

## Research Paper

# Prevalence of Vaginal Colonization by Group B Streptococcus and its Associate Factors among Pregnant Women Referring to Fatemieh Hospital in Hamadan



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## ABSTRACT



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### Key words:

Group B  
*streptococcus*,  
Pregnant women,  
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colonization

**Aims** Group B *streptococcus* (GBS) is one of the relatively common causes of vaginal infection in women of reproductive age. This infection in pregnant women is associated with adverse fetal and neonatal outcomes, such as early neonatal infection, abortion, preterm delivery, and neonatal death. Considering the different prevalence of GBS vaginal colonization in different geographical locations, this study aimed to assess the prevalence of GBS vaginal colonization among pregnant women referring to Fatemieh Hospital in Hamadan in 2021.

**Materials & Methods** In this descriptive-analytical study, 130 pregnant women at 28-37 weeks of gestation referring to Fatemiyeh Hospital in Hamadan in 2021 were examined for GBS vaginal colonization. Sampling was performed using a sterile swab from the vaginal area, and the swabs were transferred to the microbiology laboratory of the faculty of medicine after being placed in the Todd Hewitt broth medium. Bacterial diagnostic tests were performed to identify GBS. After registering in the checklist, the results were analyzed in SPSS software (version 16) at a confidence level of 95%.

**Findings** The mean age of the studied women was  $29.85 \pm 6.86$  years. In terms of employment status, most subjects were housewives (95.4 %), had high school education (34.6%), and resided in urban areas (93.8 %). The mean gestational age of participants was  $33.18 \pm 3.18$  weeks. About 70% of them had experienced at least one pregnancy. Moreover, 30%, 6.2 %, 6.9%, 16.4 %, 3.1 %, and 47.7% had a history of abortion, preterm birth, gestational hypertension, gestational diabetes, genital disease, and urinary tract infection, respectively. The prevalence of GBS vaginal colonization was 3.8%. The prevalence of GBS vaginal colonization showed no significant relationship with the age of pregnant women, gestational age, gravida, history of abortion and premature birth, and their disease records.

**Conclusion** Although GBS vaginal colonization in pregnant women referring to Fatemiyeh Hospital in Hamadan was low, its adverse effect on pregnancy outcomes highly necessitates screening for GBS infection, timely diagnosis, and treatment, as well as investigating the risk factors affecting this disease.

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## مقاله پژوهشی

# شیوع کلونیزاسیون استرپتوکوکوس گروه B در واژن زنان باردار مراجعه کننده به بیمارستان فاطمیه شهر همدان و عوامل مرتبط با آن

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## چکیده

**هدف** استرپتوکوکوس گروه B از علل نسبتاً شایع عفونت واژینال در زنان در سن باروری است که در صورت ابتلای زنان باردار، با پیامدهای ناگوار جنینی و نوزادی، از جمله عفونت زودرس نوزادی، سقط، زایمان زودرس و مرگ نوزادی همراه است. با توجه به شیوع متفاوت استرپتوکوکوس گروه B در نقاط جغرافیایی مختلف، این مطالعه با هدف تعیین شیوع کلونیزاسیون باکتریایی واژینال استرپتوکوکوس گروه B در واژن زنان باردار مراجعه کننده به بیمارستان فاطمیه شهر همدان و عوامل مرتبط با آن در سال ۱۴۰۰ انجام گرفت.

**مواد و روش ها** در این مطالعه توصیفی تحلیلی، به روش نمونه‌گیری مبتنی بر هدف، ۱۳۰ نفر از زنان باردار با سن بارداری ۲۸ تا ۳۷ هفته و مراجعه کننده به بیمارستان فاطمیه شهر همدان در سال ۱۴۰۰، انتخاب و از نظر کلونیزاسیون باکتریایی واژینال استرپتوکوکوس گروه B بررسی شدند. نمونه‌گیری با استفاده از سواب استریل از ناحیه واژن انجام شد و سواب‌ها پس از قرار گرفتن در محیط اختصاصی Todd Hewitt broth، به آزمایشگاه میکروپزشناسی دانشکده پزشکی منتقل شدند. تست‌های تشخیصی باکتریایی برای شناسایی استرپتوکوکوس گروه B انجام شد. نتایج پس از ثبت در چک‌لیست، با نرم‌افزار SPSS نسخه ۱۶ در سطح اطمینان ۹۵ درصد تحلیل شدند.

**یافته‌ها:** میانگین سن زنان مورد مطالعه  $29/85 \pm 6/86$  سال بود. از نظر وضعیت اشتغال، بیشترشان غیرشاغل و خانه‌دار (۹۵/۴ درصد)، دارای تحصیلات راهنمایی (۳۴/۶ درصد) و ساکن شهر (۹۳/۸ درصد) بودند. میانگین سن بارداری زنان مورد مطالعه  $33/18 \pm 3/18$  هفته بود. حدود ۷۰ درصد از آنان حداقل یک بار سابقه بارداری داشتند. ۳۰ درصد از آن‌ها سابقه سقط، ۶/۲ درصد سابقه زایمان زودرس، ۶/۹ درصد فشارخون حاملگی، ۱۶/۴ درصد دیابت حاملگی، ۳/۱ درصد سابقه بیماری تناسلی و ۴۷/۷ درصد سابقه عفونت ادراری داشتند. فراوانی شیوع کلونیزاسیون باکتریایی واژینال استرپتوکوکوس گروه B ۲/۸ درصد بود. بین فراوانی کلونیزاسیون باکتریایی واژینال استرپتوکوکوس گروه B و سن زنان باردار، سن بارداری، تعداد بارداری، سابقه سقط و زایمان زودرس و سوابق بیماری آنان ارتباط معنی‌دار مشاهده نشد.

**نتیجه‌گیری:** اگرچه کلونیزاسیون استرپتوکوکوس گروه B در واژن زنان باردار مراجعه کننده به بیمارستان فاطمیه شهر همدان کم بود، با توجه به تأثیر ناگوار آن بر نتیجه بارداری، غربالگری ابتلا به عفونت استرپتوکوکوس گروه B و در نتیجه، تشخیص و درمان به موقع آن و همچنین، بررسی فاکتورهای خطر مؤثر بر کلونیزاسیون باکتریایی واژینال از اهمیت ویژه‌ای برخوردار است.

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## کلیدواژه‌ها:

استرپتوکوکوس گروه B

زنان باردار

کلونیزاسیون باکتریایی واژینال

## Introduction

**G**roup B *streptococcus* (GBS) colonization of the vaginal tract affects 10% to 30% of pregnant women and is typically asymptomatic; nevertheless, it can cause endometritis, septic abortion, chorioamnionitis, urinary tract infections, and septicemia [1-3]. GBS can be carried by pregnant women in the rectum and vagina, and between 50% and 70% of them pass the bacteria on to their unborn children [4]. Pregnancy-related GBS and maternal vaginal bacterial colonization are strongly correlated with neonatal infection [5]. Neonatal infected with this bacterium develop skin or mucous membrane colonization (15–50% of newborns born to infected moms), and 1%–3% of infected newborns develop the disease [6, 7].

Early neonatal sepsis during pregnancy is related to vaginal bacterial colonization, and mother-to-child transmission happens during delivery or via the ascending channel from the mother's genital tract into the amniotic fluid [8]. Preterm birth can be related to some socio-demographic and pathological factors [9, 10]. More than half of the reported cases of GBS in neonates involve late-onset disease [11]. When it comes to late-onset illnesses, the mother is typically the source of contamination [12]. Deafness, blindness, mental impairment, and slowed growth in neonates are examples of late acute infections [13]. The rate of preterm birth in developed countries has increased from 9.7% in 1990 to 10.8% in 2004 [14, 15].

In Iran, the rate of preterm delivery is about 13.9%, and the rate of vaginal GBS colonization in mothers is estimated at 15%-18% [4, 16, 17]. Nonetheless, there are different statistics on the frequency of vaginal GBS colonization in Iran and abroad. The Centers for Disease Control and Prevention (CDC) guidelines were amended to make bacteriological screening necessary for all pregnant women 35 to 37 weeks of gestation [18]. Genital bacterial colonization in pregnant women occurs transiently, intermittently, or permanently; nonetheless, pregnant women's vaginal colonization with GBS is typically consistent throughout time. Vaginal bacterial colonization in pregnant women can be influenced by a number of factors, including ethnicity, race, maternal age, parity, marital status, socioeconomic status, education level, geographic region, occupation, smoking, presence of STDs, urinary tract infection, sexual behavior, and high body mass index [19-21]. Therefore, detection of GBS genital colonization in pregnant women is essential in order to prevent neonatal sepsis. The prevalence of GBS varies widely across geographic locations and ethnic groups.

Due to the prevalence of GBS contamination in different parts of the world, as well as the infections

caused by this bacterium in pregnant women and their neonates, it is essential to ascertain the frequency of this bacterium infection in various locations. In light of the aforementioned issues, this study aimed to assess the prevalence of GBS vaginal colonization and its associated factors in pregnant patients referring to Fatemieh-Hospital in Hamadan in 2021.

## Materials and Methods

In this descriptive-analytical study, 130 pregnant women who were referred to Fatemiyeh-Hospital in Hamedan in 2021 and had a gestational age between 28 and 37 weeks had their vaginal colonization for GBS checked. Based on the study by Yasini et al. [22] and considering the significance level of 95% and the margin error of 0.05, the required sample size was calculated at 130 cases using the following formula.

$$n = \frac{(z_{1-\frac{\alpha}{2}})^2 * p * (1 - p)}{d^2}$$

The inclusion criteria entailed willingness to participate in the research, pregnant woman between weeks 28 and 37 of gestation, and nonuse of antibiotics in the last two months. On the other hand, the exclusion criterion was pregnant women with cerclage. In this study, after obtaining written consent from the subjects, the checklist, including information on age, occupation, level of education, place of residence, gestational hypertension, parity, gravida, gestational diabetes, history of diabetes, gestational age, as well as a history of genital diseases, urinary tract infection, abortion, and preterm birth, were completed.

In order to detect GBS vaginal colonization, samples were first taken by Dacron swab, transferred to a tube containing 3 cc of Todd-Hewitt broth (THB) (with 15 mg/ml of nalidixic acid and 5 mg/ml of gentamicin), and sent to the laboratory. Thereafter, the samples were placed in an incubator at 37°C and in a candle jar (to supply 5%-10% CO<sub>2</sub> required) for 24 hours. Thereafter, the swabs were cultured on the sheep blood-agar and placed in an incubator at 37°C for 24 h. After bacterial colony growth, GBS was diagnosed based on β-hemolysis, gram staining, catalase test, bacitracin susceptibility test, sodium Hippurate-hydrolysis test, and CAMP test [17].

After collecting and entering data into SPSS software (version 16), central and dispersion indicators were used to describe quantitative variables, while frequency and frequency percentages were utilized to describe qualitative variables. In the analytical analysis, Fisher's exact test was used to investigate GBS vaginal

colonization in terms of qualitative variables. Regarding the quantitative variables, the normal distribution variables were examined using the student's t-test, and the non-normal distribution variables were examined using the Mann-Whitney test after the normality of the data was determined by the Kolmogorov-Smirnov test. The significant level was considered  $<0.05$  in all analyses.

## Results

In this study, the women who participated in the study had an average age of  $29.85 \pm 6.86$  years. (range of 16-43). The majority of patients were between the ages of 25 and 29. In terms of employment status, most pregnant women were housewives (95.4%). Regarding education, they had junior

high school (34.6%) and senior high school education (26.2%). Regarding place of residence, the majority (93.8%) lived in cities. About 70% of women had at least one prior pregnancy, and 60% of cases had at least one child. Moreover, 30% and 2.6% of cases had a history of abortion and preterm delivery, respectively. Their current mean gestational age was  $33.18 \pm 3.18$  weeks.

The prevalence rates of gestational hypertension, gestational diabetes, non-gestational diabetes, and genital disease in the studied pregnant women were reported as 6.9%, 14.6%, 2.3%, and 3.1%, respectively. Out of 130 pregnant women examined, 62 (47.7%) cases had a history of urinary tract infection (UTI), and prevalence of GBS vaginal colonization was 3.8% (Table 1).

**Table 1.** Individual characteristics of the studied pregnant women

Variable	n	%
Employment status		
Housewife	124	95.4
Employed	6	4.6
Education		
Illiterate	3	2.3
Elementary school	15	11.5
Junior high school	45	34.6
Senior high school	34	26.2
Academic education	33	25.4
Place of residence		93.8
City	122	
Village	8	6.2
Gravida		
0	41	
1	39	
2	24	31.5
3	14	
$\geq 4$	12	
Parity		
0	52	40
1	46	35.4
2	20	15.4
3	12	9.2
History of abortion		
Yes	39	30
No	91	70
History of preterm birth		6.2
Yes	8	
No	122	93.8
Gestational hypertension		6.9
Yes	9	
No	121	93.1
Gestational diabetes		
Yes	19	14.56
No	111	85.4
Non-gestational diabetes		
Yes	3	2.3
No	127	97.7
History of genital disease		
Yes	4	3.1
No	126	96.9

Based on Fisher's exact test, GBS vaginal colonization in pregnant women showed no significant relationship with

occupation, education, place of residence, history of gestational hypertension, gestational diabetes, non-

gestational diabetes, history of genital disease, history of abortion, history of preterm birth, and history of UTI (Table 2). The Kolmogorov-Smirnov test was utilized to ascertain the normalcy of the data with respect to quantitative variables. The results of the non-parametric

Mann-Whitney test and the student's t-test indicated that there was no significant correlation between GBS vaginal colonization and age, gestational age, parity, or gravida (Table 3).

**Table 2.** Correlation between group B *streptococcus* vaginal colonization and the studied variables

Variable	Group B <i>streptococcus</i> vaginal colonization			P-value
	negative n (%)	Positive n (%)	Total	
Employment status				
Housewife	120 (96.8)	4 (3.2)	124	0.213
Employed	5 (83.3)	1 (16.7)	6	
Education				
Illiterate	3 (100)	0 (0)	3	0.934
Elementary	15 (100)	0 (0)	15	
Junior high school	43 (95.6)	2 (4.4)	45	
Senior high School	43 (97.1)	1 (2.9)	34	
Academic education	1 (93.9)	2 (6.1)	33	
Place of residence				
City	117 (95.9)	5 (4.1)	122	1.00
Village	8 (100)	0 (0)	8	
Gestational hypertension				
Yes	117 (96.7)	4 (3.3)	121	0.306
No	8 (88.9)	1 (11.1)	9	
Gestational diabetes				
No	107 (96.4)	4 (3.6)	111	0.552
Yes	18 (94.7)	1 (5.3)	19	
Non-gestational diabetes				
No	123 (96.9)	4 (3.1)	127	0.552
Yes	2 (66.7)	1 (33.3)	3	
History of genital disease				
No	122 (96.6)	4 (3.2)	126	0.147
Yes	3 (75.0)	1 (25.0)	4	
History of abortion				
No	86 (94.5)	5 (5.5)	91	0.321
Yes	39 (100)	0 (0)	39	
History of preterm birth				
No	117 (95.9)	5 (4.1)	122	1.00
Yes	8 (100)	0 (0)	8	
History of urinary tract infection				
No	67 (98.5)	1 (1.5)	68	0.192
Yes	58 (93.5)	4 (6.5)	62	

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**Table 3.** Correlation of GBS vaginal colonization with age, gestational age, gravida, and parity

Variable	Group B <i>streptococcus</i> vaginal colonization		P-value
	Negative Mean $\pm$ standard deviation	Positive Mean $\pm$ standard deviation	
Age (years)	30.60 $\pm$ 2.90	25.40 $\pm$ 6.00	0.158
Gestational age (weeks)	33.30 $\pm$ 18.25	33.10 $\pm$ 4.03	0.323
Gravida	2.36 $\pm$ 1.29	1.80 $\pm$ 0.83	0.224
Parity	1.92 $\pm$ 0.95	1.60 $\pm$ 0.89	0.120

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## Discussion

The aim of the current research was to determine how common GBS vaginal colonization is in expectant

mothers. Accordingly, 130 expectant patients who were sent to Hamedan's Fatemeh Hospital in 2021 were chosen and given a check-up for standard prenatal treatment. In the present study, out of 130 pregnant

women, 5 (3.8%) cases had GBS vaginal colonization. GBS colonization of the vagina in pregnant women showed no significant relationship with occupation, education, place of residence, history of gestational hypertension, gestational diabetes, non-gestational diabetes, history of genital disease, history of abortion, history of preterm birth, history of urinary tract infection, parity, and gravida.

In the research conducted by Rostami et al. in Isfahan in 2021, Among 200 pregnant women, 13.5% had vaginal colonization caused by GBS. Moreover, GBS vaginal colonization demonstrated no significant relationship with gravida, history of abortion, history of UTI during pregnancy, history of preterm delivery, and education. Our study's sample size and vaginal bacterial colonization prevalence were smaller than those found in Rostami et al.'s study. Consistent with the findings of the aforementioned study, our investigation revealed no noteworthy correlation between GBS vaginal colonization and gravida, abortion history, history of urinary tract infection during pregnancy, or history of premature birth [23].

In the study conducted by Kabiri et al. (2015) on 403 pregnant women in the 35-37 week of gestation in Jahrom, the prevalence rates of GBS vaginal, rectal, and rectovaginal colonization were 16.4%, 5.2%, and 7%, respectively. The positive results of the culture tests showed no statistically significant correlation with the gestational hypertension, mother age, or history of genital illnesses. However, a history of abortion, diabetes, premature birth, UTI, place of residency, nationality, educational attainment, and cesarean section significantly corrected the positive results of the culture tests [24].

In addition, the prevalence of GBS in 371 pregnant women at 35 to 37 weeks of gestation in a university hospital in Korea using routine culture methods was 4.35% [25]. The current study's findings regarding the prevalence of GBS vaginal colonization in pregnant women were less than those of Kabiri's study and the research done in Korea. In accordance with the findings of the study by Kabiri et al., in our study, there was no statistically significant correlation found between the positive results of culture tests and the following: location of residence, country, education level, history of abortion, diabetes, premature birth, urinary infection, and cesarean section.

However, the results of our investigation on the correlation between positive culture tests and maternal age, genital illness history, and gestational hypertension do not align with the findings published by Kabiri et al. This discrepancy can be ascribed to different sample sizes in the two studies. In research projects carried out by Yasini et al. [22], Jahed Bazargan et al. [26], Nazer et al. [27], and Shahbazian et al. [28], the prevalence rates of

GBS vaginal colonization were reported as 9.4%, 5.3%, 14%, and 13.2%, respectively. In the study by Yasini et al., a significant relationship was seen between GBS vaginal colonization and gravida.

In the study by Jahed Bozorgan et al., positive and negative culture tests showed no significant relationship with age, nationality, education level, gravida, amniotic sac status, or mother's body temperature upon admission. In the study by Nazer et al., positive culture tests displayed no significant relationship with maternal age, gestational age, as well as a history of abortion, diabetes, and gestational hypertension. However, there was a strong correlation between gravida and vaginal bacterial colonization [27].

The prevalence of GBS vaginal colonization in Shahbazian et al.'s study was not significantly correlated with the manner of previous delivery, history of chorioamnionitis in the previous pregnancy, history of premature rupture of membranes (PROM), history of preterm delivery, age of patients, or gravida [28]. As illustrated, In the four mentioned investigations, the prevalence of GBS vaginal colonization ranged from 3.5% to 14%. Our results more closely match those of the Jahed Bozorgan et al.'s study (3.5%).

Apart from the two studies that reported a significant relationship between gravida and GBS vaginal colonization, among other demographic variables, pregnancy and delivery records did not show any association with GBS vaginal colonization, which is in line with the results of this study. In our study, less than 30% of women had a history of two or more previous pregnancies, and about 30% had no previous pregnancy history. This discrepancy can be attributed to the low prevalence of GBS vaginal colonization.

In two studies conducted by Sharifi Yazdi et al. on 250 women who were 35–37 weeks pregnant [29] and Bakhtiari et al. on 125 pregnant women at 35 to 37 weeks of gestation [30], the rates of GBS vaginal colonization using the culture and polymerase chain reaction (PCR) methods were 9.6% and 9.3%, respectively. In our study, GBS vaginal colonization was investigated by culture method. According to the higher sensitivity of PCR in the diagnosis of GBS vaginal colonization compared to the culture method [29, 30], the same problem may be partially responsible for the discrepancy in the prevalence of GBS vaginal colonization found in our study compared to that of investigations by Sharifi Yazdi et al. and Bakhtiari et al.

In a 2007 study in Rasht, 15% of 100 pregnant women at 28–37 weeks had GBS vaginal colonization, according to Amir Mozafari et al.'s findings. [17]. In their study, Zamanzad et al. examined the vaginal mucus samples of 624 mothers who were referred to Hajar Hospital in Shahrekord for term or preterm delivery or due to PROM and reported that 110 (18%) mothers were carriers of



GBS. Regarding where each of the mothers stated lived, there was no discernible difference. (urban or rural) [31]. The prevalence rate of GBS vaginal colonization obtained in both studies by Zamanzad et al. and Amir Mozafari et al. is higher than the findings of our study. This disagreement in the results can be ascribed to differences in the time of the studies, the improvement of women's health status during 15-20 years, or a difference in the geographical location of the studies, which needs further investigation.

In a study conducted in an Indian hospital at 36–37 weeks gestation, 310 pregnant women had their vaginal colonization for GBS evaluated, the prevalence of colonization was 12.9%. Among the investigated factors, a significant relationship was only found between PROM and bacterial colonization, while similar to our study, no significant relationship was observed between the other investigated factors and colonization [32]. In a study by Girma et al. in 2020 in Ethiopia, the prevalence rate of GBS rectovaginal colonization in 135 pregnant women at 35 to 37 weeks of gestation was reported as 16.3%. In the same context, in a study by Iyamba et al. in Guinea, 23.07% of 104 pregnant women at a gestational age of 35–37 weeks had GBS rectovaginal colonization. [33, 34].

In the stated study, GBS vaginal colonization had a significant relationship with the history of PROM and urinary tract infection during pregnancy. Nonetheless, there was no significant relationship with age, education, place of residence, history of contraceptive use, and pregnancy. The prevalence of GBS vaginal colonization in the this study was lower than the findings of the researches by Girma et al. and Iyamba et al. in Ethiopia and Guinea. This disparity can be ascribed to differences in geographical regions, different prevalence of GBS vaginal colonization in various geographical regions, and health status of women in different areas.

Moreover, in an investigation carried out by Tesfaye et al. in Ethiopia, 182 pregnant women were studied for antibiotic resistance and variables related to GBS vaginal colonization. In the mentioned study, the prevalence of vaginal colonization was 15.9%, and among the investigated factors, only a history of preterm birth and PROM were significantly correlated with GBS vaginal colonization [35]. In our study, the history of PROM was not examined. Our findings also agree with the results of the study by Girma et al. in terms of the lack of correlation of GBS vaginal colonization with age, education, place of residence, and pregnancy.

The low prevalence of GBS vaginal colonization can be attributed to the lack of significant correlation found between the history of UTI and GBS vaginal colonization in our study, despite the higher prevalence of GBS vaginal colonization in women with a history of UTI compared to women without such a history (6.5% vs. 1.5%). Ashary et

al. conducted a review study in India on the prevalence of GBS vaginal colonization in mothers of premature neonates with culture and immunological methods. When using culture techniques, the prevalence of GBS was 7.4%, but when using immunological techniques, it was 11.6%. Moreover, GBS vaginal colonization increased the risk of preterm delivery by 9.7 times [36].

Our study's prevalence of GBS vaginal colonization was lower than Ashary et al.'s findings utilizing both immunological and cultural approaches. This disparity in the results may be due to differences in the sample size (130 vs. 9,778 cases) or geographical location of the two studies. Contrary to the results of the study by Ashary et al., in the present research, no significant relationship was observed between preterm delivery and GBS vaginal colonization. This inconsistency in the results can be ascribed to the fact that the current research investigated the current GBS vaginal colonization of women with a history of preterm delivery in their previous deliveries. Nonetheless, the study by Ashary et al. assessed the relationship between GBS vaginal colonization in the current pregnancy and delivery of the same pregnancy.

Examining different risk variables for GBS vaginal colonization was one of the study's highlights. On the other hand, among the notable limitations of this research, we can refer to the fact that research subjects were only selected from the patients referring to one healthcare center; accordingly, we cannot generalize the results of the study to pregnant women in the whole city of Hamadan. It is suggested that future studies on GBS colonization in pregnant women and its effect on pregnancy outcomes employ a larger sample size and cover all pregnant women in Hamadan.

## Conclusion

Although the low rate of GBS vaginal colonization among pregnant patients referred to Fatemiyeh-Hospital in Hamedan, the disease's detrimental effects on pregnancy outcomes make screening for GBS infection, prompt diagnosis and treatment, and research into the risk factors contributing to the illness absolutely necessary.

## Ethical Considerations

### Compliance with ethical guidelines

The Gonabad University of Medical Sciences' regional committee on research ethics accepted this work under the ethics code IR.GMU.REC.1400.027.

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## Authors' contributions

All authors contributed to this article.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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