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REVIEW

Effects of Probiotics in Pregnant Women with Gestational Diabetes Mellitus: An Overview of Systematic Reviews

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Objective: Gestational diabetes mellitus (GDM) is a common pregnancy complication with adverse maternal and neonatal consequences. Probiotics have been proposed as a non-pharmacological intervention, but their effectiveness remains controversial. This study aimed to evaluate the methodological quality and reported efficacy evidence of systematic reviews and meta-analyses (SRs/MAs) on probiotic supplementation in pregnant women with GDM.

Methods: Four electronic databases were searched for English-language SRs/MAs published between 2017 and 2024 that examined probiotic supplementation in women with GDM. Methodological quality was assessed using PRISMA 2020, AMSTAR 2, and ROBIS, and reported efficacy outcomes were systematically synthesized.

Results: A total of 16 SRs/MAs were included. According to the AMSTAR-2 assessment, four studies were rated as low quality, while the remaining studies were rated very low. The PRISMA assessment showed that 8 of the 27 items had reporting completeness above 80%, whereas items 5, 8, and 22 showed completeness below 60%; at the study level, 12 SRs/MAs achieved overall PRISMA reporting completeness above 80%. The ROBIS scale assessment results showed that all SRs/MAs were rated as low risk of bias in Phase 1, Domain 1, Domain 3, and six items of Domain 4, as well as 12 items in Phase 3. However, in Domain 2, all SRs/MAs were rated as high risk. Regarding efficacy evaluation, probiotic supplementation significantly improved FPG, insulin-related indices (FSI, HOMA-IR, HOMA-B, QUICKI), and lipid profiles (TG, TC, HDL-C, VLDL-C). In addition, probiotics showed effects on markers of inflammation (CRP) and oxidative stress (NO, MDA, GSH, TAC) and indicated benefits in reducing neonatal risks. However, heterogeneity and overlap among primary studies were identified.

Conclusion: Probiotic supplementation demonstrates efficacy in improving metabolic biomarkers related to GDM and maternal and neonatal outcomes. Nevertheless, the overall certainty of evidence is limited by suboptimal methodological quality, heterogeneity, and overlap among primary studies.

Keywords: gestational diabetes mellitus, probiotic, pregnant women, meta-analysis, overview

Introduction

Gestational diabetes mellitus (GDM), defined as impaired glucose tolerance that first appears or is discovered during pregnancy, is one of the common pregnancy complications.[1] Wang et al found that the global standardized prevalence of GDM was 14.0%,[2] with the highest prevalence in the Middle East, North Africa, and Southeast Asia. However, a worldwide study that included 129 studies involving more than 4.46 million married women found that women with GDM were 8.3 times more likely to develop T2DM compared to pregnant women with normal blood glucose levels.[3] This transition is mainly attributed to persistent insulin resistance and β-cell dysfunction after pregnancy. Additionally, the incidence of GDM was relatively higher in Europe compared to other regions. Accordingly, GDM has become a major public health concern. Poor glycemic regulation during pregnancy can pose significant risks to both the mother

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and the newborn, such as pre-eclampsia, anxiety and depression, gestational hypertension, urinary tract infections in the mother,[4] and hypoglycaemia, jaundice, macrosomia, and respiratory distress in the newborn.[5] In the long term, GDM increases the risk of pregnant women developing T2DM, obesity, and metabolic syndrome in the future.[6,7]

Currently, the main treatment methods for GDM include dietary control, moderate exercise, oral hypoglycemic agents, and insulin injections when necessary.[8] However, some pregnant women suffer from low adherence and adverse effects such as gastrointestinal discomfort, weight gain, or hypoglycemia.[9] Consequently, this makes many doctors and patients prefer non-pharmacological therapies first. Studies have shown that gut microbiota imbalance significantly impacts insulin metabolism in GDM.[10–12] Crusell et al found that the composition of the intestinal microbiota of GDM women was already disturbed compared with that of pregnant women with normal blood glucose. Moreover, this imbalance remained detectable even eight months postpartum.[13] Dabke et al reported that gut microbiota aggravates metabolic dysfunctions such as insulin resistance by affecting intestinal permeability and triggering inflammation.[14] For this condition, probiotic supplementation can increase beneficial bacteria in the gut to help restore microbiota balance.[15] Studies suggest that probiotic supplementation may positively affect the overall health of pregnant women while also contributing to fetal development. As a result, probiotics have emerged as an emerging complementary approach for GDM patients.[15,16] Mechanistically, probiotics may exert potential benefits in GDM through multiple interrelated pathways. First, they help restore gut microbiota balance, which in turn positively modulates the composition and function of the intestinal microbiome. Second, probiotics can regulate the secretion of pro-inflammatory mediators, thereby alleviating local and systemic inflammatory responses, improving insulin resistance. Third, probiotics promote the restoration of intestinal barrier function and normalize gut permeability. These actions may act synergistically through multiple metabolic pathways, ultimately exerting beneficial effects on the onset and progression of GDM.[17]

The application of probiotics in GDM has become a research hotspot. As a potential therapeutic approach, probiotics have gained increasing scientific support and have become an important field in evidence-based medicine. Many systematic reviews and meta-analyses (SRs/MAs) have been conducted on the effects of probiotics on GDM and its complications in pregnant women and newborns. However, research findings sometimes vary and even present conflicting results. For instance, Jiajia Pan et al found that probiotic supplementation had no significant effect on fasting plasma glucose (FPG).[18] In contrast, Taylor et al reported the opposite conclusion, indicating that probiotics could significantly reduce FPG levels in GDM women.[19] Similarly, a meta-analysis by Jiayue Zhang et al concluded that probiotics had no notable impact on reducing TC and LDL-C.[20] However, research by Enav Yefet et al demonstrated that probiotic therapy could lower these lipid markers.[21] These inconsistencies highlight the ongoing controversy surrounding the clinical application of probiotic therapy in GDM patients.

To date, numerous SRs/MAs have evaluated the effects of probiotic supplementation on glycemic control and metabolic parameters in GDM. However, these studies have not yet been systematically assessed. The quality of the original studies included in different SRs/MAs may vary, with some studies suffering from methodological limitations, such as poor study design or small sample sizes, which could compromise the overall quality of the SRs/MAs and undermine the credibility of their findings. To the best of our knowledge, no comprehensive overview has been conducted on the effects of probiotic supplementation on glycemic control and metabolic parameters in GDM. Therefore, we performed a thorough and systematic search and evaluation of relevant SRs/MAs to help clinical decision-makers better understand the impact of probiotic supplementation on GDM. Our goal is to provide more reliable evidence to ensure the scientific validity and effectiveness of its clinical application.

Methods

Registration

This study is a quality assessment of meta-analyses and does not involve the direct participation of human subjects or data collection. Therefore, ethical approval is not required. The study has been registered in the PROSPERO database with the registration number [CRD420250651897] to ensure transparency and standardization of the study.[22]

Inclusion and Exclusion Criteria

Study Type

This study includes all SRs/MAs on probiotic supplementation for the treatment of GDM, regardless of intervention duration or study location. However, only studies published in English, full-text articles, and those exclusively include RCTs are considered for inclusion. The probiotics investigated in the included reviews mainly involved Lactobacillus and Bifidobacterium species, either alone or in combination.

Subjects

The study population consists of pregnant women who have been clinically diagnosed with GDM and have not used any hypoglycemic medications. There will be no restrictions on age, gestational stage, or weight status.

Interventions

The experimental group received probiotic-containing foods or supplements, while the control group received a placebo without probiotics or no intervention. Since probiotics, prebiotics, and synbiotics are often used together and have complementary effects, and many food products and supplements contain all three, this study does not strictly distinguish between them.

Outcome Measures

The primary outcome included fasting plasma glucose (FPG). Secondary outcomes included: (1) Insulin metabolism related biomarkers: fasting serum insulin (FSI), homeostatic model assessment of insulin resistance (HOMA-IR), homeostatic model assessment of β-cell function (HOMA-B, calculated from fasting glucose and insulin to estimate β-cell function), quantitative insulin sensitivity check index (QUICKI), and glycated hemoglobin (HbA1c); (2) Fasting lipid profiles: fasting total cholesterol (TC), fasting low-density lipoprotein cholesterol (LDL-C), fasting high-density lipoprotein cholesterol (HDL-C), fasting very-low-density lipoprotein cholesterol (VLDL-C), and fasting triglycerides (TG); (3) Inflammatory biomarkers: high-sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α); (4) Oxidative stress biomarkers: malondialdehyde (MDA), total antioxidant capacity (TAC), nitric oxide (NO), and glutathione (GSH); (5) pregnancy outcomes: gestational hypertension (GHTN), preeclampsia (PE), and polyhydramnios, etc; (6) neonatal outcomes: neonatal hyperbilirubinemia (NHB), large for gestational age (LGA), neonatal hospitalization (NH), neonatal hypoglycemia (NHG), and neonatal intensive care unit (NICU) admission.

Exclusion Criteria

(i) duplicate publications from the same team; (ii) expert consensus statements, abstracts, reviews, conference papers, and animal studies; (iii) studies lacking primary outcome indicators; (iv) studies with less than 20 participants in the original study included in the SRs/MAs; (v) studies that do not explicitly diagnose participants with GDM.

Retrieval Strategy

Two authors (RHX and ZNJ) searched four databases, including PubMed, Web of Science, EMBASE, and the Cochrane Library, to collect relevant literature on the effects of probiotic supplementation on metabolic outcomes in pregnant women with GDM from the time the databases were created until December 30, 2024. Keywords included “probiotics”, related strains such as “lactobacilli” and “bifidobacteria”, as well as gestational diabetes-related terms and their synonyms. In Appendix 1, we present the search strategy for each database in detail.

Literature Screening and Data Extraction

The retrieved literature was imported into NoteExpress 3.9 software, and duplicates were removed. Two researchers (LYH and YY) reviewed the titles and abstracts of the remaining studies independently, first excluding studies that did not meet the inclusion criteria and then reading the full text of the literature that passed the initial screening to exclude further studies that did not meet the inclusion criteria. Any discrepancies were resolved by a third researcher (BHE). A PRISMA 2020 flowchart was used to clearly present the literature search, screening, and inclusion process, as shown in Figure 1. After the final studies were determined, two authors (LYH and YY) independently extracted data to ensure

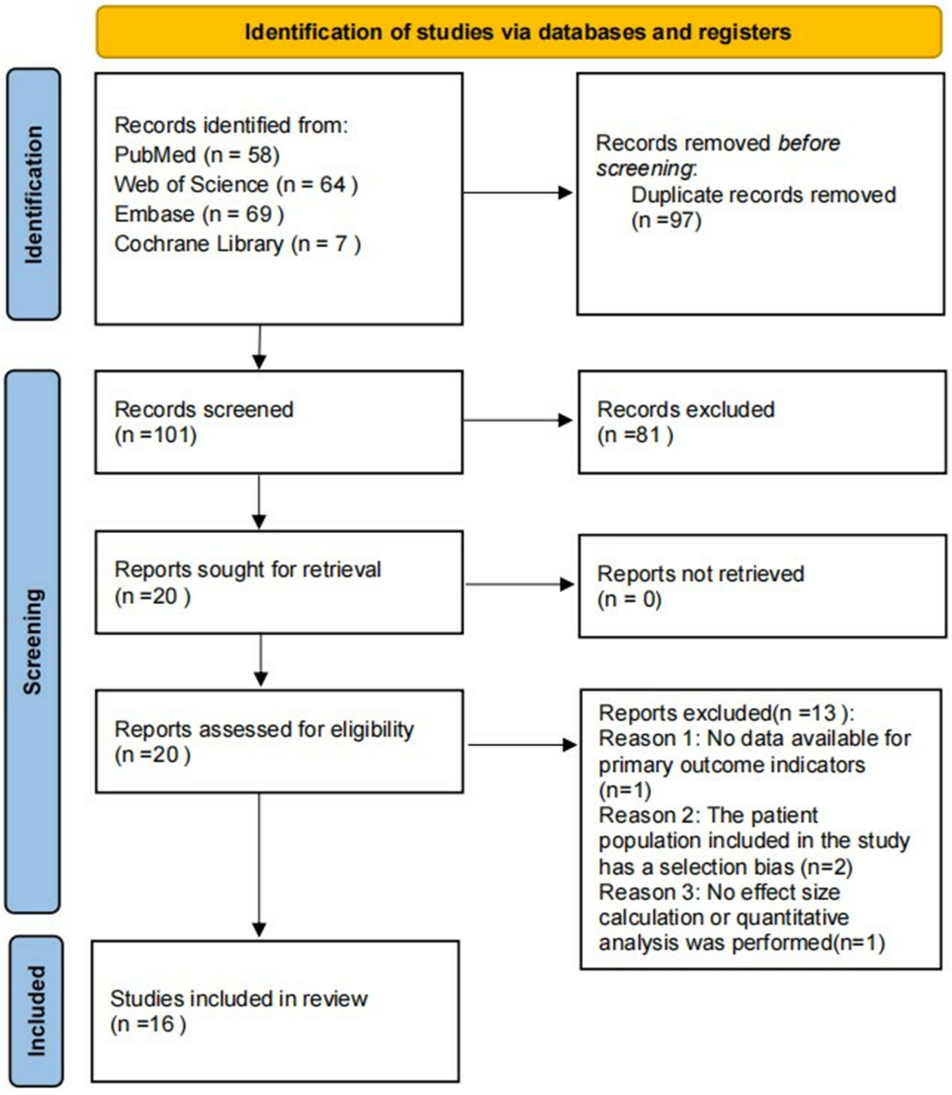

Figure 1 Flowchart of the screening process.

accuracy and consistency. The extracted information included the first author, year of publication, study region, study type, number of included studies, sample size, intervention and control measures, outcome indicators, and methodological assessment tools; heterogeneity statistics (I^2), as reported in the original meta-analyses, were also extracted.

Assessment Methods

Quality Assessment Using PRISMA 2020 (Appendix 2)

This study evaluates the reporting quality of systematic reviews and meta-analyses based on the PRISMA 2020 guidelines.[23,24] The PRISMA 2020 checklist consists of 27 items, and each item is scored as "yes", "partially yes", or "no" according to the degree of compliance, with a score of 1, 0.5, or 0. Studies meeting over 21 criteria are high-quality, 15–20 criteria are medium-quality, and fewer than 15 are low-quality. Two authors (RHX and ZNJ) independently evaluated each study, and the third researcher (BHE) resolved any discrepancies in scoring.

Methodological Quality Assessment Tool — AMSTAR 2 Scale (Appendix 3)

AMSTAR 2 is a validated tool for assessing the quality of systematic reviews,[25] consisting of 16 items that cover aspects such as the development and registration of the research protocol, comprehensiveness of the search strategy,

methodological rigor, and robustness of the results. Each item is rated as "yes," "no," or "partial yes" according to the level of compliance. Two independent reviewers (RHX and ZNJ) assessed the quality of all included studies. If discrepancies occurred, the third reviewer (BHE) participated in discussions to reach a consensus.

Risk of Bias Assessment Tool — ROBIS Scale (Appendix 4)

This study used the three-stage assessment process of the ROBIS tool to evaluate the risk of bias in the included studies.[26] In each domain, researchers scored the SRs/MAs based on the questions in the scale and classified the risk of bias as "low," "unclear," or "high." Two authors (RHX and ZNJ) individually evaluated the included SRs/MAs. If disagreements could not be resolved, a third reviewer (BHE) made the final decision.

Assessment of Overlap of Primary Studies

To evaluate the overlap of primary randomized controlled trials among the included systematic reviews, a citation matrix was created and the Corrected Covered Area (CCA) was calculated following established methods. The CCA quantifies the extent of shared primary studies across reviews, with higher values reflecting a greater degree of overlap.[27]

Results

Literature Retrieval Results

198 articles were retrieved from multiple databases, including 58 from PubMed, 64 from Web of Science, 7 from Cochrane Library, and 69 from Embase. After excluding duplicate articles, 97 articles remained. According to the pre-set screening criteria, 16 articles were ultimately included for analysis. The literature screening process and reasons for exclusion are shown in Figure 1. The SRs/MAs that were not included with their reasons for exclusion are listed in Appendix 5.

The Basic Characteristics of the Included Literature

The 16 SRs/MAs included in this study collectively encompassed 183 original studies, all of which were RCTs published between 2017 and 2024.[18–21,28–39] Most of the studies were conducted in Iran, contributing 129 studies, with the remaining research distributed across Ireland, New Zealand, the United Kingdom, the United States, Australia, Finland, Denmark, Turkey, Israel, Thailand, and China. The number of studies included in each SR/MA ranged from 4 to 28, with participant numbers ranging from 288 to 4865. The treatment methods of the observation group mainly involved probiotics, probiotic yogurt, or synbiotics, while the comparison group used a placebo or ordinary yogurt. The probiotics used mainly included Lactobacillus and Bifidobacterium species, administered alone or in combination, with some formulations also containing Streptococcus thermophilus or prebiotics. Regarding quality assessment, 8 studies used the Cochrane Risk of Bias Assessment tool, 2 studies applied both GRADE and the Cochrane Risk of Bias tool, 1 study used only GRADE, 1 study used the Cochrane Risk of Bias tool along with the Newcastle-Ottawa scale, 2 studies applied the Jadad scale, 1 study used the JBI tool, and 1 study followed the CONSORT guidelines. The primary outcome measure of this study was FPG, with further details provided in Table 1.

Report on the Quality Evaluation Results

PRISMA Reporting Quality Results

According to the PRISMA evaluation guidelines, at the item level, 8 of the 27 PRISMA items were reported with a completeness rate exceeding 80% across the included SRs/MAs, particularly those related to the title, structured abstract, introduction, and inclusion/exclusion criteria. However, the reporting completeness of items 5, 8, and 22 was below 60%.

At the study level, the survey results indicated that only 9 SRs/MAs were registered before the study, and only 5 SRs/MAs provided the complete search strategy for at least one electronic database. Furthermore, 6 SRs/MAs did not address potential biases in the study reports, and only 10 SRs/MAs provided a detailed description of the strength of evidence for the primary outcomes. Overall, 12 SRs/MAs demonstrated an overall PRISMA reporting completeness of over 80%. Further details can be found in Figure 2.

Table 1 Characteristics of Included Reviews

First Author/Year	Country	Study Size/ Number of Cases	Probiotic Strains	Control Group	Methodology Evaluation Tools	Outcomes
Pan et al 2019[18]	Iran (4), Ireland (1), New Zealand (1)	6/830	Mainly Lactobacillus (eg, L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. reuteri, L. salivarius, L. delbrueckii) and Bifidobacterium spp., sometimes with S. thermophilus, inulin/FOS, or selenium.	Placebo	The Jadad Scale	①②③⑨㉒
Taylor et al 2017[19]	Iran (3), Ireland (1)	4/288	Predominantly Lactobacillus (L. acidophilus, L. casei, L. delbrueckii, L. salivarius, L. plantarum, L. paracasei) and Bifidobacterium spp., occasionally with S. thermophilus.	Placebo	Cochrane Collaboration Risk of Bias tool	①②④⑨⑩⑪⑫⑬⑭⑮⑯⑰
Zhang et al 2019[20]	Iran (8), Turkey (1), Ireland (1), Thailand (1)	11/719	Multi-strain combinations of Lactobacillus (eg, L. acidophilus, L. casei, L. fermentum, L. gasseri, L. reuteri, L. salivarius, L. plantarum, L. paracasei, L. delbrueckii) and Bifidobacterium spp., with some trials including S. thermophilus.	Placebo	Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑧⑲⑳㉑㉓㉔㉕㉖
Ramanathan et al 2020[28]	Iran (6), Ireland (1), New Zealand (1), Turkey (1)	9/1053	Not Reported	Placebo	Cochrane Collaboration Risk of Bias tool	②③
Mahdizade et al 2021[29]	Iran (13), Ireland (2), New Zealand (2), UK (2), Australia (4), USA (1), Finland (2), Denmark (1), China (1)	28/4865	Mainly Lactobacillus (L. acidophilus, L. casei, L. rhamnosus, L. plantarum, L. fermentum, L. salivarius, L. reuteri, L. paracasei, L. bulgaricus) and Bifidobacterium spp., occasionally with S. thermophilus.	Placebo	JBI	①②③④⑤⑥⑦⑧⑲⑳㉔㉕㉖㉘㉙㉚㉛㉜㉝
Zhou et al 2021[30]	Iran (10), Ireland (1), Thailand (1)	12/894	Predominantly Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. reuteri, L. salivarius, L. delbrueckii) and Bifidobacterium spp., with S. thermophilus and prebiotics (inulin/FOS).	Placebo/ ordinary yoghurt	Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑦⑧⑯⑰⑱⑲㉑㉒㉓㉔㉕㉖㉗㉘㉞㉟㊱㊲㊳㊵㊶
Chen et al 2021[31]	Iran (5), Ireland (1), Thailand (1)	7/462	Mainly Lactobacillus (L. acidophilus, L. casei, L. reuteri, L. fermentum, L. salivarius, L. paracasei, L. delbrueckii, L. plantarum) and Bifidobacterium spp., sometimes with S. thermophilus.	Placebo	The Jadad Scale	②⑦⑧㉕㉖
Pan et al 2021[32]	Iran (12), Ireland (2), New Zealand (2), Australia (1), Finland (2), Thailand (1)	2972	Multi-strain formulations with Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. rhamnosus, L. salivarius, L. delbrueckii) and Bifidobacterium spp. (B. bifidum, B. lactis, B. longum, B. breve, B. infantis, B. animalis), occasionally with S. thermophilus or prebiotics.	Placebo/ ordinary yoghurt	Cochrane Collaboration Risk of Bias tool	①②③⑳㉘㉚㊸㊹
Hasain et al 2021[33]	Iran (8), Ireland (1), Thailand (1)	10/594	Mainly Lactobacillus (L. acidophilus, L. casei, L. fermentum, L. plantarum, L. salivarius, L. paracasei, L. delbrueckii) and Bifidobacterium spp., sometimes with S. thermophilus.	placebo	Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑧⑰⑱㉖㉛㉜㉞㉟㊴㊷
Çetinkaya et al 2022[34]	Iran (7), Thailand (1)	8/551	Predominantly Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri) and Bifidobacterium spp., occasionally with S. thermophilus and prebiotics (inulin/FOS).	Placebo/ ordinary yoghurt	Cochrane Collaboration Risk of Bias tool	①②③⑳㉚
Suastika et al 2023[35]	Iran (12), Thailand (1)	13/896	Mainly Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum) and Bifidobacterium spp., sometimes with S. thermophilus or L. delbrueckii.	Placebo	GRADE, Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑦⑧

Yefet et al 2023[21]	Iran (9), Ireland (1), Thailand (1), Turkey (1), Finland (1)	14/854	Predominantly Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. salivarius, L. reuteri, L. rhamnosus, L. gasseri, L. delbrueckii) and Bifidobacterium spp., with some formulations including S. thermophilus.	placebo	CONSORT guidelines	①②③④⑤⑥⑨⑲⑳㉑㉒
Mu et al 2023[36]	Iran (8), Turkey (1), Ireland (1), Thailand (1)	11/779	Multi-strain mixtures of Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. reuteri, L. salivarius, L. delbrueckii) and Bifidobacterium spp., sometimes with S. thermophilus and prebiotics.	placebo	Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑨⑱⑳
Tabatabaeizadeh et al 2023[37]	Iran (3), China (1)	4/533	Mainly L. acidophilus (La-5) and B. lactis (Bb-12).	Ordinary yoghurt	Cochrane Collaboration Risk of Bias tool/the Newcastle-Ottawa Scale	②
Wu et al 2024[38]	Iran (11), Ireland (1), Israel (1), Turkey (1), Thailand (1)	15/1006	Multi-strain probiotics with Lactobacillus spp. (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. reuteri, L. paracasei, L. rhamnosus, L. salivarius, L. delbrueckii) and Bifidobacterium spp., sometimes with S. thermophilus or prebiotics/micronutrients.	Placebo	GRADE	①②③⑪⑯⑰⑳㉑㉓㉞㉟㊱㊲㊳㊴
Lan et al 2024[39]	Iran (10), Thailand (1)	11/713	Predominantly Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. fermentum, L. gasseri, L. delbrueckii) and Bifidobacterium spp., sometimes with S. thermophilus.	Placebo	GRADE/ Cochrane Collaboration Risk of Bias tool	①②③④⑤⑥⑧⑰⑱⑳㉑㉓㉔㉕㉖㊲㊴㊵㊶㊷

Notes: ① HOMA-IR; ②FPG; ③FSI; ④LDL; ⑤HDL; ⑥TG; ⑦CRP; ⑧NO; ⑨Gestational weight gain(GWG); ⑩Pregnancy-induced hypertension(PIH); ⑪Induction of labor(IOL); ⑫Commencement of glucose-lowering medications; ⑬Blood loss at delivery; ⑭Postpartum hemorrhage; ⑮Fetal anomalies; ⑯NICU; ⑰Delivery by caesarian section; ⑱TC; ⑲vLDL; ⑳QUICKI; ㉑Neonatal birth weight; ㉒Gestational age; ㉓Newborns' hyperbilirubinemia; ㉔TAC; ㉕GSH; ㉖MDA; ㉗BMI; ㉘HOMA-B; ㉙C-peptide; ㉚HbA1c; ㉛IL-6; ㉜TNF-α; ㉝Colostrum adiponectin; ㉞Preeclampsia; ㉟Premature birth; ㊱Polyhydramnios; ㊲Macrosomia; ㊳Newborn weight; ㊴Neonatal hypoglycemia; ㊵Newborn length; ㊶Newborn head circumference; ㊷Neonatal hospitalization; ㊸1 h and 2 h OGTT; ㊹Incidence of GDM.

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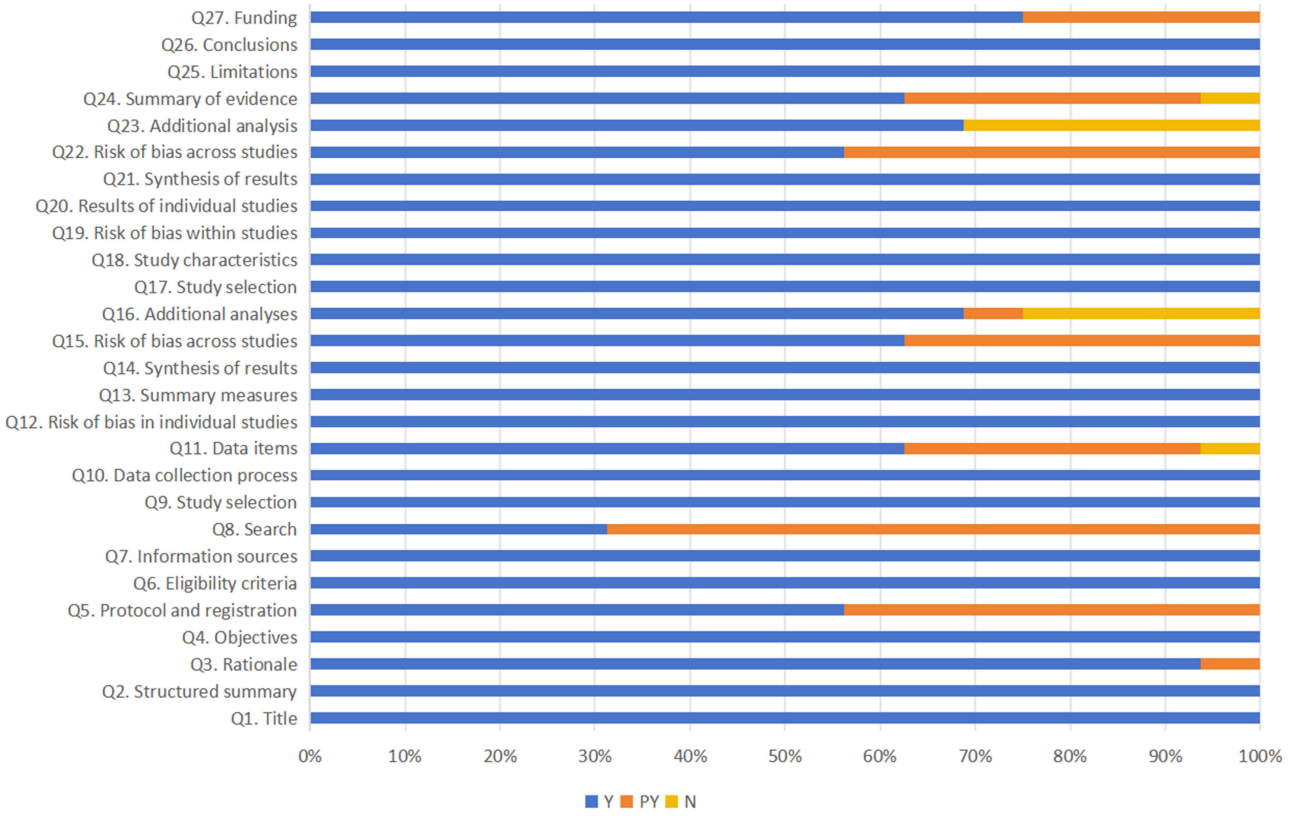

Figure 2 Report the quality evaluation results.

Methodological Quality Evaluation Results

Based on the AMSTAR-2 assessment, 4 SRs/MAs were rated low quality, while the remaining studies were classified as very low quality. The second item noted that only 5 studies had developed a pre-study protocol and were registered. In contrast, all studies failed to provide a list of excluded studies or explain the reasons for exclusion. All included studies utilized appropriate assessment tools to evaluate the risk of bias in the primary literature, including the Cochrane Collaboration Risk of Bias tool, the Newcastle-Ottawa Scale, CONSORT guidelines, and GRADE. The risk of bias in the included studies was discussed in the results of 11 studies. Among the SRs/MAs in this study, the relevant assessments of non-critical items were relatively complete. Further details are provided in Table 2.

Risk of Bias Evaluation Results

Table 3 presents the results of the risk of bias assessment for SRs/MAs using the ROBIS scale. All SRs/MAs were assessed as low risk in Phase 1, as well as in Domain 1 and 3 of Phase 2. However, all were rated as high risk in Domain 2. In Domain 4, only 6 studies (37.50%) were evaluated as low risk of bias in data synthesis and result presentation. In Phase 3, 12 SRs/MAs (75.00%) were assessed as low overall risk of bias.

Overlap of Primary Studies

The overlap of primary randomized controlled trials across the included systematic reviews was evaluated using the CCA. The CCA was 26.31%, indicating a high degree of overlap among the primary studies, with detailed information provided in Appendix 6.

Efficacy Evaluation Results

We summarized the findings from the tables and forest plots of each SR/MA, focusing on the impact of probiotics on the primary and secondary outcomes of GDM. In addition to effect estimates, heterogeneity statistics (I^2) reported in the original meta-analyses were extracted and summarized in Appendix 7.

Table 2 Result of the AMSTAR-2 Assessments

Author/Year	Q1	Q2*	Q3	Q4*	Q5	Q6	Q7*	Q8	Q9*	Q10	Q11*	Q12	Q13*	Q14	Q15*	Q16	Quality
Pan et al 2019[18]	Y	N	Y	PY	N	N	N	Y	Y	N	Y	Y	Y	Y	N	Y	CL
Taylor et al 2017[19]	Y	Y	Y	PY	Y	Y	N	Y	Y	N	Y	Y	N	Y	N	Y	CL
Zhang et al 2019[20]	Y	N	Y	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	CL
Ramanathan et al 2020[28]	Y	N	Y	PY	Y	Y	N	Y	Y	N	Y	N	Y	N	N	Y	CL
Mahdizade et al 2021[29]	Y	Y	Y	PY	Y	N	N	Y	Y	N	Y	Y	Y	Y	Y	Y	L
Zhou et al 2021[30]	Y	N	Y	PY	N	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	CL
Chen et al 2021[31]	Y	N	Y	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	Y	CL
Pan et al 2021[32]	Y	N	Y	PY	N	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	CL
Hasain et al 2021[33]	Y	Y	Y	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	L
Çetinkaya et al 2022[34]	Y	N	Y	PY	N	Y	N	Y	Y	N	Y	Y	Y	N	Y	Y	CL
Suastika et al 2023[35]	Y	N	Y	PY	Y	Y	N	Y	Y	N	Y	N	N	Y	N	Y	CL
Yefet et al 2023[21]	Y	N	Y	PY	N	Y	N	Y	Y	N	Y	N	N	Y	N	Y	CL
Mu et al 2023[36]	Y	N	Y	PY	N	N	N	Y	Y	N	Y	Y	N	Y	Y	Y	CL
Tabatabaeizadeh et al 2023[37]	Y	N	Y	PY	N	N	N	Y	Y	N	Y	Y	Y	Y	Y	Y	CL
Wu et al 2024[38]	Y	Y	Y	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	L
Lan et al 2024[39]	Y	Y	Y	PY	N	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	L

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Table 3 Result of the ROBIS Assessments

Review	Phase 1	Phase 2				Phase 3
	Assessing Relevance	Domain 1. Study Eligibility Criteria	Domain 2. Identification and Selection of Studies	Domain 3. Data Collection and Study Appraisal	Domain 4. Synthesis and Findings	Risk of Bias in the Review
Pan et al 2019[18]	L	L	H	L	L	L
Taylor et al 2017[19]	L	L	H	L	H	L
Zhang et al 2019[20]	L	L	H	L	L	L
Ramanathan et al 2020[28]	L	L	H	L	H	H
Mahdizade et al 2021[29]	L	L	H	L	H	L
Zhou et al 2021[30]	L	L	H	L	L	L
Chen et al 2021[31]	L	L	H	L	L	L
Pan et al 2021[32]	L	L	H	L	L	L
Hasain et al 2021[33]	L	L	H	L	L	L
Çetinkaya et al 2022[34]	L	L	H	L	H	L
Suastika et al 2023[35]	L	L	H	L	H	H
Yefet et al 2023[21]	L	L	H	L	H	H
Mu et al 2023[36]	L	L	H	L	H	L
Tabatabaeizadeh et al 2023[37]	L	L	H	L	H	H
Wu et al 2024[38]	L	L	H	L	H	L
Lan et al 2024[39]	L	L	H	L	H	L

FPG

Overall, all SRs/MAs reported the effects of probiotic supplementation on the primary outcome of FPG, and 14 studies showed that probiotic supplementation significantly improved FPG levels ($P<0.05$). However, considerable heterogeneity was observed: only two meta-analyses showed low heterogeneity ($I^2 < 50\%$), whereas most reported at least moderate heterogeneity ($I^2 \geq 50\%$), and nearly half exhibited high heterogeneity ($I^2 \geq 75\%$), with the highest I^2 value exceeding 90%.

Insulin Metabolism and Insulin Sensitivity Indicators

In terms of insulin metabolism and insulin sensitivity indicators, 13 SRs/MAs reported the effect of probiotic supplementation on FSI, 14 SRs/MAs reported changes in HOMA-IR, and 2 SRs/MAs reported changes in HOMA-B, and the results consistently showed that the probiotic group had a significant advantage in improving these three indicators ($P<0.05$). Additionally, 9 SRs/MAs mentioned the effect of probiotic supplementation on the QUICKI index, with 7 SRs/MAs indicating that the probiotic group significantly increased this indicator ($P<0.05$).

Lipid Profiles

Among lipid profiles, 7 SRs/MAs reported the effects of probiotic supplementation on TG in pregnant women with GDM, of which 3 studies pointed out that this therapy could significantly reduce TG ($P<0.05$). 6 SRs/MAs reported the effect on TC, with 2 studies showing that the probiotic group could reduce TC ($P<0.05$). 7 SRs/MAs reported on HDL-Cholesterol, with 2 studies indicating that the probiotic group significantly increased this indicator ($P<0.05$). 3 SRs/MAs reported on VLDL-Cholesterol, and 2 studies showed that the probiotic group could lower its levels ($P<0.05$). In addition, 8 SRs/MAs reported on LDL-Cholesterol, but no statistically significant changes were found between the probiotic group and the control group ($P>0.05$). However, some primary studies included in these SRs/MAs suggested that probiotic supplementation could significantly reduce LDL-C levels, although this effect did not remain statistically significant after pooling, which may be attributable to methodological and clinical heterogeneity across the included studies.

Inflammation Biomarkers

5 SRs/MAs reported changes in CRP, and 3 studies revealed that the CRP in the probiotic group were significantly lower than those in the control group ($P<0.05$).

Oxidative Stress Biomarkers

8 SRs/MAs reported the effects of probiotic supplementation on NO, with 5 studies showing that the probiotic group significantly increased NO levels ($P<0.05$). 6 SRs/MAs reported on MDA and GSH, of which 5 studies showed a significant decrease in MDA ($P<0.05$), and 3 studies demonstrated a increase in GSH levels ($P<0.05$). Additionally, 5 SRs/MAs assessed TAC, with 3 studies indicating a significant increase in TAC in the probiotic group ($P<0.05$).

Long-Term Blood Glucose Control Biomarkers

For HbA1c, 3 SRs/MAs mentioned the effect of probiotics on it, but no significant difference was found between the two groups ($P>0.05$).

Maternal and Neonatal Outcome Indicators

Regarding maternal and neonatal outcome indicators, 3 SRs/MAs reported the incidence of macrosomia, and 2 SRs/MAs showed that probiotic intervention significantly reduced the risk of macrosomia ($P<0.05$). 2 SRs/MAs reported on newborn hyperbilirubinemia and 2 on newborn weight, all showing beneficial effects of probiotic supplementation ($P<0.05$). In addition, 2 SRs/MAs assessed neonatal hospitalization rates, with 1 reporting a significant reduction in the treatment groups. For other maternal and neonatal outcome indicators, such as gestational age and weight gain, no obvious changes were found between the two groups ($P>0.05$). Further details can be found in Appendix 7.

Discussion

Main Findings

This review evaluated 16 SRs/MAs published between 2017 and 2024, aiming to compare the effects of probiotic supplementation on the improvement of various aspects in pregnant women with GDM, including FPG, lipid profiles, inflammation and oxidative stress, pregnancy outcomes, and neonatal outcomes. Among the included studies, 13 were published in the last five years, indicating that research in this field has rapidly developed and gained widespread attention in recent years. The findings of this review suggest that probiotic supplementation can significantly improve the FPG in GDM pregnant women, reduce the risk of insulin resistance, and positively impact biomarkers such as blood lipids and inflammation. However, the strength and statistical significance of these effects varied across outcomes and SRs/MAs. Notably, heterogeneity varied across outcome categories. Glycemic and insulin-related outcomes demonstrated a broad distribution of I^2 values, with moderate to high heterogeneity accounting for a substantial proportion of pooled analyses. Lipid-related outcomes more frequently showed low heterogeneity, although moderate or high I^2 values were observed in some analyses. Inflammatory biomarkers were predominantly associated with high heterogeneity, whereas oxidative stress biomarkers were mainly characterized by low heterogeneity, with only a limited number of analyses reporting very high I^2 values (Appendix 7). Accordingly, the pooled findings should be interpreted with caution. A structured summary of outcome indicators and consistency of results across SRs/MAs is provided in Table 4.

In the 16 SRs/MAs reviewed in this study, according to the AMSTAR-2 assessment, 4 SRs/MAs were rated as low quality. In comparison, the quality of the remaining 12 SRs/MAs was very low, especially regarding insufficient responses in items 2, 4, 5, 7, and 10. This indicates obvious methodological flaws in the included studies, primarily due to the lack of pre-study plans and registration, the absence of a complete literature search strategy that did not include gray literature, failure to provide a list of excluded studies with reasons for exclusion, as well as inadequate analysis of potential biases. In the PRISMA assessment, only one SR/MA fully reported all PRISMA items, and the reporting completeness of 12 SRs/MAs exceeded 80%. These issues are consistent with those identified in the AMSTAR-2 assessment. The evaluation using the ROBIS tool revealed that all included SRs/MAs showed high risk of bias in Domain 2 and Domain 4 of Phase 2, indicating biases in study design and literature selection. Furthermore, some studies

Table 4 Summary of Outcomes Reported Across Included SRs/MAs

No.	Outcome Indicator	No. of SRs/MAs Reporting	No. of SRs/MAs with Significant Effect	Main Effect Observed	Consistency Across SRs/MAs
1	FPG	16	14	↓	High
2	FSI	13	13	↓	High
3	HOMA-IR	14	14	↓	High
4	HOMA-B	2	2	↑	High
5	QUICKI	9	7	↑	Moderate–High
6	TG	7	3	↓	Moderate
7	TC	6	2	↓	Low–Moderate
8	HDL-C	7	2	↑	Low–Moderate
9	VLDL-C	3	2	↓	Low–Moderate
10	LDL-C	8	0	↔	Low
11	CRP	5	3	↓	Moderate
12	NO	8	5	↑	Moderate
13	MDA	6	5	↓	Moderate
14	GSH	6	3	↑	Moderate
15	TAC	5	3	↑	Moderate
16	HbA1c	3	0	↔	Low
17	Macrosomia	3	2	↓	Moderate
18	Newborns' hyperbilirubinemia	2	2	↓	High
19	Newborn weight	2	2	↓	High
20	Neonatal hospitalization	2	1	↓	Low–Moderate

Notes: ↓= reduction; ↑= increase; ↔ = no significant change.

improperly synthesized results from different studies and lacked sensitivity analysis and explanations for the sources of heterogeneity.

At the same time, it is worth noting that most of the studies originated from Iran, which may raise concerns about the regional differences affecting the generalizability of the results. Furthermore, there were significant differences between RCTs in the form of probiotic supplementation (such as yogurt, capsules, and milk), strains used, dosage, intervention duration, and participant characteristics. For example, Sahhaf Ebrahimi F used probiotic milk containing L. acidophilus and B. lactis with a 1×10^6 CFU/strain for 8 weeks.[40] The average BMI of the probiotic group was 31.7 kg/m^2, while that of the control group was 29.7 kg/m^2, with 42 GDM patients recruited in each group. Jafarnejad S used probiotic capsules containing multiple strains (S. thermophilus, B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii subsp. Bulgaricus) with a dosage of 15×10^9 CFU/g for 8 weeks.[41] The mean BMI of the probiotic group was 26.8 kg/m^2, while the control group had a mean BMI of 27.4 kg/m^2, with 82 participants. Jamilian M used a probiotic supplement containing L. acidophilus, B. bifidum, L. reuteri, and L. fermentum with a dosage of 8×10^9 CFU/g, with 2×10^9 CFU/g of each strain for 6 weeks.[42] The average BMI of the probiotic group was 31.2 kg/m^2, while that of the control group was 29.9 kg/m^2, with 57 participants. As such, the differences in study design across these studies inevitably result in high heterogeneity and bias during the combined analysis of the results.

In terms of efficacy evaluation, most SRs/MAs consistently concluded that probiotic supplementation can significantly reduce FPG in women with GDM and improve insulin metabolism and insulin sensitivity indicators (such as FSI, HOMA-IR, HOMA-B, and QUICKI) as well as lipid profiles (such as TG, TC, HDL-Cholesterol, and VLDL-Cholesterol). Meanwhile, probiotics have also shown significant positive effects on oxidative stress and inflammation-related biomarkers (such as NO, MDA, TAC, GSH, and CRP). Regarding neonatal outcomes, studies suggest that probiotic intervention can significantly reduce the risk of fetal macrosomia,[20,30] newborn hyperbilirubinemia,[30] and neonatal hospitalization rate,[33] while the newborn weight in the probiotic group was generally lower compared to the placebo group.[30] However, due to the lack of high-quality evidence, the significant heterogeneity of the included studies,

and the potential risks of bias, these flaws may affect the reliability of the research results and the credibility of the conclusions. Therefore, we should be cautious when recommending probiotic supplementation for women with GDM.

Significance for Future Research

Probiotic supplementation is a highly regarded health intervention with potential benefits in the management of GDM.[43,44] World Health Organization defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host."[45] Current research indicates that the benefits of probiotics are mainly derived from their ability to regulate the gut microbiota, restore the balance of intestinal flora, enhance intestinal barrier function, improve insulin sensitivity, and regulate the secretion of pro-inflammatory mediators.[46,47] Based on the evidence presented in this review, we posit that probiotic supplementation is a promising adjunctive therapeutic tool for GDM.

Based on the quality analysis of the 16 included SRs/MAs, we believe that future research should focus on enhancing the scientific rigor and reliability of probiotic interventions for GDM. First, a clear research plan should be established and registered before publication to ensure transparency and standardization in study design. Second, the literature search strategy should be improved, especially the comprehensive inclusion of gray literature and providing a detailed search process along with a list of excluded literature with its reasons for exclusion to reduce selective bias. In addition, the analysis and reporting of research bias and heterogeneity should be strengthened through methods such as subgroup analysis and sensitivity analysis to ensure the robustness of the results. It is also important to control for potential conflicts of interest arising from funding sources to reduce related biases. Finally, studies also need to focus on clarifying the optimal dosage, strain combinations, and intervention duration, as well as segmenting participant characteristics to reduce heterogeneity and improve the applicability of conclusions.

Strength and Limitations

This study systematically evaluated the latest evidence on the effects of probiotic supplementation on GDM, revealing its potential benefits in improving glycemic control, lipid metabolism, inflammation and oxidative stress, as well as maternal and neonatal outcomes. By searching four English databases, we identified and included 16 SRs/MAs related to the research topic and conducted a comprehensive analysis. However, the study also uncovered several limitations. First, there were significant methodological and evidence quality differences among the included studies, with overall low quality and evident risk of bias in literature selection and result synthesis. In addition, substantial between-study heterogeneity was observed across multiple outcomes, together with a lack of sensitivity analyses and insufficient explanation of heterogeneity. Second, most RCTs originated from Iran, which may lead to regional differences affecting the generalizability of the results. Third, there were notable variations in the types of probiotics, optimal doses, supplementation forms, intervention duration, participant characteristics, and sample sizes across studies. Yet we were unable to conduct an in-depth analysis of the specific impact of these differences on clinical value. Moreover, our search was restricted to English-language publications and did not include grey literature or trial registries, which may have introduced selection bias. Finally, substantial overlap of primary randomized controlled trials was observed across the included systematic reviews, with a CCA of 26.31%, indicating that a large share of the synthesized evidence was derived from overlapping primary studies. Consequently, similarities across reviews may partly reflect shared underlying data rather than fully independent evidence, and should be considered when interpreting the overall conclusions.

Conclusion

This study suggests that probiotic supplementation shows significant potential benefits in improving FPG, lipid profiles, inflammation-related biomarkers and oxidative stress, as well as maternal and neonatal outcomes in pregnant women with GDM. However, limitations in methodological design, quality of evidence, and heterogeneity of results may weaken the reliability of study findings and the credibility of results. Nevertheless, we should interpret these conclusions with caution in clinical applications.

From a clinical and public health perspective, the current evidence is not yet sufficient to support the routine use of probiotics in the management of GDM. While probiotics appear safe and well-tolerated, the inconsistency and overall low quality of evidence mean that they should be considered only as an adjunctive option rather than a standard therapy. Future large-scale, rigorously designed RCTs are needed to clarify optimal strains, dosages, and intervention durations, and to provide stronger evidence that could inform clinical guidelines and public health recommendations.

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Author Contributions

Huixia Ren and Naijin Zhang contributed equally to this work and share first authorship. Hongwu Wang and Huaien Bu are co-corresponding authors and contributed to study conception and supervision. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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