

# Detection of group B streptococcus colonization in cervical and lower vaginal secretions of pregnant women

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## Summary

**Objective:** To examine the efficacy of cervical and lower vaginal secretions in the detection of group B streptococcus (GBS) colonization in women in early and late pregnancy. **Materials and Methods:** Two-thousand women in early pregnancy (8-12 weeks of gestation) aged approximately 27 years who visited our outpatient clinic from December 2016 to December 2018 were enrolled. Cervical and lower vaginal secretions were obtained at 8-12 weeks (early pregnancy) and then at 37 weeks (late pregnancy) of gestation and screened for GBS infection. Patients were classified as follows: (i) cervical and lower vaginal secretions were GBS-positive (group A), (ii) only cervical secretions were GBS-positive (group B), (iii) only lower vaginal secretions were GBS-positive (group C), and (iv) both cervical and lower vaginal secretions were GBS-negative (group D). **Results:** No difference in the GBS-positive rate was observed between women in early and late pregnancy. The GBS-positive detection rate was lower in women from which cervical secretions were screened than that in women from which lower vaginal secretions were assayed; however, the incidence rates of adverse pregnancy events, such as abortion, preterm delivery, premature rupture of membranes and neonatal infection, were higher in women in which cervical secretions were assayed. **Conclusion:** Women in early pregnancy should be screened for GBS infection using cervical secretions.

**Key words:** Pregnancy; Group B streptococcus; Cervical secretions; Vaginal secretions; Neonate.

## Introduction

Prenatal infections can seriously threaten the safety of the mother and the baby [1-4]. Group B streptococcus (GBS) is a conditional pathogen; it is a component of the microbial environment in approximately 15-35% of healthy individuals but it can also cause disease [5]. Additionally, the rate of GBS transmission via the vagina and rectum of pregnant women is approximately 10-30% in the United States and approximately 12.7% in developing countries [6, 7], and GBS infections can also threaten neonates by causing septicemia [8], meningitis [9], and pneumonia [10]. Other studies revealed that GBS-positive pregnant woman are at an increased risk of premature rupture of membranes [11, 12], preterm delivery [13-15], and neonatal infection [16, 17]. Therefore, it is important to detect GBS colonization in pregnant women.

Most medical centers and clinics screen for GBS infection during the third trimester of pregnancy (37-40 weeks of gestation) [18-21]; however, the United States Centers for Disease Control recommend screening all women at 35-37 weeks of gestation and treating GBS-positive women with antibiotics [2], whereas the American College of Obstetricians and Gynecologists recommend screening women at 36-37 weeks and confirming results by bacterial cultures [22]. However, serious conditions, such as postpartum tricuspid endocarditis, which associates with GBS infection, as well as premature rupture of membranes and abortion, are common during the first trimester of pregnancy [5], in-

dicating that it is important to screen for GBS infection in women in early pregnancy [2, 23-25].

Rectal and lower vaginal secretions are often used in assays that screen for GBS infection [26, 27]; however, women with neonatal GBS colonization may be misdiagnosed if lower vaginal secretions are GBS-negative. Furthermore, the cervix is in close proximity to the uterus, indicating that GBS in the cervix may be transmitted to neonates during vaginal delivery. Few studies have examined the clinical significance of cervical secretions, and further investigations are needed.

In this study, we used cervical and lower vaginal secretions from women in the first and third trimester of pregnancy to assess GBS colonization and demonstrated that women in early pregnancy should be screened, because GBS colonization can increase the risks of abortion and preterm delivery. We also present a new method to screen for GBS colonization using cervical and lower vaginal secretions.

## Materials and Methods

### Patient information and ethical consent

Two-thousand women in early pregnancy (8-12 weeks of gestation) aged approximately 27 years who visited our outpatient clinic from December 2016 to December 2018 were enrolled. Patients with a history of antibiotic use 3 months prior to the commencement of the study were excluded. Cervical and lower vaginal (lower one-third of the

Table 1. — General information of pregnant women.

Group	Cases (n)	Age (years)	BMI (Kg/m <sup>2</sup> )	Gestational Weeks (w)	Medical Illness (n)	Parity (n)
A	62	29.58 ± 5.79	22.75 ± 2.71	10.0 ± 1.5	3.72 ± 2.01	1.83 ± 0.93
B	69	28.98 ± 4.92	22.82 ± 2.83	9.8 ± 1.3	4.83 ± 2.34	2.03 ± 1.02
C	123	29.50 ± 5.77	22.28 ± 3.11	9.6 ± 1.1	6.77 ± 3.12	1.95 ± 0.85
D	1746	28.95 ± 5.12	23.01 ± 3.01	9.7 ± 1.2	69.84 ± 11.45	2.01 ± 1.05
<i>F</i>	—	0.696	2.41	1.693	2650	0.727
<i>p</i>	—	0.5545	0.0652	0.1664	0.9958	0.5361

Note: Group A: both cervical secretions and the lower 1/3 of the vaginal secretions showing GBS-positive; Group B: only cervical secretions showing GBS-positive; Group C: only the lower 1/3 of the vaginal secretions showing GBS-positive; Group D: both cervical and the lower 1/3 of the vaginal secretions showing GBS-negative.

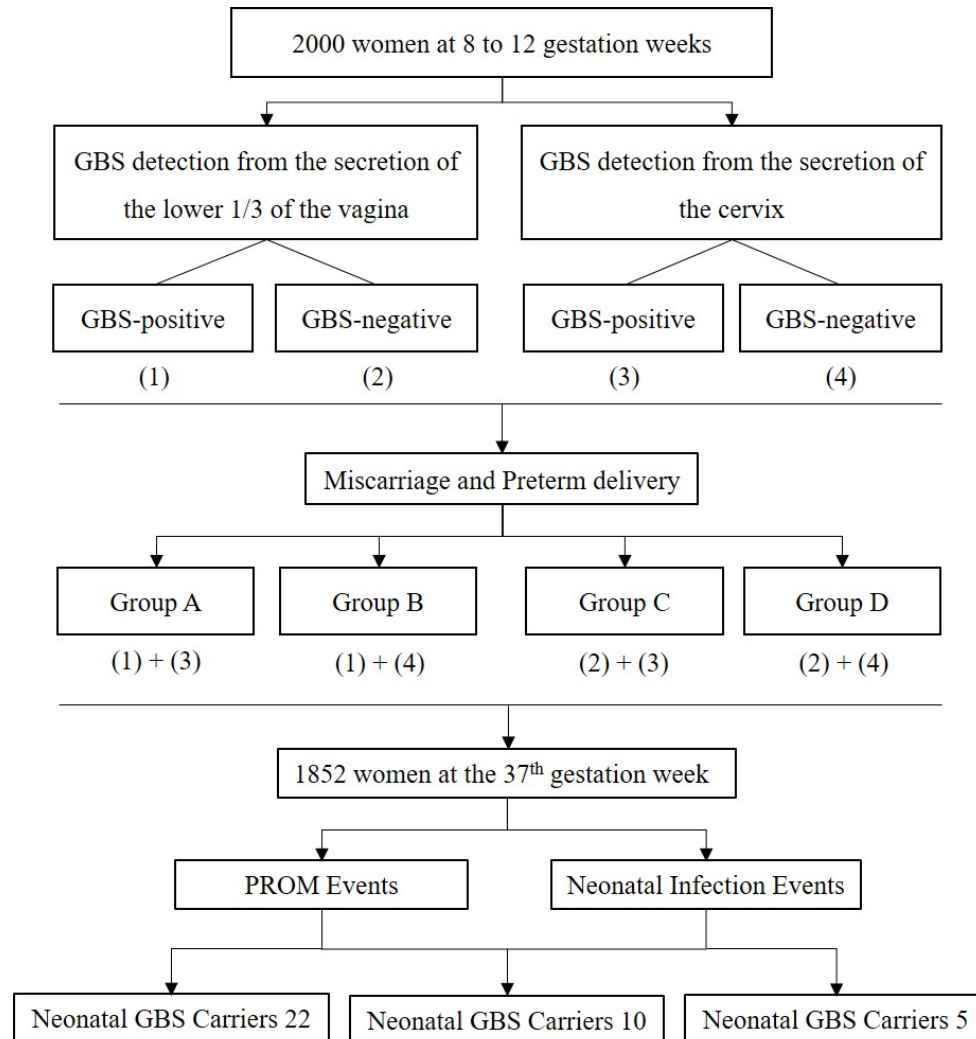


Figure 1. — The flow chart of this study.

vagina) secretions were obtained at 8-12 and 37 weeks of gestation and screened for GBS. Postoperative results were analyzed, and patients were classified as follows: (i) cervical and lower vaginal secretions were GBS-positive (group A), (ii) only cervical secretions were GBS-positive (group B), (iii) only lower vaginal secretions were GBS-positive (group C), and (iv) both cervical and lower vaginal secre-

tions were GBS-negative (group D). Pregnancy outcomes, such as a history of abortion and preterm delivery, were evaluated. The GBS was examined using the same method for the cases except abortion women at the 37<sup>th</sup> gestation week (the third trimester of pregnancy). Pregnancy outcomes, such as premature rupture of membranes and neonatal infections, were also evaluated.

Table 2. — Comparison of the rate of pregnant women, abortion and premature delivery among the four groups women in early pregnancy.

Group	Pregnant women n (%) (n = 2000)	Abortion n (%) (n = 33)	Premature delivery n (%) (n = 115)
A	62 (3.1)	3 (4.48)	9 (14.52)
B	69 (3.45)	4 (5.80)	9 (13.04)
C	123 (6.15)	3 (2.44)	8 (6.50)
D	1746 (87.3)	23 (1.32)	89 (5.10)
$\chi^2$	—	12.0514	14.2272
<i>p</i>	—	0.0072	0.0026

Group A: both cervical secretions and the lower 1/3 of the vaginal secretions showing GBS-positive; Group B: only cervical secretions showing GBS-positive; Group C: only the lower 1/3 of the vaginal secretions showing GBS-positive; Group D: both cervical and the lower 1/3 of the vaginal secretions showing GBS-negative.

Table 3. — Cases of four groups women at the 37<sup>th</sup> gestation week and consequent PROM and neonatal infection events.

Group	Pregnant Women n (%) (n = 1852)	PROM Events n (%) (n = 187)	Neonatal Infection Events (GBS infection) n (%) (n = 4)
A	56 (3.02)	13 (23.21)	1 (1.79)
B	57 (3.08)	14 (24.56)	1 (1.75)
C	123 (6.64)	20 (16.26)	2 (1.63)
D	1616 (87.26)	140 (8.63)	0 (0.00)
$\chi^2$	—	24.2339	27.0466
<i>p</i>	—	0	0

Group A: both cervical secretions and the lower 1/3 of the vaginal secretions showing GBS-positive; Group B: only cervical secretions showing GBS-positive; Group C: only the lower 1/3 of the vaginal secretions showing GBS-positive; Group D: both cervical and the lower 1/3 of the vaginal secretions showing GBS-negative.

The study was approved by the Women and Children Health Hospital of Futian Shenzhen. The hospital obtained written informed consent from each patient (Ethical approval number: 20160116).

#### Collection of cervical and lower vaginal secretions from pregnant women

Cervical and lower vaginal secretions were collected with sterile swabs using a vaginal speculum during a routine gynecological examination. Specimens were transferred to sterile tubes, sealed, and delivered to the laboratory.

#### Collection of mucus and blood from neonates

Mucus was collected from the mouth, eyes, and ears of neonates, and blood was collected from GBS-positive neonates.

#### Isolation and detection of the GBS antigen

The GBS antigen was isolated from secretions collected at 8-12 and 37 weeks of gestation using Colloidal gold im-

munochromatography according to the manufacturer's instructions. The GBS antigen was detected by colloidal gold test paper analyzer (No. srs1709026, Hebei Senyue Biotechnology Co., Ltd.) for 10-15 min. The detection was carried out automatically by the GBS detector. Values  $\geq 5$  cfu were considered positive. GBS-positive pregnant women were prescribed prophylactic intravenous antibiotics, which were given once or twice. The GBS antigen was also isolated from neonates and used as indicated above.

#### Statistical analysis

Results of GBS detection assays from women at 8-12 and 37 weeks of gestation, as well as neonates, were analyzed. Pregnancy outcomes and other parameters, such as the GBS-positive rate in pregnant women, abortion rate, preterm delivery rate (gestational age was between 28 weeks and < 37 weeks), premature rupture of membranes, number of neonatal carriers (mucus specimens were GBS-positive, but blood specimens were GBS-negative), number of neonatal infectors (mucus and blood specimens were GBS-positive), and number of neonatal intensive care cases, were analyzed. SPSS 21.0 software was used to calculate means and standard deviations ( $\pm$  SD). Count data were converted to rates (%). Categorical variables with low frequencies were evaluated using chi-square test or Fisher's exact test. *p*-values less than 0.05 were considered significant.

## Results

#### Clinical results of women in early pregnancy

No significant differences in patient characteristics (*p* > 0.05), including maternal age, primiparity, body mass index, gestational age, and a history of medical conditions, were observed (Table 1). Cervical and lower vaginal secretions from women in early pregnancy were screened for GBS. There were 62 (3.10%) GBS-positive women in group A, 69 (3.45%) in group B, and 123 (6.15%) in group C (Table 2). The percentage of GBS-positive women in all groups was 12.70%, with 3.10% in group A, 3.45% in group B, and 6.15% in group C, suggesting that lower vaginal secretions are superior to cervical secretions in the detection of GBS.

#### Association between the GBS-positive rate and adverse pregnancy outcomes in early pregnancy

The abortion rates of women in groups A through D were 4.84%, 5.80%, 2.44% and 1.32%, respectively, indicating that GBS-positive women are more likely to undergo abortions. A comparison of the abortion rate between group A and group B revealed no significant difference, suggesting that the high rate is attributed to GBS positive results as determined by cervical secretions. Therefore, cervical secretions should be collected from women in early pregnancy and screened for GBS infection.

The preterm delivery rates of women in groups A through D were 14.52%, 13.04%, 6.50% and 5.10%, respectively, revealing that GBS-positive women are also more likely to undergo preterm delivery. These results in-

Table 4. — Comparison of GBS positive rate of pregnant women in early and late pregnancy.

Gestational Weeks (w)	Cases (n)	GBS Positive Cases n (%)	GBS Negative Cases n (%)	$\chi^2$	<i>p</i>
8-12	2000	254 -12.7	1746 -87.3	0.0016	0.9681
37	1852	236 -12.74	1616 -87.26		

Note: Values are percentages (numbers). Categorical variables: chi-square test or Fisher's exact test. There is no statistically significant difference in early and late pregnancy ( $p > 0.05$ )

Table 5. — The final outcome of four groups women.

Group	Cases (n)	Termination of Gestational Weeks (w)	Neonatal Weight (Kg)	Neonatal GBS Carriers n (%)	Neonatal GBS Infectors n (%)	Neonatal Intensive Care Cases n (%)
A	62	38.4 ± 0.65	3.296 ± 0.4268	6 (9.68)	2 (3.23)	2 (3.23)
B	69	38.3 ± 0.47	3.312 ± 0.5716	6 (8.70)	2 (2.90)	1 (1.45)
C	123	38.5 ± 0.39	3.2302 ± 0.5605	10 (8.13)	6 (4.88)	2 (1.63)
D	1746	38.6 ± 0.55	3.310 ± 0.4989	0 (0)	7 (4.01)	5 (2.86)
$F/\chi^2$	—	18.23	0.025	—	33.812	14.8094
<i>p</i>	—	0.9205	0.9947	< 0.01	< 0.01	0.002

Group A: both cervical secretions and the lower 1/3 of the vaginal secretions showing GBS-positive; Group B: only cervical secretions showing GBS-positive; Group C: only the lower 1/3 of the vaginal secretions showing GBS-positive; Group D: both cervical and the lower 1/3 of the vaginal secretions showing GBS-negative.

indicate the GBS-positive determined by lower vaginal secretions leads to increasing preterm delivery rate. The preterm delivery rates of group A and group B are almost 2-3 times that of group C and group D, indicating the GBS-positive determined by cervical secretions leading women to suffer more risk of preterm delivery.

#### Clinical findings of women at 37 weeks of gestation

Cervical and lower vaginal secretions from women at 37 weeks of gestation were screened for GBS. There were 56 (3.02%) GBS-positive women in group A, 57 (3.08%) in group B, 123 (6.64%) in group C, and 1616 (87.26%) in group D. The increase in GBS-positive women at 37 weeks of gestation was due to abortion and preterm delivery events and new GBS positive cases. There were significant differences in the GBS-positive detection rate among all groups, except between group A and group B ( $p > 0.05$ ). The percentage of GBS-positive women in all groups was 12.47%, with 3.02% in group A, 3.08% in group B, and 6.64% in group C (Table 4).

#### Association between the GBS-positive rate and adverse pregnancy outcomes at the 37 weeks of gestation

The rates of premature rupture of membranes were 23.21%, 24.56%, 16.26%, and 8.63% for groups A through D, indicating that GBS-positive women are more likely to experience premature rupture of membranes. These results suggest that the high rates of premature rupture of membranes in GBS-positive women were attributed to the screening of cervical secretions rather than lower vaginal secretions. More than half of GBS-positive women could only be confirmed by lower vaginal secretions. Therefore, it is great importance to screen GBS by cervical secretions.

The rates of neonatal infection, which was acquired from GBS-positive mothers, were 1.79%, 1.75%, 1.63%, and 0%

for group A through D. Compared with premature rupture of membranes, neonatal infection events are much more serious for GBS-positive women. As shown in Table 3, pregnancy outcomes of GBS-positive women showed high rates of neonatal infection (group A, 1.79%; group B, 1.75%; group C, 1.62%; 4/1852 = 2.2% for all pregnant women), while that of GBS-negative women show no one form GBS infection. Group B had a higher neonatal infection rate than that of group C, indicating that neonates are at increased risk of GBS infection. Therefore, it is important to collect cervical secretions and to screen for GBS infection. The final outcome of four groups women (Table 5).

## Discussion

In this study, we investigated GBS colonization in pregnant women at 8-12 and 37 weeks of gestation using cervical and lower vaginal secretions. Patients had no history of antibiotic use 3 months prior to the commencement of the study. No significant differences in patient characteristics, including maternal age, primiparity, body mass index (BMI), gestational age, and a history of medical conditions, was observed. The positive rate of GBS colonization in this study was lower than that reported in previous reports [2-4], which may be related to the population living in the Futian District of Shenzhen.

GBS has been successfully detected by many methods, including bacterial cultures, antigen-antibody assays [28, 29], DNA probe detection [30], fluorescence *in situ* hybridization [31], pulsed gel electrophoresis [32], polymerase chain reactions [29], and Gram staining [33]. Bacterial cultures are most commonly used, although 72 h are needed for clinical results. Colloidal gold immunochromatography is a more rapid method, requiring only 5-15

min, and it has been in clinical settings for 6 years due to its sensitivity and specificity. In this study, we used this method to screen cervical and lower vaginal secretions from women in early and late pregnancy.

GBS colonization in pregnant women was examined using cervical and lower vaginal secretions. Lower vaginal secretions are more commonly sampled because they are easier to obtain than cervical secretions, which require insertion of a speculum, exposure of the cervix, and collection of the specimen by an experienced gynecologist. There is also an increased risk of pain and bleeding. The small specimen volume should also be taken into consideration when screening for GBS.

GBS-positive women experienced more adverse pregnancy outcomes than GBS-negative women, indicating that all pregnant women should be screened for GBS colonization. In addition, more than half of cervix GBS-positive women were confirmed with cervical secretions, indicating that pregnant women would be misdiagnosed if only lower vaginal secretions were used. Therefore, cervical secretions should be collected and screened for GBS colonization.

We also found that GBS-positive women in early pregnancy had higher rates of abortion and preterm delivery compared to GBS-negative women. Moreover, there was a risk of GBS transmission from mothers to neonates. The rates of preterm delivery (5.10%) and premature rupture of membranes (8.63%) were low for GBS-negative women, and there were no GBS-positive neonates, indicating that neonatal GBS colonization is mainly dependent on maternal GBS colonization.

In conclusion, we used cervical and lower vaginal secretions from women in the first and third trimester of pregnancy to assess GBS colonization and demonstrated that women in early pregnancy should be screened, because GBS colonization can increase the risks of abortion and preterm delivery. We also presented a new method to screen for GBS colonization using cervical and lower vaginal secretions. It is noteworthy that more than half of cervical GBS-positive women would be misdiagnosed if only lower vaginal secretions are used. Furthermore, the rate of adverse pregnancy outcomes was higher for cervical GBS-positive women than that for lower vaginal GBS-positive women. These results should be confirmed in further studies using more specimens from pregnant women in early and late pregnancy.

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## Conflict of Interest

The authors declare no conflict of interest.

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