Association between maternal rectovaginal group B streptococcus and the risk of stillbirth: a meta-analysis

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ABSTRACT

Background: Group B streptococcus (GBS) colonization in pregnant women is associated with adverse perinatal outcomes, including stillbirth. This meta-analysis investigated the relationship between maternal rectovaginal GBS colonization and the risk of stillbirth.

Methods: We conducted a comprehensive literature search across several databases, including PubMed, Embase, Web of Science, Wanfang, and China National Knowledge Infrastructure, covering studies published from the inception of the database until September 9, 2024. The search focused on observational studies comparing the risk of stillbirth in pregnant women with and without rectovaginal GBS colonization. Results were summarized using odds ratios (ORs) and 95% confidence intervals (CIs), and a random-effects model was used to account for potential heterogeneity.

Results: A total of ten studies comprising 121,195 pregnant women were included in the analysis. The pooled results indicated no significant overall association between maternal rectovaginal GBS colonization and the risk of stillbirth (OR: 1.66, 95% CI [0.95–2.91], $p = 0.08$; $I^2 = 84\%$). However, sensitivity analyses revealed a significant association in studies that included intrapartum antibiotic prophylaxis (IAP) (OR: 1.36, 95% CI [1.02–1.80], $p = 0.03$). Subgroup analyses demonstrated a significant association between maternal rectovaginal GBS colonization and stillbirth risk in retrospective studies (OR: 2.62, $p = 0.04$) and in studies employing multivariate analysis (OR: 2.11, $p = 0.04$).

Conclusions: While the meta-analysis did not find a significant overall association between maternal rectovaginal GBS colonization and stillbirth, significant associations were noted under specific conditions, such as studies using IAP, retrospective designs, and multivariate analyses. Further research is needed to clarify these associations.

Subjects Developmental Biology, Gynecology and Obstetrics, Infectious Diseases
Keywords Group B streptococcus, Meta-analysis, Pregnancy, Rectovaginal, Stillbirth

INTRODUCTION

Stillbirth, defined as the death of a fetus at or after 20 weeks of gestation, is a tragic event for families and a significant public health concern (*McClure et al., 2022*; *Page & Silver, 2020*; *Smith & Fretts, 2007*). The global incidence of stillbirth, estimated at approximately

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one in 160 pregnancies, varies widely, with higher rates observed in low- and middle-income countries (*Hug et al., 2021*). Factors contributing to stillbirth are multifaceted, encompassing maternal, fetal, and environmental influences (*Escañuela Sánchez, Meaney & O.'Donoghue, 2019*; *Gardosi et al., 2013*; *Lawn et al., 2016*). Recognized risk factors include advanced maternal age, obesity, smoking, pre-existing medical conditions such as diabetes and hypertension, and certain infections (*Escañuela Sánchez, Meaney & O.'Donoghue, 2019*; *Gardosi et al., 2013*; *Lawn et al., 2016*). Understanding these risk factors is crucial for the development of effective prevention strategies aimed at reducing the incidence of stillbirth (*Silver & Reddy, 2024*).

Identifying novel risk factors, particularly reversible ones, is essential for enhancing maternal and fetal health. Group B streptococcus (GBS), a bacterium commonly found in the gastrointestinal and genitourinary tracts, is among these potential risk factors (*Dotters-Katz et al., 2022*; *Furfaro, Chang & Payne, 2018*). It is estimated that 10% to 30% of pregnant women are colonized with GBS rectovaginally, although this prevalence varies based on geographic and demographic factors (*Mei & Silverman, 2023*; *Russell et al., 2017*). The prevalence of GBS colonization varies by geographic region and demographic factors, with higher rates reported in African and Asian populations compared to Western countries (*van Kassel et al., 2021*). The diagnosis of GBS colonization typically involves culture methods or nucleic acid amplification tests from rectovaginal swabs taken during pregnancy, usually between 35 to 37 weeks of gestation (*Russell et al., 2017*). Despite the common nature of this bacterium, its influence on pregnancy outcomes, particularly stillbirth, has been a subject of ongoing research and debate (*Yuan et al., 2021*).

Maternal GBS colonization has been associated with several adverse pregnancy outcomes, including preterm labor, chorioamnionitis, and neonatal sepsis (*Bianchi-Jassir et al., 2017*; *Patras & Nizet, 2018*; *Puopolo, Lynfield & Cummings, 2019*). The mechanisms by which GBS influences these outcomes may involve the inflammatory response triggered by the bacterium, which can lead to premature rupture of membranes and other complications (*Vornhagen, Adams Waldorf & Rajagopal, 2017*). Additionally, there are concerns about the impact of intrapartum antibiotic prophylaxis (IAP) on the relationship between GBS colonization and adverse outcomes, including stillbirth (*Ohlsson & Shah, 2014*). Although IAP has significantly reduced the incidence of neonatal GBS disease, its impact on stillbirth associated with maternal colonization remains uncertain (*Seale et al., 2017*). This uncertainty highlights the importance of understanding the potential risks posed by maternal GBS colonization to guide prevention strategies beyond neonatal outcomes. While some studies suggest a potential link between maternal rectovaginal GBS colonization and increased risk of stillbirth (*Seale et al., 2016*; *Yadeta et al., 2018*; *Zhu et al., 2019*), the evidence remains inconsistent (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Hastings et al., 1986*; *Regan et al., 1996*; *Sweet et al., 1987*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*). Various studies have reported differing results, leading to confusion regarding the significance of GBS colonization as a risk factor for stillbirth. In view of this knowledge gap, this meta-analysis aims to clarify the association between maternal rectovaginal GBS colonization and the risk of stillbirth by synthesizing available observational studies. The

intended audience for this meta-analysis includes healthcare professionals, researchers, and policymakers focused on maternal-fetal medicine, infectious disease, and public health. It aims to inform clinicians and researchers about the potential link between maternal rectovaginal GBS colonization and stillbirth risk, supporting improved screening and preventive strategies in pregnancy care.

METHODS

The study adhered to PRISMA 2020 (*Page et al., 2021a*, *2021b*) and the Cochrane Handbook for Systematic Reviews and Meta-analyses (*Higgins et al., 2021*) guidelines for conducting this meta-analysis, including the study design, data collection, statistical analysis, and results interpretation. Additionally, the meta-analysis protocol was registered in the International Prospective Register of Systematic Reviews under registration identifier CRD42024594867.

Literature search

To identify studies pertinent to this meta-analysis, we searched the PubMed, Embase, Web of Science, Wanfang, and China National Knowledge Infrastructure databases using an extensive array of search terms, which included: ("Group B Streptococci" OR "GBS" OR "Streptococcus agalactia" OR "Group b Streptococcus" OR "*Streptococcus agalactiae*") AND ("maternal" OR "pregnancy" OR "pregnant") AND ("perinatal mortality" OR "perinatal death" OR "neonatal mortality" OR "neonatal death" OR "stillbirth" OR "fetal death" OR "stillborn"). The search was limited to research involving human subjects, and we only included studies published in English or Chinese as full-length articles in peer-reviewed journals. Additionally, we manually reviewed the references of relevant original and review articles to identify further pertinent studies. The literature was assessed from the inception of the searched databases up to September 9, 2024.

Inclusion and exclusion criteria

The inclusion criteria for potential studies were defined according to the PICOS framework:

P (patients): Pregnant women without significant comorbidities. For this analysis, comorbidities were defined as pre-existing maternal medical conditions such as diabetes mellitus, hypertension, autoimmune disorders, chronic infections (*e.g.*, HIV), or conditions known to significantly affect pregnancy outcomes.

I (exposure): Maternal rectovaginal GBS colonization confirmed *via* rectovaginal or vaginal swab tests during pregnancy.

C (comparison): Pregnant women without rectovaginal GBS colonization.

O (outcome): Incidence of stillbirth, compared between women with and without GBS colonization. Stillbirth is generally defined as the delivery of a fetus that has reached at least 28 weeks of gestation and exhibits no signs of life following complete separation from the mother.

S (study design): Observational studies with longitudinal follow-up, such as cohort studies, nested case-control studies, and *post-hoc* analysis of clinical trials.

The exclusion criteria included reviews, editorials, meta-analyses, preclinical studies, cross-sectional studies, studies including non-pregnant women, studies that did not include women with rectovaginal GBS colonization as the exposure, or studies that did not report the outcome of stillbirth. If two or more studies with overlapping populations were found, the study with the largest sample size was enrolled for the meta-analysis.

Study quality evaluation and data extraction

The literature search, study identification, quality assessment, and data extraction were conducted independently by two authors (Yujue Wang and Jingjing Liu), and any disagreements were resolved through a discussion with the corresponding author (Teng Zhang). The quality of included studies was evaluated using the Newcastle-Ottawa Scale (NOS) (*Wells et al., 2010*). The NOS assesses study quality across three domains: (1) Selection (0–4 points): Representativeness of the study population, selection of controls, and ascertainment of exposure; (2) Comparability (0–2 points): Adjustment for confounding factors such as maternal age, parity, or socioeconomic status; and (3) Outcome (0–3 points): Assessment of outcomes, adequacy of follow-up, and outcome measurement methods. Each study was assigned a total score ranging from 0 to 9, with higher scores indicating better methodological quality. Studies scoring ≥6 were considered of moderate to high quality. This detailed evaluation allowed us to reliably assess the robustness of the included studies and their potential influence on the meta-analysis results.

The data collected for analysis included the study details (author, year, country, and design), participant characteristics (number of pregnant women, mean age, timing and methods for evaluating rectovaginal GBS, the number of women with rectovaginal GBS colonization, and the number of women with IAP for GBS), the number of women who had stillbirth in the index pregnancy, variables adjusted or matched when the association between maternal GBS colonization and the risk of stillbirth was observed, and the overall incidence of stillbirth in the observed cohorts.

Statistical analyses

The association between maternal rectovaginal GBS colonization and the risk of stillbirth was analyzed using odds ratios (ORs) and 95% confidence intervals (CIs), which were calculated based on the events rate of stillbirth in women with and without GBS colonization. To assess heterogeneity, we used the Cochrane Q test and I^2 statistics (*Higgins & Thompson, 2002*), with $I^2 > 50\%$ indicating significant statistical heterogeneity. A random-effects model was applied to integrate the results to account for study variability (*Higgins et al., 2021*). By excluding individual studies sequentially, a sensitivity analysis was performed to evaluate the robustness of the findings. Predefined subgroup analyses were performed to explore the effects of various factors, such as geographic region, study design, methods for determination of GBS colonization (rectovaginal or vaginal swabs), overall incidence of stillbirth in the studied cohort, methods for analyzing the association between GBS colonization and stillbirth (univariate or multivariate analyses), and the Newcastle–Ottawa Scale (NOS) scores of the included studies. The medians of the

continuous variables were selected as the cutoff values for defining subgroups. Publication bias was evaluated using funnel plots and visual inspection for asymmetry, as supplemented by Egger's regression test (*Egger et al., 1997*). Analyses were performed using RevMan (Version 5.1; Cochrane Collaboration, Oxford, UK) and Stata software (version 12.0; Stata Corporation, College Station, TX, USA).

RESULTS

Study inclusion

The study inclusion process is illustrated in Fig. 1. Initially, 766 potentially relevant records were identified from the five searched databases, with 157 excluded due to duplication. A subsequent screening of the titles and abstracts led to the further exclusion of 580 studies, primarily because they did not align with the objectives of the meta-analysis. The full texts of the remaining 29 records were reviewed by two independent authors, resulting in the exclusion of 19 more studies for various reasons, as detailed in Fig. 1. Finally, ten cohort studies remained and were deemed appropriate for inclusion in the quantitative analysis (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Hastings et al., 1986*; *Regan et al., 1996*; *Seale et al., 2016*; *Sweet et al., 1987*; *Yadeta et al., 2018*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*; *Zhu et al., 2019*).

Overview of the study characteristics

Table 1 shows the summarized characteristics of the available studies included in the meta-analysis. Overall, four prospective cohort studies (*Hastings et al., 1986*; *Regan et al., 1996*; *Seale et al., 2016*; *Sweet et al., 1987*) and six retrospective studies (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Yadeta et al., 2018*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*; *Zhu et al., 2019*) were included in the meta-analysis. These studies were published from 1986 to 2023, and were conducted in the United Kingdom, the United States, Australia, Kenya, China, and Ethiopia. Overall, 121,195 pregnant women were included. The mean ages of these women at enrollment ranged from 22.7 to 30.7 years. The timing for evaluating rectovaginal GBS colonization was from gestational age (GA) of 23 to 26 weeks to the day of admission for delivery. Rectovaginal swabs were used to evaluate GBS colonization in six studies (*Chen et al., 2023*; *Hastings et al., 1986*; *Seale et al., 2016*; *Yadeta et al., 2018*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*), whereas vaginal swabs were used in the other four studies (*Garland, Kelly & Ugoni, 2000*; *Regan et al., 1996*; *Sweet et al., 1987*; *Zhu et al., 2019*), and the GBS colonization was confirmed by standard bacterial culture and identification among all the included studies. Accordingly, 18,062 (14.9%) women had rectovaginal GBS colonization during pregnancy. The IAP was used for all women during labor in five studies (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Seale et al., 2016*; *Zhou & Mou, 2023*; *Zhu et al., 2019*) and for partial women in two studies (*Regan et al., 1996*; *Yadeta et al., 2018*), whereas the use of IAP was not reported in three studies (*Hastings et al., 1986*; *Sweet et al., 1987*; *Zhang, Lu & Yuan, 2017*). Overall, 806 (0.67%) of the included women had stillbirth during the index pregnancy, and the overall incidence of stillbirth ranged from 0.06% to 8.53% among the studied cohorts. Potential confounding factors were not adjusted or matched between women with and without

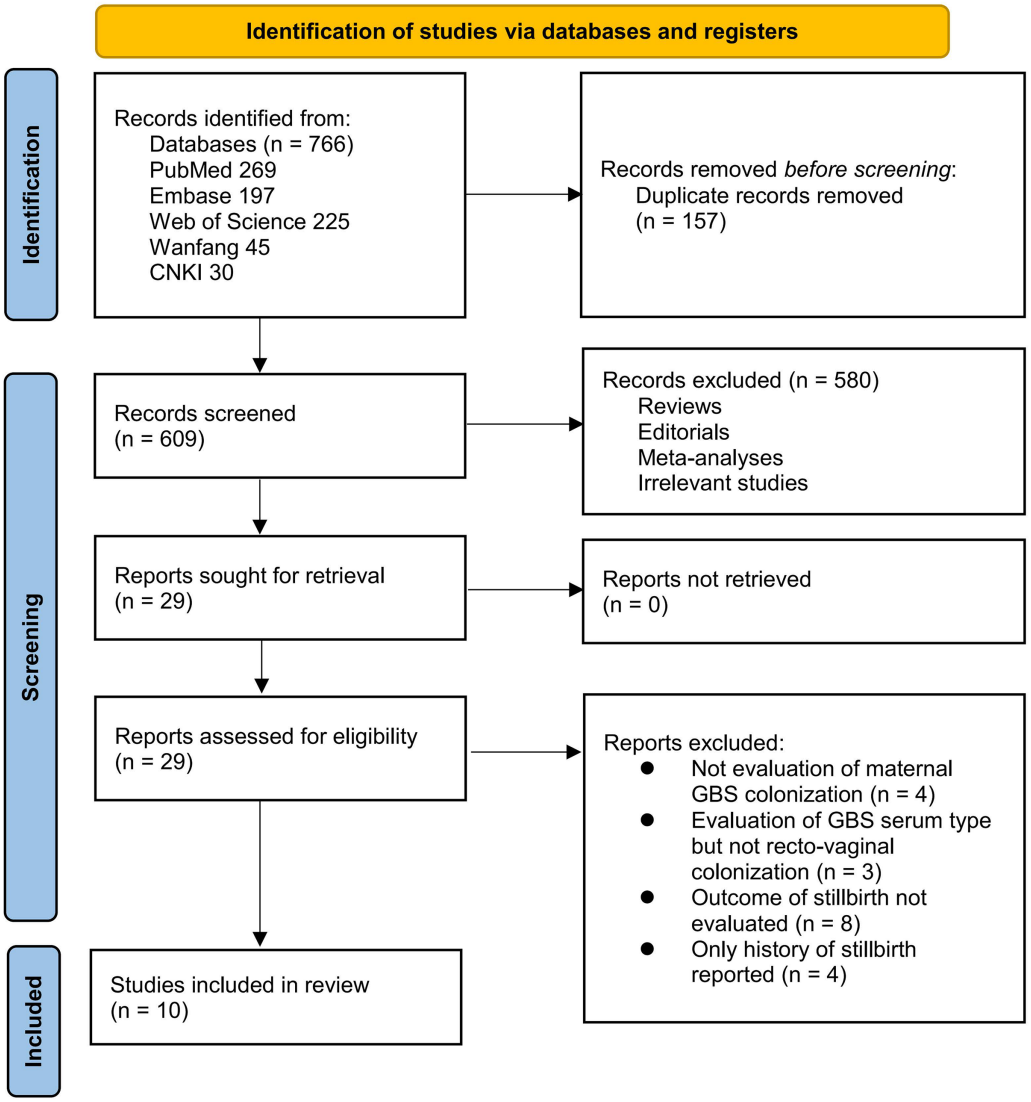

Figure 1 **Flowchart of database search and study inclusion.**
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rectovaginal GBS colonization in four early studies published until 2000 (*Garland, Kelly & Ugoni, 2000*; *Hastings et al., 1986*; *Regan et al., 1996*; *Sweet et al., 1987*), while six later studies (*Chen et al., 2023*; *Seale et al., 2016*; *Yadeta et al., 2018*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*; *Zhu et al., 2019*) controlled factors such as maternal age, parity, and socioeconomic status of the subjects, *etc*. The NOS scores of the included studies were six to nine, suggesting an overall moderate to good study quality (Table 2).

Results of the meta-analysis

Overall, the pooled results of the ten included studies (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Hastings et al., 1986*; *Regan et al., 1996*; *Seale et al., 2016*; *Sweet et al., 1987*; *Yadeta et al., 2018*; *Zhang, Lu & Yuan, 2017*; *Zhou & Mou, 2023*; *Zhu et al., 2019*) showed that maternal rectovaginal GBS colonization in pregnancy was not significantly related to

Table 1 Characteristics of the included studies.

Study	Country	Study design	No. of pregnant women	Mean age (years)	Timing for evaluating GBS colonization	Methods for evaluating GBS colonization	No. of women with GBS colonization	IAP use for women with GBS colonization	No. of stillbirth	Variables matched or adjusted	Overall incidence of stillbirth (%)
Hastings et al. (1986)	UK	PC	1,184	NR	GA: 28~36 weeks	Recto-vaginal swabs and bacterial culture	338	NR	8	None	0.68
Sweet et al. (1987)	USA	PC	3,341	22.7	GA: 30~34 weeks	Dacron swab from the lateral vaginal wall and bacterial culture	478	NR	48	None	1.44
Regan et al. (1996)	USA	PC	13,646	NR	GA: 23~26 weeks	Vaginal and endocervical swab and bacterial culture	2,877	17% received	82	None	0.60
Garland, Kelly & Ugoni (2000)	Australia	RC	1,541	NR	GA: 28~32 weeks	Vaginal swab and bacterial culture	583	100% received	1	None	0.06
Seale et al. (2016)	Kenya	PC	7,967	NR	At admission for delivery	Recto-vaginal swabs and bacterial culture	934	100% received	278	Maternal age, parity, education, SES, nutritional status, HIV infection, and multiple delivery	3.49
Zhang, Lu & Yuan (2017)	China	RC	403	27.9	GA: 37 weeks	Recto-vaginal swabs and bacterial culture	135	NR	1	Maternal age and parity	0.25
Yadeta et al. (2018)	Ethiopia	RC	1,688	26.6	At admission for delivery	Recto-vaginal swabs and bacterial culture	231	Partially received	144	Maternal age, parity, education, SES, anemia, hypertension, and GA at delivery, and IAP received	8.53
Zhu et al. (2019)	China	RC	49,908	29.5	GA: 35~37 weeks	Vaginal swab and bacterial culture	6,933	100% received	161	Maternal age, parity, SES, education, and previous abortion history	0.32
Chen et al. (2023)	China	RC	40,905	30.7	GA: 35~37 weeks	Recto-vaginal swabs and bacterial culture	5,502	100% received	81	Maternal age, parity, SES, GA at delivery, eclampsia, and thyroid dysfunction	0.20
Zhou & Mou (2023)	China	RC	612	26.3	GA: 35~37 weeks	Recto-vaginal swabs and bacterial culture	51	100% received	2	Maternal age, parity, BMI, GDM, and previous abortion history	0.33

Note:
BMI, body mass index; GA, gestational age; GBS, group B streptococcus; GDM, gestational diabetes mellitus; HIV, human immunodeficiency virus; IAP, intrapartum antibiotic prophylaxis; NR, not reported; PC, prospective cohort; RC, retrospective cohort; SES, socioeconomic status.

Table 2 Study quality evaluation *via* the Newcastle–Ottawa Scale.

Study	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome not present at baseline	Control for maternal age	Control for other confounding factors	Assessment of outcome	Enough long follow-up duration	Adequacy of follow-up of cohorts	Total
Hastings et al. (1986)	1	1	1	1	0	0	1	1	1	7
Sweet et al. (1987)	1	1	1	1	0	0	1	1	1	7
Regan et al. (1996)	1	1	1	1	0	0	1	1	1	7
Garland, Kelly & Ugoni (2000)	0	1	1	1	0	0	1	1	1	6
Seale et al. (2016)	1	1	1	1	1	1	1	1	1	9
Zhang, Lu & Yuan (2017)	0	1	1	1	1	0	1	1	1	7
Yadeta et al. (2018)	0	1	1	1	1	1	1	1	1	8
Zhu et al. (2019)	0	1	1	1	1	1	1	1	1	8
Chen et al. (2023)	0	1	1	1	1	1	1	1	1	8
Zhou & Mou (2023)	0	1	1	1	1	1	1	1	1	8

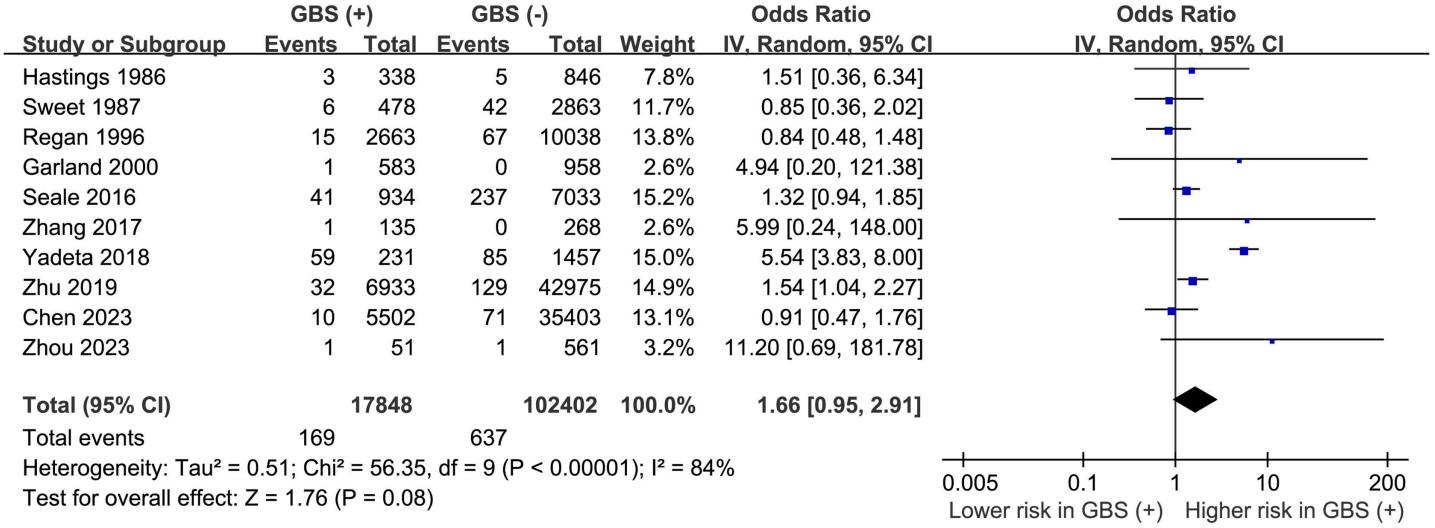
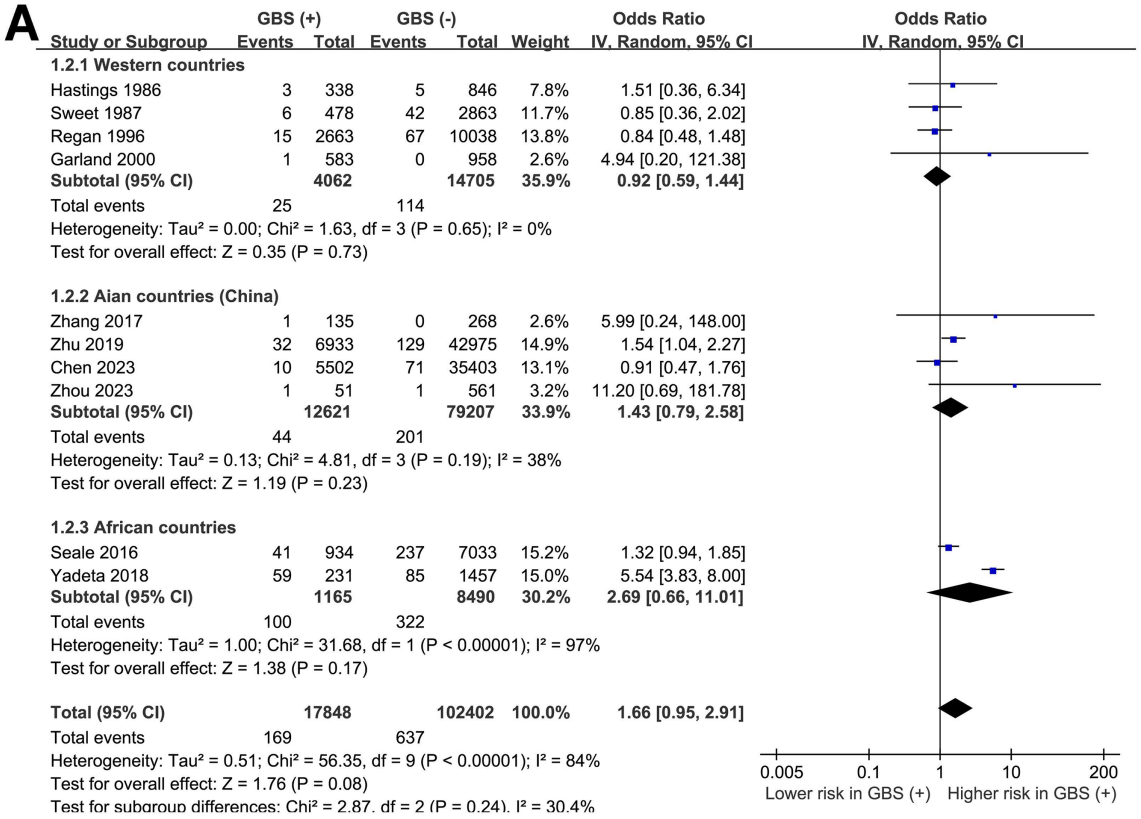


Figure 2 **Forest plots for a meta-analysis of the association between maternal rectovaginal GBS colonization and the risk of stillbirth.** GBS, group B streptococcus; OR indicates the odds of stillbirth associated with maternal GBS colonization; values greater than 1 suggest increased risk, whereas values less than 1 indicate a reduced risk. Full-size DOI: 10.7717/peerj.18834/fig-2

an overall increased risk of stillbirth (OR: 1.66, 95% CI [0.95–2.91], $p = 0.08$; $I^2 = 84\%$; Fig. 2). The sensitivity analyses were performed by excluding one dataset at a time, but did not significantly change the results (OR: 1.24–1.85, p all > 0.05). Interestingly, a further sensitivity analysis that was limited to the five studies including women all receiving IAP (*Chen et al., 2023*; *Garland, Kelly & Ugoni, 2000*; *Seale et al., 2016*; *Zhou & Mou, 2023*; *Zhu et al., 2019*) suggested an association between rectovaginal GBS colonization and an increased risk of stillbirth (OR: 1.36, 95% CI [1.02–1.80], $p = 0.03$; $I^2 = 15\%$). The subgroup analyses indicated that there was not a significant association between maternal rectovaginal GBS colonization in pregnancy and the risk of stillbirth in studies from western, Asian, or African countries ($p = 0.73$, 0.23, and 0.17; Fig. 3A). The association



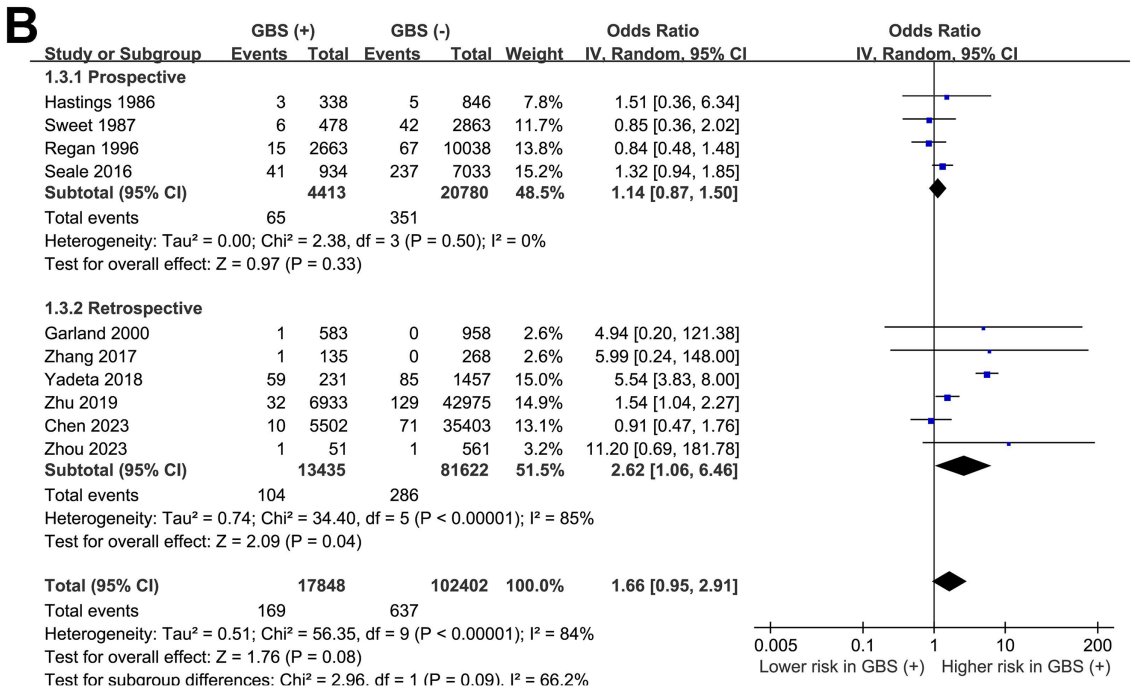


Figure 3 Forest plots of subgroup analyses of the association between maternal rectovaginal group B streptococcus colonization and the risk of stillbirth. (A) Subgroup analysis according to geographic regions; (B) subgroup analysis according to study design. OR indicates the odds of stillbirth associated with maternal GBS colonization; values greater than 1 suggest increased risk, whereas values less than 1 indicate a reduced risk.

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between maternal rectovaginal GBS colonization and the increased risk of stillbirth was not significant in prospective studies (OR: 1.14, $p = 0.33$), but significant in retrospective studies (OR: 2.62, $p = 0.04$). Although the difference between these subgroups was not statistically significant (p for subgroup difference = 0.09; Fig. 3B). Further subgroup analysis did not show a significant association between maternal GBS colonization and stillbirth in studies with vaginal or rectovaginal swab tests (p for subgroup effect = 0.50 and 0.07; Fig. 4A), or in cohorts with an overall incidence of stillbirth > or ≤ 0.05% (p for subgroup effect = 0.31 and 0.16; Fig. 4B). Subsequent analysis suggested that maternal rectovaginal GBS colonization was related to an increased risk of stillbirth in studies with multivariate analysis, but not in studies with univariate analysis (OR: 2.11 *vs.* 0.92, p for subgroup effect = 0.04 and 0.73; p for subgroup difference = 0.06; Fig. 5A). Finally, the subgroup analysis showed that maternal rectovaginal GBS colonization in pregnancy was not significantly related to an overall increased risk of stillbirth in studies with NOS scores of 6 to 7, or those of 8 to 9 (p for subgroup effect = 0.85 and 0.07; Fig. 5B).

Publication bias

Upon visual inspection, the funnel plots for a meta-analysis of the association between maternal rectovaginal GBS colonization and the risk of stillbirth were symmetrical, indicating a low likelihood of publication bias (Fig. 6). Additionally, Egger's regression test results ($p = 0.29$) also supported this conclusion by suggesting a low risk of publication bias.

DISCUSSION

This meta-analysis aimed to elucidate the relationship between maternal rectovaginal GBS colonization and the risk of stillbirth. The pooled analysis from ten studies, comprising 121,195 pregnant women, revealed no significant overall association. However, the observed p-value (0.08) approaching significance indicates the possibility of an association that might not have been detected due to the low event rates of stillbirth across the included studies. Further sensitivity and subgroup analyses revealed a significant association in studies involving IAP, in retrospective studies, and in studies employing multivariate analysis. These findings underline the need for cautious interpretation, as the limited number of stillbirth occurrences may have rendered the studies underpowered for identifying a clear relationship, although significant associations were observed under specific conditions. Further research is still needed to elucidate the association between maternal rectovaginal GBS colonization and the risk of stillbirth.

Hypothetically, GBS colonization could contribute to adverse pregnancy outcomes through several pathophysiological mechanisms. The primary mode involves the inflammatory response elicited by GBS colonization in maternal and fetal tissues (*Afsari, White & Adhikari, 2024*). Upon colonization, GBS can provoke an immune response characterized by the release of pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-alpha (*Vadeboncoeur et al., 2003*). This inflammatory cascade can lead to chorioamnionitis, an infection or inflammation of the fetal membranes, which is known to be associated with adverse outcomes, including preterm labor and fetal hypoxia (*Conde-Agudelo et al., 2020*). The inflammatory mediators released during this process can

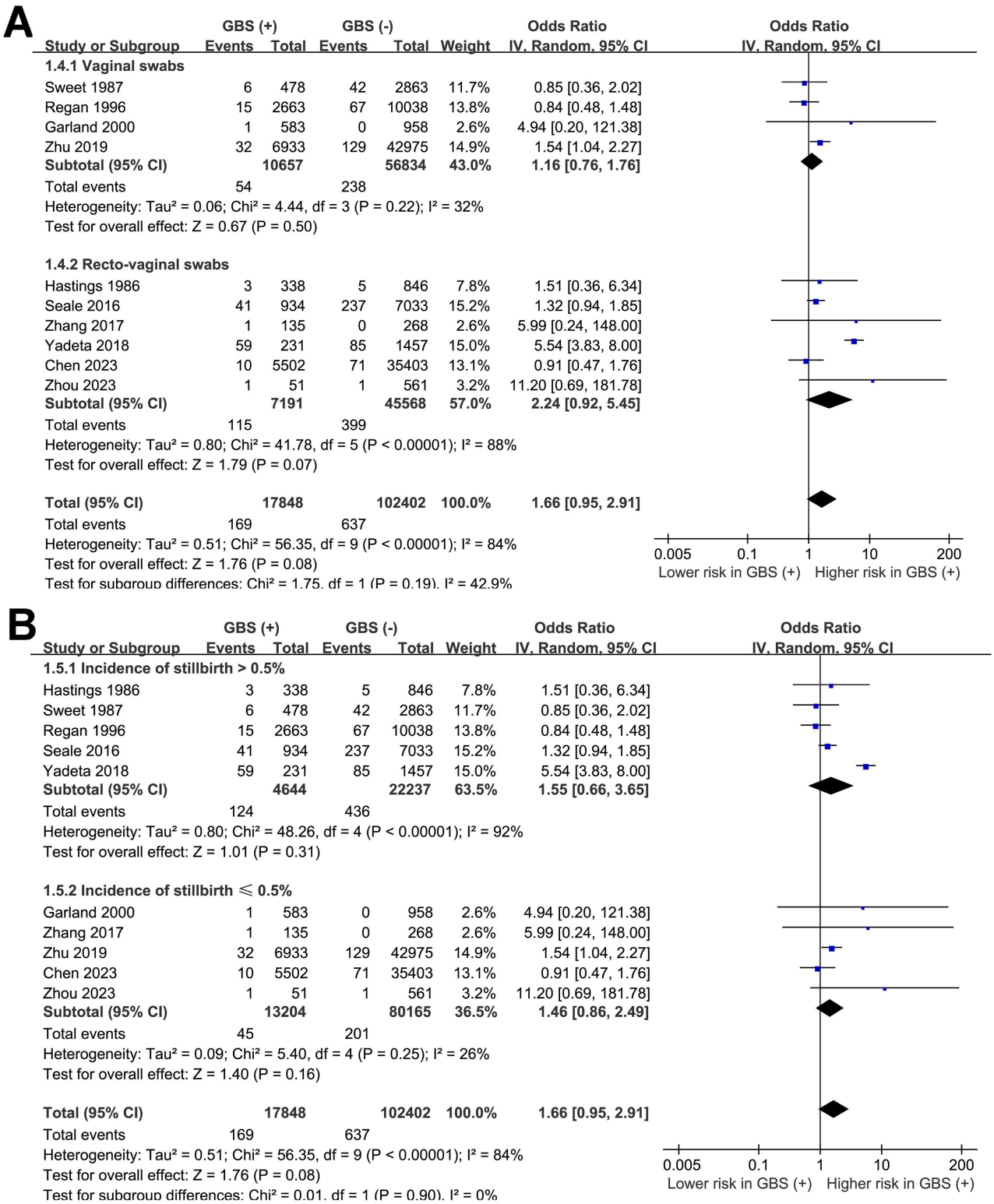

Figure 4 Forest plots for subgroup analyses of the association between maternal rectovaginal GBS colonization and the risk of stillbirth. (A) Subgroup analysis according to methods for determining rectovaginal GBS colonization; (B) subgroup analysis according to overall incidence of stillbirth among the studied cohorts. GBS, group B streptococcus; OR indicates the odds of stillbirth associated with maternal GBS colonization; values greater than 1 suggest increased risk, whereas values less than 1 indicate a reduced risk. Full-size DOI: 10.7717/peerj.18834/fig-4

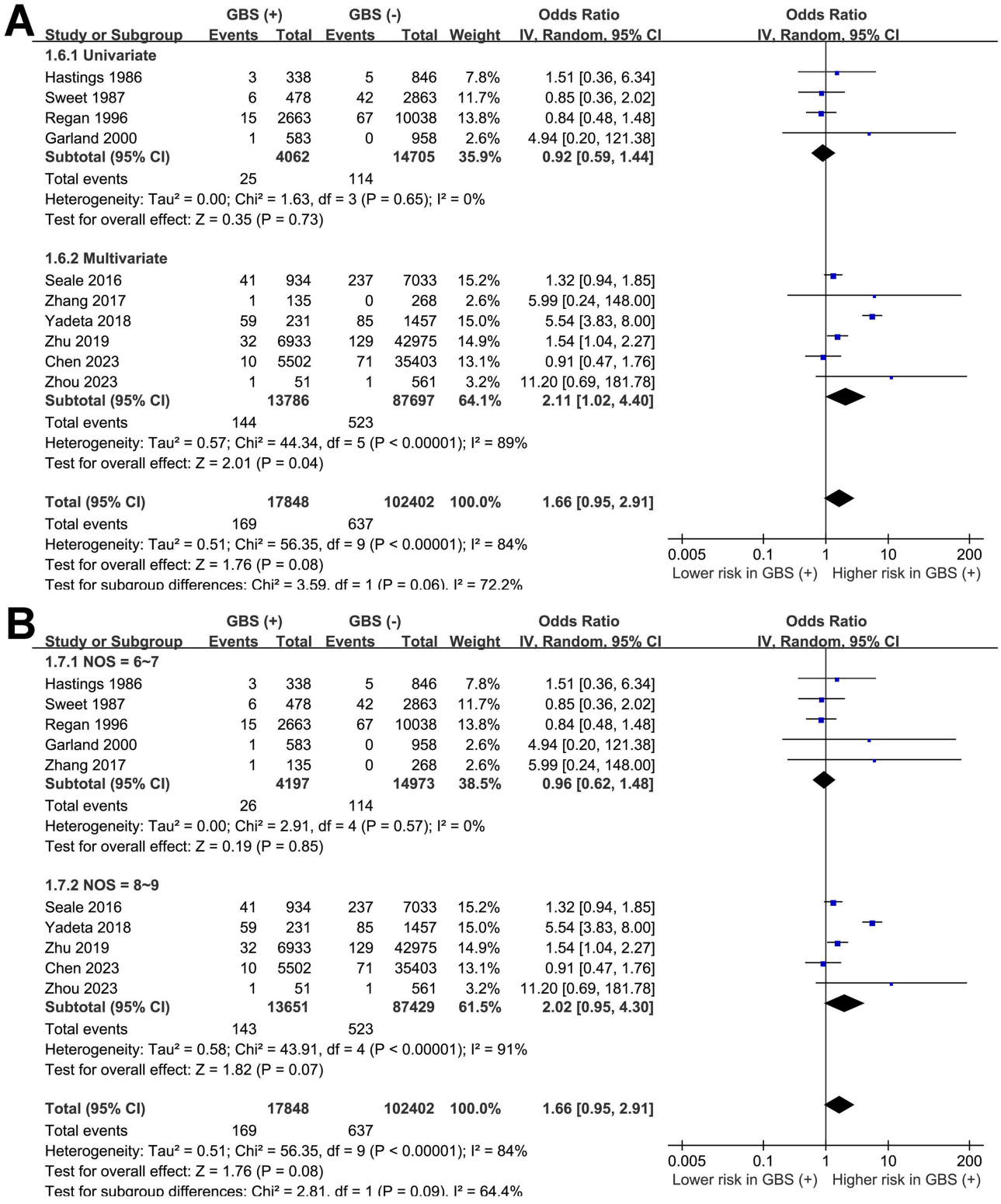

Figure 5 Forest plots for subgroup analyses of the association between maternal rectovaginal GBS colonization and the risk of stillbirth. (A) Subgroup analysis according to analytic model for the association between GBS colonization and the risk of stillbirth; (B) subgroup analysis according to the NOS scores of the included studies. GBS, group B streptococcus; OR indicates the odds of stillbirth associated with maternal GBS colonization; values greater than 1 suggest increased risk, whereas values less than 1 indicate a reduced risk.

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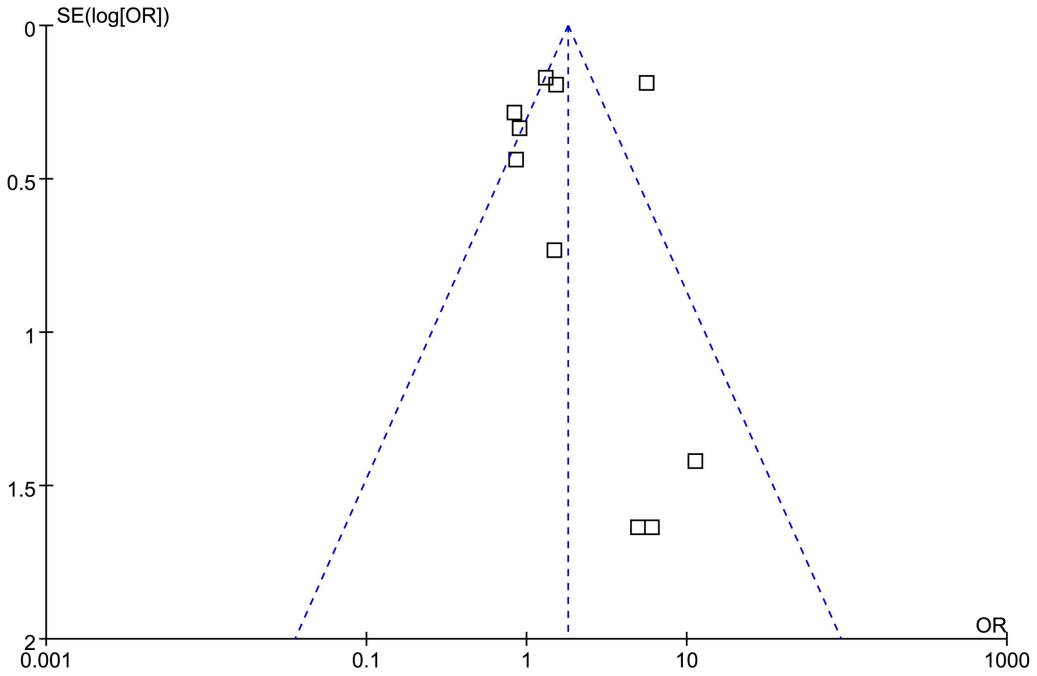

Figure 6 Funnel plots evaluating the publication bias for meta-analysis of the association between maternal rectovaginal GBS colonization and the risk of stillbirth. GBS, group B streptococcus.
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compromise placental function, impairing blood flow and oxygen delivery to the fetus, ultimately leading to fetal distress and demise (*Jain et al., 2022*). Moreover, GBS colonization may induce mechanical stress on the membranes and placenta, thereby weakening their structural integrity (*Surve et al., 2016*). This could lead to premature rupture of membranes (PROM), a known risk factor for preterm labor and stillbirth (*Regan, Chao & James, 1981*). The inflammatory process can also trigger uterine contractions, further increasing the risk of preterm birth and related complications. The fetal response to maternal GBS colonization is critical, as the developing fetus is particularly vulnerable to maternal infections (*Kumar, Saadaoui & Al Khodor, 2022*). The inflammatory response can activate fetal stress responses, and elevated maternal inflammatory markers can result in fetal heart rate abnormalities and reduced fetal movements, ultimately contributing to fetal demise in severe cases (*Goldstein et al., 2020*).

The sensitivity analyses performed in this meta-analysis yielded a significant association in studies in which all women received IAP. This finding underscores the importance of contextual factors when evaluating the risk of stillbirth in the presence of GBS colonization. The subgroup analysis also highlighted significant associations between maternal rectovaginal GBS colonization and stillbirth risk in retrospective studies and in studies employing multivariate analyses controlling for maternal age and parity. These findings suggest that the context of GBS colonization—such as the use of IAP and study design—can significantly influence the observed associations and may provide insights into the mechanisms linking GBS colonization to stillbirth.

While this meta-analysis provides valuable insights into the association between maternal rectovaginal GBS colonization and stillbirth risk, significant heterogeneity was observed. Several factors may contribute to this heterogeneity, including variations in study design and population characteristics, such as geographic location and socioeconomic status, which can influence GBS colonization rates and outcomes. Methodological differences, including the timing and type of specimens used for GBS assessment, as well as the definitions and reporting of stillbirth, may also introduce discrepancies. The use of IAP in some studies could further complicate the relationship between GBS colonization and stillbirth. Additionally, differences in statistical analysis techniques and adjustments for confounding factors may lead to varying effect estimates. To mitigate these sources of heterogeneity in future research, it is essential to adopt standardized protocols for GBS screening and stillbirth definitions, along with robust statistical methods to control for potential confounders. Exploring the impact of GBS genotypes and their associated virulence factors on stillbirth risk could also enhance understanding of this complex relationship.

Despite the lack of significant results in the overall analysis, the strengths of this meta-analysis include a comprehensive literature search across multiple databases, ensuring a broad representation of available studies, and the incorporation of sensitivity analyses that provide insights into how different factors influence the results. However, several limitations must be acknowledged. First, six of the included studies were retrospective, which may be prone to recall and selection biases (*Talari & Goyal, 2020*). These biases could lead to overestimation or underestimation of the association between GBS colonization and stillbirth, particularly if data on key confounding factors, such as maternal comorbidities or socioeconomic status, were incomplete. Future studies employing prospective designs and rigorous data collection methods are needed to reduce such biases. Second, variability in population characteristics, definitions of stillbirth, and methods for assessing GBS colonization (vaginal *vs*. rectovaginal swabs) likely contributed to heterogeneity in the results. This heterogeneity may have attenuated the observed association. Standardized definitions and protocols for evaluating GBS colonization should be adopted to enhance comparability across studies. Besides, genotypic variations in GBS may play a role in its pathogenicity and association with adverse pregnancy outcomes, including stillbirth. Specific GBS serotypes, such as Ia, Ib, and III, have been linked to increased virulence and invasive disease in neonates (*Huebner et al., 2022*; *Liu & Ai, 2024*). These differences could influence the inflammatory response, placental invasion, or fetal immune tolerance, potentially exacerbating the risk of stillbirth. However, none of the included studies in this meta-analysis provided genotypic data, limiting our ability to explore this factor further. Future studies should investigate the impact of GBS genotypes on pregnancy outcomes to better understand these mechanisms and refine prevention strategies. Moreover, the observational nature of the included studies precludes causal inferences. Residual confounding, even in studies employing multivariate analyses, may have influenced the findings. This could result in either overestimation (if unmeasured confounders are positively associated with both GBS colonization and stillbirth) or underestimation (if key protective factors were not accounted for). Large-scale studies with

individual participant data meta-analyses could help overcome this limitation by enabling more precise adjustments for confounders. Lastly, the low event rate of stillbirth in most included studies may have reduced statistical power, potentially leading to an underestimation of the association. Collaborative studies pooling data from multiple centers or regions could increase the sample size and improve statistical precision.

Although the overall analysis did not reveal a strong association between GBS colonization and stillbirth, the significant associations observed in specific subgroups may provide useful information when conducting future studies. For example, the influence of IAP use, study design, and possible confounding factors should be considered when designing future studies for an evaluation of the association between maternal rectovaginal GBS colonization and stillbirth. In addition, understanding the mechanisms by which GBS colonization may contribute to adverse pregnancy outcomes could guide future research and clinical practices aimed at mitigating these risks.

CONCLUSIONS

In conclusion, while this meta-analysis did not establish a statistically significant association between maternal rectovaginal GBS colonization and stillbirth, the underlying pathophysiological mechanisms underscore the complexity of this relationship. The potential for GBS to induce inflammatory responses, disrupt the vaginal microbiome, and compromise placental integrity suggests that it may still pose a risk for adverse outcomes in certain contexts. Further research should focus on identifying and mitigating risk factors associated with GBS colonization, particularly in high-risk populations, to improve maternal and fetal health outcomes. Understanding these mechanisms will be critical for developing interventions that can enhance pregnancy outcomes, especially for vulnerable populations. Future studies should aim to address the limitations identified in this meta-analysis and explore the interplay between GBS colonization, IAP, possible confounding factors, and stillbirth risk in greater detail.

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The authors declare that they have no competing interests.

Author Contributions

- Yujue Wang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jingjing Liu performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Jinlian Song analyzed the data, prepared figures and/or tables, and approved the final draft.

- Teng Zhang conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a systematic review/meta-analysis.

Supplemental Information

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