were provided by the Keimyung Human Bio-Resource Bank.

2. Inclusion and exclusion criteria

The observational cohort study was conducted at High-Risk Maternal and Newborn Integrated Care Center, Keimyung University Dongsan Hospital, Daegu, Republic of Korea. As shown in Figure 2, three hundred fourteen amniotic fluids were collected from 257 pregnant women from January 2017 to December 2019. The clinical information of pregnant women on the amniotic fluid was investigated through medical records, including cervical *ureaplasma* colonization and histologic chorioamnionitis. First, we excluded 12 cases with no investigation of cervical *ureaplasma* colonization. To avoid the

skewed outcomes by other microorganisms colonized in the cervix, we also excluded 20 cases with *mycoplasma* colonization, 5 cases with *chlamydia* colonization, and 2 cases with *trichomonas* colonization. Considering the clinical situation that causes intraamniotic infection, we divided into three categories of preterm labor with intact membrane, preterm premature rupture of membrane (PPROM), and control group without preterm labor or preterm premature rupture of membrane.

For the further comparison, we also investigated other clinical characteristics, including maternal age, parity, cerclage intervention, gestational diabetes, pregnancy-induced hypertension, placenta previa, placenta abruptio, oligohydramnios, gestational age at delivery, and birth weight. Gestational age was estimated on the basis of the mother's last menstrual period and ultrasonography findings. Cerclage intervention was defined as a prophylactic operative intervention to treat painless cervical dilation in the second trimester. Preterm labor was defined as regular contractions of the uterus resulting in changes in the cervix. PPROM was defined as the rupture of membranes earlier than 24 hours before the onset of labor.

3. Amniotic fluid analysis

Five inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) and two inflammatory regulators (MMP-8 and MMP-9) of amniotic fluid were quantitatively measured with a Human Magnetic Luminex screening assay (R&D Systems, Minneapolis, MN, USA) on the Bio-Plex 200 (Bio-rad, CA, USA). All the measurements were carried out strictly according to the manufacturer's instructions, and all samples were measured in duplicate at the same time. For the IL-1β assay, a standard curve was developed from 39.0 to 9488.0 pg/mL with a sensitivity of 1.6 pg/mL; for the IL-6 assay, the curve was linear from 9.6 to 2308.0 pg/mL with a sensitivity of 3.4 pg/mL; for the IL-8 assay, the curve was linear from 10.4 to 2510.0 pg/mL with a sensitivity of 3.6 pg/mL; for the IL-10 assay, the curve was linear from 9.6 to 2324.0 pg/mL with a sensitivity of 3.2 pg/mL; for the TNF-α assay, the curve was linear from 19.4 to 4718.0 pg/mL with a sensitivity of 2.4 pg/mL; for the MMP-8 assay, the curve was linear from 490.2 to 119124.0 pg/mL with a sensitivity of 68.4 pg/mL; and for the MMP-9 assay, the curve was linear from 6,705.0 to 1629800.0 pg/mL with a sensitivity of 680.0 pg/mL.

4. Pathological investigation of placenta

Placentas were subjected to histologic evaluation; representative sections included the chorioamnion, 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. Histologic chorioamnionitis was diagnosed when acute inflammation was observed in any of the placental tissues.

5. Drtection of cervical *ureaplasma* colonization

Detection of *ureaplasma* colonization in the cervix was performed by means of the Anyplex II Sexually Transmitted Infections-7 Kit (STI-7 Seegene, Seoul, Republic of Korea), a commercially available multiplex real-time polymerase chain reaction Prelying on newly developed Tagging Oligonucleotide Cleavage and

Extension technology which allows for the simultaneous detection of seven microorganisms (*ureaplasma urealyticum, ureaplasma parvum, mycoplasma hominis, mycoplasma genitalium, chlamydia trachomatis, neisseria gonorrhoeae, trichomonas vaginalis*).

6. Statistical analysis

Prior to statistical analysis, values of inflammatory cytokines and regulators were log2 transformed. Continuous variables were expressed as means ± standard deviation and categorical variables as numbers and proportions. The clinical characteristics and mean values of the inflammatory cytokines and regulators were compared according to the presence or absence of histological chorioamnionitis. Comparisons between categorical variables were performed using the chi-square test or Fisher's exact test, and those between continuous variables were performed using the independent t-test. To compare the mean values of the inflammatory cytokines and regulators between the three clinical categories, a one-way analysis of variance (ANOVA) was used. Post-hoc analysis was performed with Bonferroni method. Previous studies reported that the earlier the gestational age, the more intense the intraamniotic inflammation in women with preterm labor and PPROM [7-9, 24, 25]. Considering the aforementioned studies, we investigated the Pearson correlation coefficient between gestational age at delivery and inflammatory cytokines and regulators of amniotic fluid. Finally, multivariate logistic regression analysis was conducted to investigate whether cervical *ureaplasma* colonization is an independent risk factor for the histologic chorioamnionitis, including previously known associated factors, such as preterm labor with intact membrane, preterm premature rupture of membrane, and gestational age at delivery [6-9, 24, 25]. Statistical analysis was done using SPSS version 25.0 (IBM Co., Armonk, NY, USA). A P value of <0.05 was considered statistically significant.

Results

Of the 276 total cases included for analysis, histologic chorioamnionitis was present in 76 cases (27.5%) while cervical *ureaplasma* colonization was present in 136 cases (49.3%). Table 1 shows the comparison of clinical characteristics and mean values of inflammatory cytokines and regulators according to the presence or absence of cervical *ureaplasma* colonization. At first, the incidence of cervical *ureaplasma* colonization in the histologic chorioamnionitis group was also significantly lower than that in the control group (64.5% [49/76] vs 43.5% [87/200], $P = 0.002$). In addition, gestational age at delivery and birth weight in the histologic chorioamnionitis group was significantly lower than that in the control group (30.1 ± 2.7 vs 31.7 ± 2.2 weeks, $P < 0.001$). The incidence of pregnancy-induced hypertension in the histologic chorioamnionitis group was also significantly lower than that in the control group (15.8% [12/76] vs 32.5% [65/200], $P = 0.006$). However, there were no differences in the preterm labor with intact membrane, and PPROM rates between the two groups. In the comparison of inflammatory cytokines and regulators, all were significantly increased in the histologic chorioamnionitis group ($P < 0.001$)

Table 1
Comparison of clinical characteristics and mean values of inflammatory cytokines and regulators according to the presence or absence of histologic chorioamnionitis.

Variables	Histologic chorioamnionitis (n = 76)	Control (n = 200)	*P* value
Maternal age, years	32.8 ± 4.1	33.3 ± 4.1	0.353
Cerclage intervention, n (%)	12 (15.8)	26 (13.0)	0.548
Pregnancy-induced hypertension, n (%)	12 (15.8)	65 (32.5)	0.006
Gestational diabetes, n (%)	6 (7.9)	27 (13.5)	0.200
Oligohydramnios, n (%)	5 (6.6)	29 (14.5)	0.074
Preterm labor with intact membrane, n (%)	46 (60.5)	107 (53.5)	0.294
Premature rupture of membrane, n (%)	21 (27.6)	35 (17.5)	0.062
Placenta previa, n (%)	3 (3.9)	9 (4.5)	0.841
Cervical ureaplasma colonization, n (%)	49 (64.5)	87 (43.5)	0.002
Gestational age, weeks	30.1 ± 2.7	31.7 ± 2.2	< 0.001
Birth weight, *g*	1480 ± 483	1651 ± 508	0.012
Apgar score, 1min	6.2 ± 1.6	6.4 ± 1.4	0.249
Apgar score, 5min	8.0 ± 1.0	8.2 ± 0.8	0.168
Inflammatory cytokines and regulators (Logarithymically)			
IL-1ß	6.6 ± 4.8	1.5 ± 2.2	< 0.001
IL-6	13.8 ± 3.5	9.3 ± 2.7	< 0.001
IL-8	12.1 ± 1.3	10.2 ± 1.6	< 0.001
IL-10	4.4 ± 2.3	2.3 ± 0.7	< 0.001
TNF-α	5.6 ± 3.1	3.0 ± 0.9	< 0.001
MMP-8	15.9 ± 3.6	11.2 ± 2.4	< 0.001

Continuous variables are expressed as mean ± standard deviation.

IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteins.

Variables	Histologic chorioamnionitis (n = 76)	Control (n = 200)	*P* value
MMP-9	12.4 ± 3.5	9.7 ± 1.2	< 0.001
Continuous variables are expressed as mean ± standard deviation.			
IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteins.			

Figure 3 shows the comparison of mean values of inflammatory cytokines and regulators among the three clinical categories that causes intra-amniotic infection by one-way ANOVA. All of inflammatory cytokines and regulators concentrations in preterm labor with intact membrane or PPROM were significantly higher than those in control without preterm labor or PPROM ($P < 0.05$). However, Log IL-10 and MMP-9 concentrations in PPROM were significantly higher than those in preterm labor with intact membrane.

Table 2 shows the correlation between gestational age at delivery and inflammatory cytokines and regulators of amniotic fluid. In preterm labor with intact membrane, IL-1ß, IL-6, IL-10, and MMP-8 concentrations of amniotic fluid collected at delivery were shown a weakly or moderately negative correlation with gestational age at delivery ($P < 0.05$). In PPROM, IL-1ß, IL-6, IL-10, and MMP-8 concentrations of amniotic fluid were shown a weakly negative correlation with gestational age at delivery ($P < 0.05$) However, none of the inflammatory cytokines and regulators were significantly correlated with gestational age at delivery in control without preterm labor or PPROM.

Table 2
Correlation between gestational age at delivery and inflammatory cytokines and regulators of amniotic fluid.

Variables	Preterm labor with intact membrane (n = 153)		PPROM (n = 66)		Control (n = 74)	
	Pearson's coefficient	*P* value	Pearson's coefficient	*P* value	Pearson's coefficient	*P* value
IL-1β	-0.412	< 0.001	-0.334	0.006	-0.082	0.488
IL-6	-0.375	< 0.001	-0.295	0.016	0.014	0.905
IL-8	-0.279	< 0.001	-0.119	0.340	-0.047	0.691
IL-10	-0.307	< 0.001	-0.280	0.023	-0.117	0.319
TNF-α	-0.376	< 0.001	-0.231	0.062	-0.182	0.121
MMP-8	-0.382	< 0.001	-0.364	0.003	-0.230	0.049
MMP-9	-0.251	0.001	-0.208	0.094	-0.150	0.201

IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteins.

Figure 4 shows the comparison of inflammatory cytokines and regulators according to the presence or absence of cervical *ureaplasma* colonization. Of the 153 cases that experienced preterm labor with intact membrane, IL-10, MMP-8, and MMP-9 levels in the *ureaplasma* positive group were significantly higher than those of the *ureaplasma* negative group. Of the 56 cases with PPROM and 67 cases with control group, there were no significant differences.

Figure 5 shows the comparison of inflammatory cytokines and regulators between *Ureaplasma parvum* and *ureaplasma urealyticum*. Except for 3 cases in which both *Ureaplasma parvum* and *ureaplasma urealyticum* were colonized in cervix, *Ureaplasma parvum* and *ureaplasma urealyticum* were present in 100 and 30 cases, respectively. There were no significant differences in any inflammation-related cytokines and regulators between the two groups.

Table 3 shows the independent risk factors associated with histologic choriamnionitis by logistic regression analysis. The adjusted odd ratios (95% CI) of cervical *ureaplasma* colonization, preterm labor with intact membrane, and gestational age at delivery were 2.622 (1.443–4.766), 3.243 (1.545–6.807), and 0.731 (0.649–0.825), respectively.

Table 3
Multivariate logistic regression analysis of the clinical factors with histological chorioamnionitis

Variables	Odds ratios	95% Confidence interval	*P* value
Cervical *ureaplasma* colonization	2.622	1.443–4.766	0.002
Preterm labor with intact membrane	3.243	1.545–6.807	0.002
Premature rupture of membrane	1.853	0.946–3.632	0.072
Gestational age at delivery	0.731	0.849 – 0.825	< 0.001

Discussion

In the quantitative analysis of inflammatory cytokines and regulators of amniotic fluid collected at delivery, cervical *ureaplasma* colonization was significantly associated with intraamniotic inflammation in preterm labor with intact membrane.

According to recommendations of the American College of Obstetricians and Gynecologists, intraamniotic infection or chorioamnionitis, which is defined as an infection with resulting inflammation of any combination of amniotic fluid, placenta, fetal membranes, or decidua, can be established by amniotic fluid culture, gram stain, or both accompanied with biochemical analysis [26]. However, chorioamnionitis can be subclinical, which is defined histologically by inflammation of the chorion, amnion, and placenta [13]. In consideration of these limitations, we further analyzed the histologically proven chorioamnionitis as well as inflammatory cytokines and regulators derived in amniotic fluid.

The microorganisms that were most commonly associated with infection of the amniotic cavity were the species of *ureaplasma urealyticum*, *ureaplasma parvum*, *mycoplasma hominis*, et al [27, 28]. To investigate the association between inflammatory cytokines and regulators of the amniotic fluid and cervical *ureaplasma* colonization, 27 cases which were colonized with other organisims (*mycoplasma, chlamydia, and trichomonas*) were excluded from the present study.

Previous studies have already reported a positive relationship between inflammatory cytokines (IL-6, MMP-8) of the amniotic fluid and the stage of histologic inflammation of the placenta in preterm birth [5, 29, 30]. In the present study, cervical *ureaplasma* colonization was significantly associated with the incidence of histologic chorioamnionitis in preterm birth. In particular, increased IL-10, MMP-8, and MMP-9 concentrations in the amniotic fluid of the *ureaplasma* positive group were significantly increased in those who experienced preterm labor with intact membrane. These findings might partially support the role of cervical *ureaplasma* colonization as a risk factor for chorioamnionitis and preterm birth [31, 32]. However, other studies that have demonstrated that *ureaplasma* colonization in the lower genital tract is

not a significant predictor of preterm birth or chorioamnionitis [33–35]. In these reasons, it was difficult to confirm the role of *ureaplasma* as a causative agent of chorioamnionitis [21].

Unlike pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNF-α, IL-10 dampens inflammation. Gotsch et al. reported that IL-10 concentration in amniotic fluid is elevated in spontaneous term and preterm labor as well as intraamniotic infection [8]. MMPs lead to degradation of extracellular matrix components and modulation of cytokines, which may play an important role in spontaneous labor as well as intraamniotic infection [10, 11]. In the present study, intraamniotic infection-associated biomarkers of amniotic fluid collected at delivery, including IL-10, MMP-8, and MMP-9, was significantly different according to the presence or absence of cervical *ureaplasma* colonization.

There was inversely proportional between intraamniotic inflammation and gestational age [7–9, 24, 25]. In the present study, mean gestational age at delivery in histologic chorioamnionitis was lower, mean values of inflammatory cytokines and regulators was negatively correlated with gestational age at delivery.

Similar to the findings in previous studies [19, 20], *ureaplasma parvum* was more frequent in lower genital tract than *ureaplasma urealyticum*. *ureaplasma parvum* was more frequent in the lower genital tract than *ureaplasma urealyticum*. In the present study, there were no significant differences in the concentrations of chorioamnionitis-associated cytokines between cases with *ureaplasma parvum* and those with *ureaplasma urealyticum*. However, due to the small number of cases with *ureaplasma urealyticum* included in our study, it is impossible to determine a difference between *ureaplasma parvum* and *ureaplasma urealyticum* solely from our results.

Apart from biochemical chorioamnionitis by quantitative analysis of inflammatory cytokines and regulators of amniotic fluid, we performed a multivariate logistic regression analysis to determine whether cervical *ureaplasma* colonization is a risk factor for histologic chorioamnionitis. Although there was no significant difference between histologic chorioamnionitis and preterm labor with intact membrane or PPROM in two-by-two analysis, preterm labor with intact membrane and PPROM were included in the multivariate logistic regression analysis, considering previous studies [6–9, 24, 25]. After adjusted by preterm labor with intact membrane, PPROM, and gestational age at delivery, cervical ureaplasma colonization was an independent risk factor of histologic chorioamnionitis in the present study.

A particular strength of the present study includes the method of amniocentesis. In previous studies, amniocentesis was conducted via ultrasound-guided transabdominal approach [7, 9, 29, 30, 36–38]. Although ultrasound guidance for amniocentesis is not associated with amniocentesis-attributable complications, such as preterm labor, placental abruption, PPROM, and fetal heart rate abnormality [39], the possibility of emergent cesarean delivery after amniocentesis is a major complication to be considered during third-trimester amniocentesis [40, 41]. Considering the possibility of complications caused by ultrasound-guided transabdominal amniocentesis, we collected amniotic fluid when the amniotic sac was exposed during cesarean section. In a previous study on pregnant women who made it

to full term with intact membranes, amniotic fluid was retrieved by puncture under direct visualization after uterine incision at the time of cesarean section [42]. However, in our study, more than 50% of enrolled pregnant women experienced preterm labor, and the mean gestational age at delivery was 31.5 weeks.

The limitations of the present study are as follows: At first, although Administration of intrapartum antibiotics is recommended whenever an intraamniotic infection is suspected, there were no investigations related to antibiotic use. This study was conducted in a single tertiary institution, but it was difficult to investigate the use of antibiotics, the type of antibiotics, and the duration of antibiotic administration in the pregnant women who were recruited from secondary medical institutions. Second, other microorganisms including *gardnerella vaginalis*, *group B Streptococcus*, *candida albicans* were not investigated. Additional studies with larger population sizes are needed to clarify the association between chorioamnionitis-associated cytokines in amniotic fluid and cervical *ureaplasma* colonization in preterm delivery.

Although a causative relationship between cervical *ureaplasma* colonization and intraamniotic infection in preterm birth is impossible to conclusively determine, cervical *ureaplasma* colonization augments biochemically intraamniotic inflammation in preterm labor with intact membrane, and was an independent risk factor of histologic chorioamnionitis.

Declarations

Ethics approval and consent to participate: After the approval of the Dongsan Medical Center Institutional Review Board (approval No. DSMC 2020-01-001-001), amniotic fluids were provided by the Keimyung Human Bio-Resource Bank.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no conflicts of interest.

Funding: This work was supported by the research promoting grant from the Keimyung University Dongsan Medical Center in 2016

Authors' contributions: JGB, SK, and JHP analyzed and interpreted the patient data. ISH and JMP performed the histological examination of the placenta. JGB and JHP were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements: The biospecimens and data used for this study were provided by the Biobank of Keimyung University Dongsan Hospital Biobank, a member of the Korea Biobank Network.

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Figures

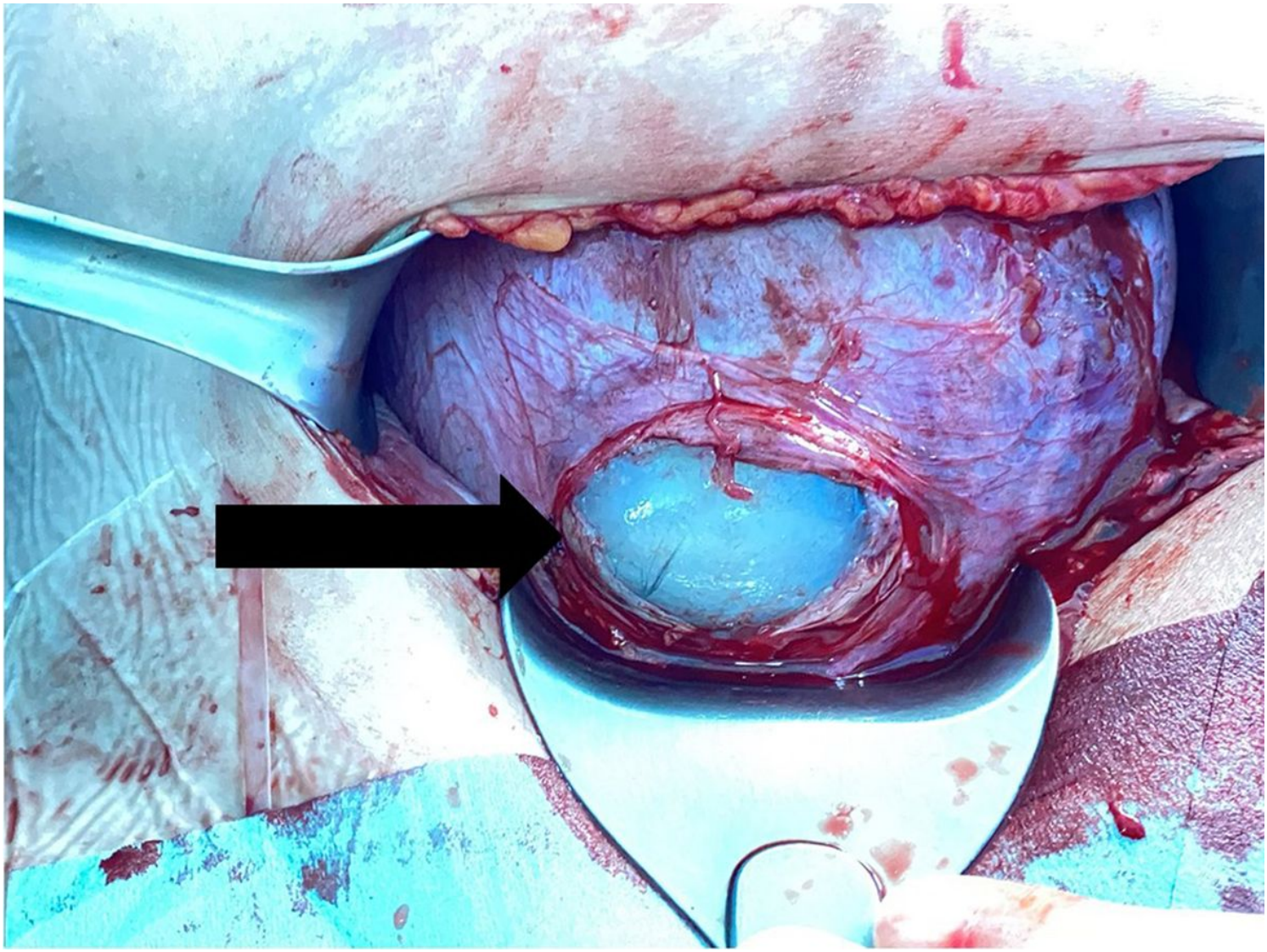

Figure 1

Amniotic membrane (arrow) exposed after the myometrium was incised with shallow strokes during cesarean section

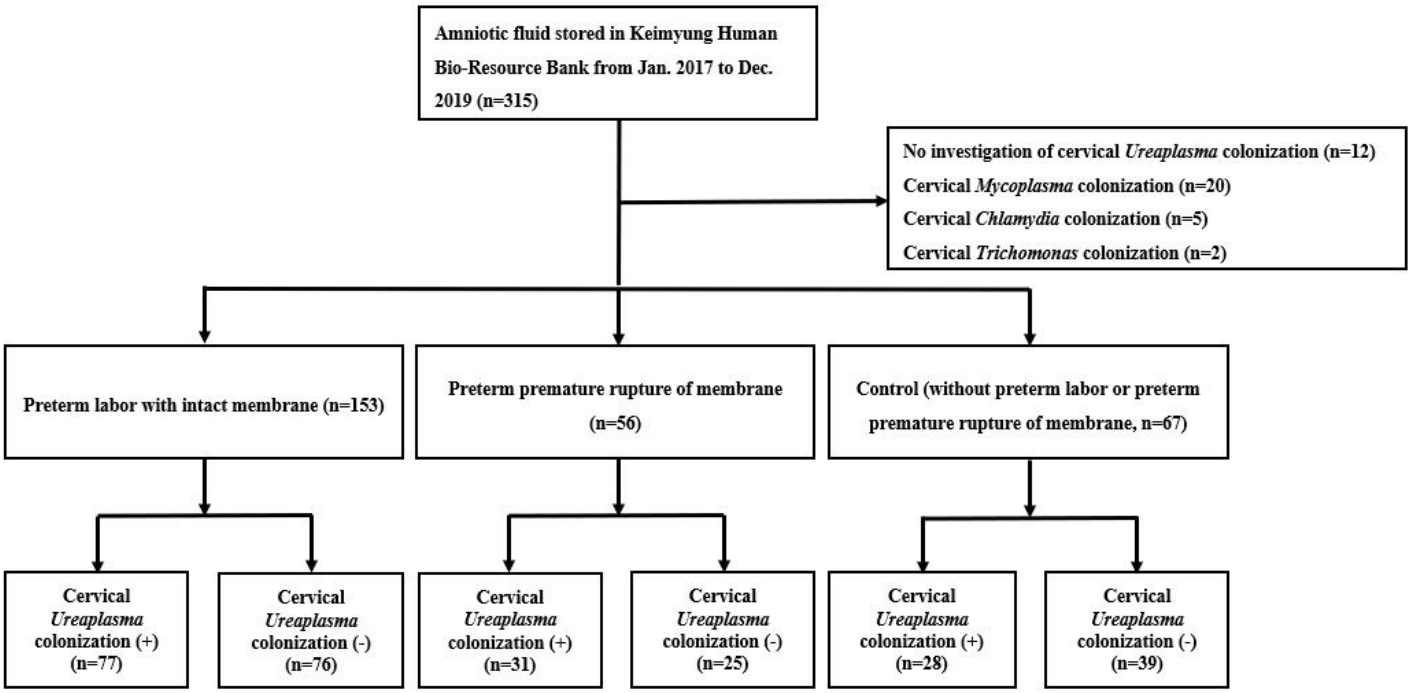

Figure 2

Relative mean values of inflammatory cytokines and regulators among the three clinical categories. *a one-way ANOVA and post-hoc comparisons, $p < 0.05$. PPROM, preterm premature rupture of membrane; TNF, tumor necrosis factor; MMP, matrix metalloproteins.

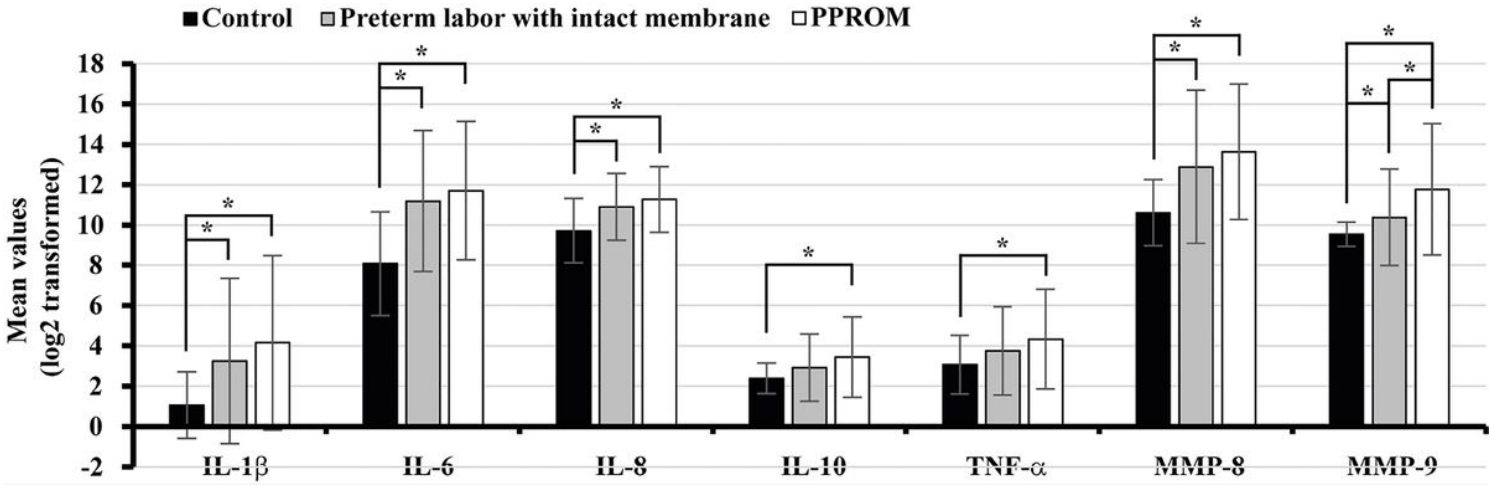

Figure 3

Relative mean values of inflammatory cytokines and regulators in the presence or absence of cervical ureaplasma colonization. (A) Control. (B) Preterm labor with intact membrane. (C) PPROM. *Independent t-test, $p < 0.05$. PPROM, preterm premature rupture of membrane; TNF, tumor necrosis factor; MMP, matrix metalloproteins.

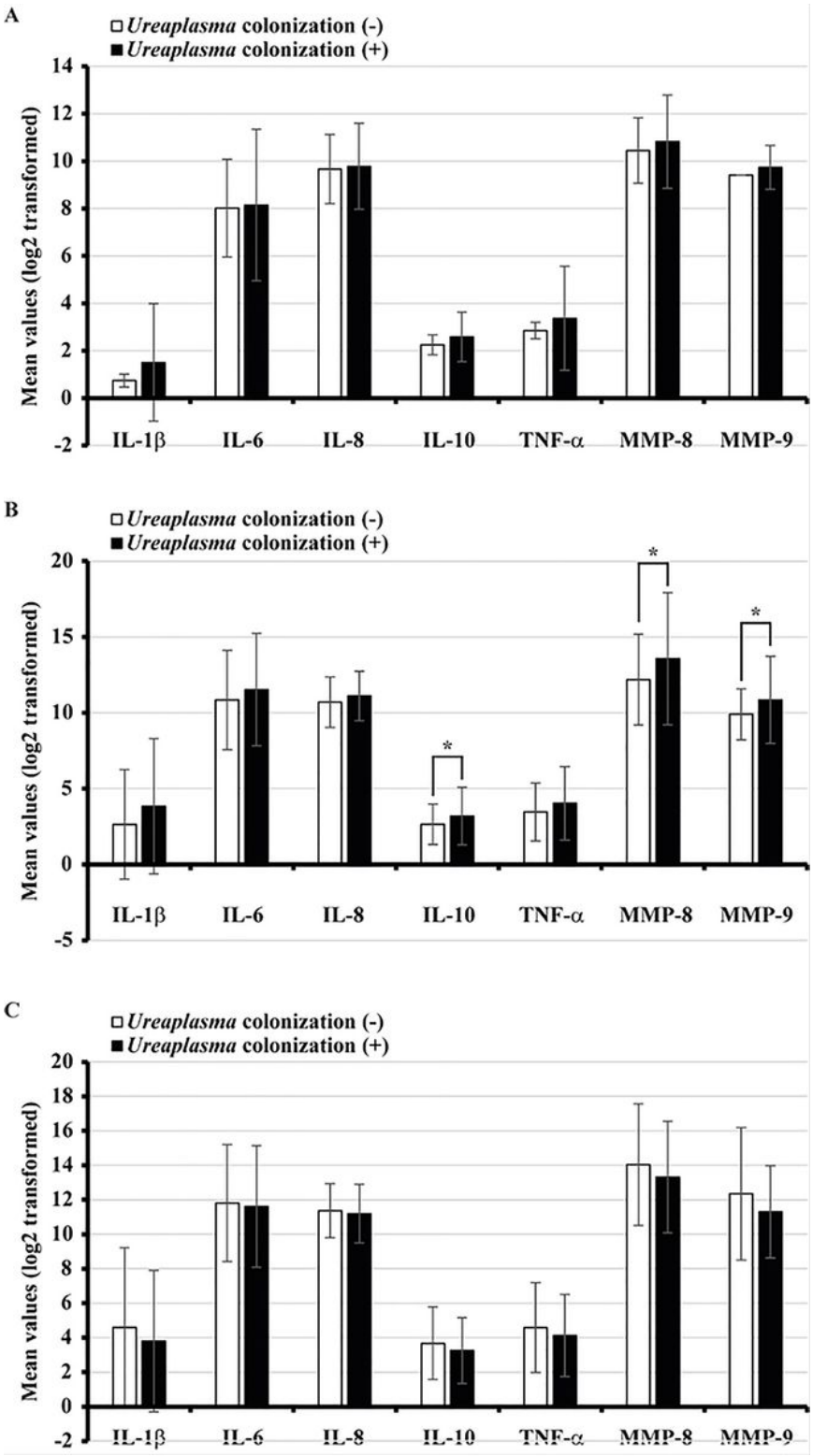

Figure 4

Relative mean values of inflammatory cytokines and regulators between Ureaplasma parvum and ureaplasma urealyticum. *Independent t-test, $p < 0.05$. TNF, tumor necrosis factor; MMP, matrix metalloproteins.

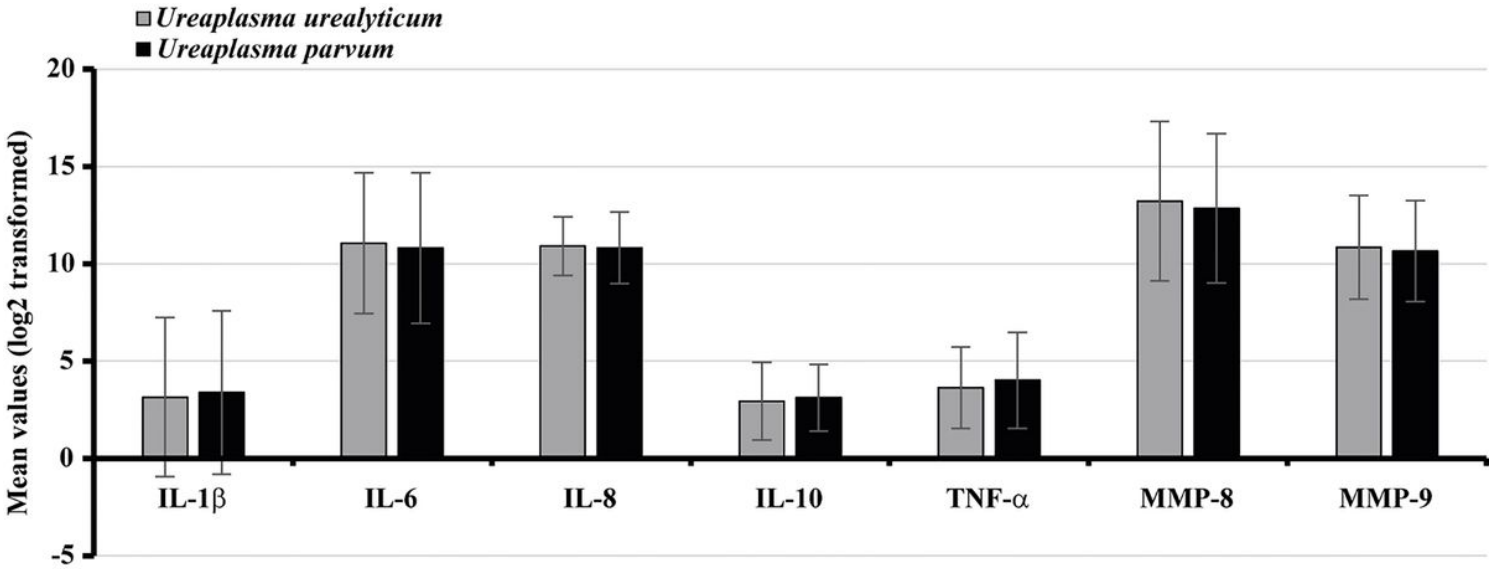

Figure 5

comparison of inflammatory cytokines and regulators between Ureaplasma parvum and ureaplasma urealyticum.