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Probiotics for preventing gestational diabetes (Review)

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[Intervention Review]

Probiotics for preventing gestational diabetes

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ABSTRACT

Background

Gestational diabetes mellitus (GDM) is associated with a range of adverse pregnancy outcomes for mother and infant. The prevention of GDM using lifestyle interventions has proven difficult. The gut microbiome (the composite of bacteria present in the intestines) influences host inflammatory pathways, glucose and lipid metabolism and, in other settings, alteration of the gut microbiome has been shown to impact on these host responses. Probiotics are one way of altering the gut microbiome but little is known about their use in influencing the metabolic environment of pregnancy. This is an update of a review last published in 2014.

Objectives

To systematically assess the effects of probiotic supplements used either alone or in combination with pharmacological and non-pharmacological interventions on the prevention of GDM.

Search methods

We searched Cochrane Pregnancy and Childbirth's Trials Register, ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP) (20 March 2020), and reference lists of retrieved studies.

Selection criteria

Randomised and cluster-randomised trials comparing the use of probiotic supplementation with either placebo or diet for the prevention of the development of GDM. Cluster-randomised trials were eligible for inclusion but none were identified. Quasi-randomised and cross-over design studies were not eligible for inclusion in this review. Studies presented only as abstracts with no subsequent full report of study results were only included if study authors confirmed that data in the abstract came from the final analysis. Otherwise, the abstract was left awaiting classification.

Data collection and analysis

Two review authors independently assessed study eligibility, extracted data and assessed risk of bias of included studies. Data were checked for accuracy.

Main results

In this update, we included seven trials with 1647 participants. Two studies were in overweight and obese women, two in obese women and three did not exclude women based on their weight. All included studies compared probiotics with placebo. The included studies were at low risk of bias overall except for one study that had an unclear risk of bias. We excluded two studies, eight studies were ongoing and three studies are awaiting classification.

Six included studies with 1440 participants evaluated the risk of GDM. It is uncertain if probiotics have any effect on the risk of **GDM** compared to placebo (mean risk ratio (RR) 0.80, 95% confidence interval (CI) 0.54 to 1.20; 6 studies, 1440 women; low-certainty evidence). The evidence was low certainty due to substantial heterogeneity and wide CIs that included both appreciable benefit and appreciable harm.

Probiotics increase the risk of **pre-eclampsia** compared to placebo (RR 1.85, 95% CI 1.04 to 3.29; 4 studies, 955 women; high-certainty evidence) and may increase the risk of **hypertensive disorders of pregnancy** (RR 1.39, 95% CI 0.96 to 2.01, 4 studies, 955 women), although the CIs for hypertensive disorders of pregnancy also indicated probiotics may have no effect.

There were few differences between groups for other primary outcomes. Probiotics make little to no difference in the risk of **caesarean section** (RR 1.00, 95% CI 0.86 to 1.17; 6 studies, 1520 women; high-certainty evidence), and probably make little to no difference in **maternal weight gain during pregnancy** (MD 0.30 kg, 95% CI −0.67 to 1.26; 4 studies, 853 women; moderate-certainty evidence). Probiotics probably make little to no difference in the incidence of **large-for-gestational age infants** (RR 0.99, 95% CI 0.72 to 1.36; 4 studies, 919 infants; moderate-certainty evidence) and may make little to no difference in **neonatal adiposity** (2 studies, 320 infants; data not pooled; low-certainty evidence). One study reported adiposity as fat mass (MD −0.04 kg, 95% CI −0.12 to 0.04), and one study reported adiposity as percentage fat (MD −0.10%, 95% CI −1.19 to 0.99). We do not know the effect of probiotics on **perinatal mortality** (RR 0.33, 95% CI 0.01 to 8.02; 3 studies, 709 infants; low-certainty evidence), a **composite measure of neonatal morbidity** (RR 0.69, 95% CI 0.36 to 1.35; 2 studies, 623 infants; low-certainty evidence), or **neonatal hypoglycaemia** (mean RR 1.15, 95% CI 0.69 to 1.92; 2 studies, 586 infants; low-certainty evidence). No included studies reported on perineal trauma, postnatal depression, maternal and infant development of diabetes or neurosensory disability.

Authors' conclusions

Low-certainty evidence from six trials has not clearly identified the effect of probiotics on the risk of GDM. However, high-certainty evidence suggests there is an increased risk of pre-eclampsia with probiotic administration. There were no other clear differences between probiotics and placebo among the other primary outcomes. The certainty of evidence for this review's primary outcomes ranged from low to high, with downgrading due to concerns about substantial heterogeneity between studies, wide CIs and low event rates.

Given the risk of harm and little observed benefit, we urge caution in using probiotics during pregnancy.

The apparent effect of probiotics on pre-eclampsia warrants particular consideration. Eight studies are currently ongoing, and we suggest that these studies take particular care in follow-up and examination of the effect on pre-eclampsia and hypertensive disorders of pregnancy. In addition, the underlying potential physiology of the relationship between probiotics and pre-eclampsia risk should be considered.

PLAIN LANGUAGE SUMMARY

Probiotics to prevent gestational diabetes mellitus

We analysed evidence from randomised controlled trials (clinical studies where people are randomly put into one of two or more treatment groups) investigating probiotic supplements alone or in combination with drug or non-drug interventions for preventing gestational diabetes mellitus (GDM).

What is the issue?

GDM is a condition where the mother develops high blood sugar levels, usually after 13 weeks of pregnancy. GDM is different from type 2 diabetes in that blood sugar levels are normal before pregnancy, and the levels usually return to normal after pregnancy. GDM is associated with an increased risk of developing type 2 diabetes later in life. Women with GDM are at increased risk of high blood pressure with protein in the urine (pre-eclampsia) and instrumental delivery or caesarean section. Their infants are more likely to be born large for their gestational age. Probiotics are 'good bacteria' that are usually taken in the form of capsules or drinks to add to the gut bacteria. We are dependent on our gut bacteria to help digest our food, produce certain vitamins, regulate our immune system and keep us healthy by protecting us against disease-causing bacteria. Probiotics could change a person's metabolism and play a role in the prevention of GDM.

Why is this important?

Women who are overweight or obese, had GDM in a previous pregnancy or have an immediate family member with diabetes are at increased risk of GDM. Current treatment for GDM includes diet with or without medication but does not always prevent the problems

associated with GDM. Probiotics could be a simple method for preventing GDM. This review looked at whether there is evidence to show if this is true.

What evidence did we find?

We searched for evidence from randomised controlled trials in March 2020 and identified seven studies with 1647 pregnant women comparing probiotics with inactive placebo (pretend treatment). Two studies were in overweight and obese women, two in obese women and three did not exclude women based on their weight. The overall risk of bias was low except for one study where the risk of bias was unclear.

It is unclear how probiotics affect the risk of developing **GDM** due to the wide variation in the results of six studies (1440 women, low-quality evidence). Probiotics increase the risk of developing **pre-eclampsia** (4 studies, 955 women; high-quality evidence). Probiotics make little to no difference to the risk of needing a **caesarean section** (6 studies, 1520 women; high-quality evidence), and probably make little to no difference to weight gain during pregnancy (4 studies, 853 women; moderate-quality evidence) or to the risk of giving birth to a **big baby** (4 studies, 919 women; moderate-quality evidence). None of the studies reported information about the risk of perineal trauma (tears during vaginal birth or a surgical incision (episiotomy)), postnatal depression or developing subsequent diabetes.

We do not know if probiotics affect the infant having **medical problems after birth** because of the variation in results between studies (2 studies, 623 infants; low-quality evidence). It is also uncertain how probiotics affect **infant death** (either before birth or as a newborn) (3 studies, 709 infants; low-certainty evidence), **low blood sugar** (2 studies, 586 infants; low-certainty evidence) or **body fat** (2 studies, 320 infants; low-certainty evidence). None of the studies reported information about the risk of infants developing diabetes or long-term conditions that affect brain development.

What does this mean?

Low-quality evidence from six trials has not clearly identified the effect of probiotics on the risk of GDM. However, high-quality evidence suggests that probiotics probably increase the risk of pre-eclampsia. Therefore, there is currently evidence of possible harm with little observed benefit for widespread use of probiotics in pregnancy.

There are eight studies currently ongoing that may help to provide more clarity on the effects of probiotics. It is also important to explore the relationship between probiotics and pre-eclampsia further.

Summary of findings 1. Probiotics compared to placebo for preventing gestational diabetes (maternal outcomes)

Probiotics compared to placebo for preventing gestational diabetes (maternal outcomes)

Patient or population: preventing gestational diabetes

Setting: Australia, Finland, Iran, Ireland, and New Zealand

Intervention: probiotics

Comparison: placebo

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo	Risk with Probiotics				
Diagnosis of gestational diabetes mellitus	**Study population**		**Mean RR 0.80** (0.54 to 1.20)	1440 (6 RCTs)	⊕⊕⊖⊖ **Low** [a,b]	—
	191 per 1000	153 per 1000 (103 to 229)				
Hypertensive disorders of pregnancy (pre-eclampsia)	**Study population**		**RR 1.85** (1.04 to 3.29)	955 (4 RCTs)	⊕⊕⊕⊕ **High**	—
	35 per 1000	65 per 1000 (37 to 116)				
Caesarean section	**Study population**		**RR 1.00** (0.86 to 1.17)	1520 (6 RCTs)	⊕⊕⊕⊕ **High**	—
	285 per 1000	285 per 1000 (245 to 333)				
Perineal trauma	—	—	—	—	—	Not reported
Weight gain during pregnancy	The mean weight gain during pregnancy was 9.4–14.8 kg	**MD 0.30 kg** higher (0.67 lower to 1.26 higher)	—	853 (4 RCTs)	⊕⊕⊕⊖ **Moderate** [a]	—
Postnatal depression	—	—	—	—	—	Not reported
Development of subsequent diabetes	—	—	—	—	—	Not reported

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded one level for serious concerns about inconsistency due to substantial unexplained heterogeneity between studies.
[b]Downgraded one level for serious concerns about imprecision due to a wide CI.

Summary of findings 2. Probiotics compared to placebo for preventing gestational diabetes (infant outcomes)

Probiotics compared to placebo for preventing gestational diabetes (infant outcomes)

Patient or population: preventing gestational diabetes

Setting: Australia, Finland, Iran, Ireland, and New Zealand

Intervention: probiotics

Comparison: placebo

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo	Risk with probiotics				
Large-for-gestational age	**Study population**		**RR 0.99** (0.72 to 1.36)	919 (4 RCTs)	⊕⊕⊕⊖ **Moderate** [a]	—
	142 per 1000	141 per 1000 (102 to 193)				
Perinatal mortality (stillbirth and neonatal mortality)	**Study population**		**RR 0.33** (0.01 to 8.02)	709 (3 RCTs)	⊕⊕⊖⊖ **Low** [b]	—
	3 per 1000	1 per 1000 (0 to 22)				
Mortality or morbidity composite	**Study population**		**RR 0.69** (0.36 to 1.35)	623 (2 RCTs)	⊕⊕⊖⊖ **Low** [b]	—

	61 per 1000	42 per 1000 (22 to 83)				
Hypoglycaemia as defined by trialists	**Study population**		**Mean RR 1.15** (0.69 to 1.92)	586 (2 RCTs)	⊕⊕⊝⊝ **Low** [a,c]	—
	135 per 1000	155 per 1000 (93 to 259)				
Adiposity	The included studies showed no appreciable difference in fat mass or % fat between groups. 1 study reported adiposity as fat mass (MD −0.04 kg, 95% CI −0.12 to 0.04) 1 study reported adiposity as % fat (MD −0.10%, 95% CI −1.19 to 0.99)		—	320 (2 RCTs data not pooled)	⊕⊕⊝⊝ **Low** [d]	—
Diabetes	—	—	—	—	—	Not reported
Neurodisability	—	—	—	—	—	Not reported

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded one level for serious concerns about imprecision due to a wide confidence intervals that included both appreciable benefit and harm.
[b]Downgraded two levels for very serious concerns about imprecision due to a very small number of events and a wide confidence intervals that included both appreciable benefit and harm.
[c]Downgraded one level for serious concerns about inconsistency due to unexplained heterogeneity between studies.
[d]Downgraded two levels for very serious concerns about imprecision due to the small sample sizes of the included studies and wide confidence intervals that included both appreciable benefit and harm.

BACKGROUND

Description of the condition

According to the American Diabetes Association (ADA), gestational diabetes mellitus (GDM) is diabetes in pregnancy that is diagnosed during the second or third trimester and was not clearly present prior to pregnancy (ADA 2019). There are multiple sets of diagnostic criteria that are used worldwide, which has caused estimates of prevalence to vary greatly (Buchanan 2012). According to the International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria, approximately 14% of pregnancies worldwide were affected by GDM in 2017 (Cho 2018), and multiple studies have observed that the incidence is rising (Noh 2021; López-de-Andrés 2020; Abouzeid 2014; Dabelea 2005).

GDM is associated with a number of maternal and fetal adverse outcomes, and the risk of these outcomes increases with higher fasting plasma glucose levels (HAPO 2008). Women with GDM have higher rates of pre-eclampsia and need for a caesarean section, and their infants have higher rates of macrosomia, shoulder dystocia, neonatal hypoglycaemia and respiratory distress syndrome (Carr 2011; Dodd 2007; Esakoff 2009; HAPO 2008). In addition, there is an increased risk for metabolic dysfunction for both mother and infant in the long term including diabetes, obesity and metabolic syndrome (Malcolm 2012). Large randomised controlled trials have demonstrated the benefits of treating GDM for preventing many of the associated adverse outcomes (Crowther 2005; Landon 2009), but it is not known if treatment prevents the long-term adverse effects. In addition, there are substantial costs associated with the treatment of GDM, and cost-effectiveness has not been clearly demonstrated (Fitria 2019). Therefore, prevention of GDM is favourable.

Prevention efforts have primarily focused on lifestyle interventions such as diet and exercise. The Cochrane Review evaluating the combination of diet and exercise interventions for the prevention of GDM concluded that there is moderate-quality evidence that this combination of lifestyle interventions can reduce the risk of GDM (Shepherd 2017). However, the Cochrane Reviews evaluating diet and exercise for GDM prevention independently were inconclusive and suggested the need for higher-quality evidence (Han 2012; Tieu 2017). All these reviews reported that studies involving these interventions were difficult to interpret given heterogeneity and small sample sizes. In addition, there was concern about adherence to these interventions on a population level (Sui 2013).

Due to these concerns, dietary supplements such as probiotics and myo-inositol are being studied. The Cochrane Review evaluating myo-inositol use for the prevention of GDM concluded there may be a reduction in GDM risk with its use, but the review authors ultimately suggested the need for further research due to low-quality evidence (Crawford 2015). One overview of Cochrane Reviews for the prevention of gestational diabetes looked at all these interventions, and they found that no studied intervention resulted in clear benefit or harm, and many of these interventions did not have enough high-quality evidence to determine an effect (Griffith 2020).

Description of the intervention

According to the World Health Organization, probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2006). The health effects provided by probiotics vary depending on the specific species and strain of probiotic used, and, therefore, have been investigated in a wide variety of health conditions. Among the most common probiotics used are members of the genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus*, but products differ greatly in the strains and concentrations used (Syngai 2016; FAO/WHO 2006). Probiotics are available in a variety of food products, such as yoghurt or fermented milks, or as dietary supplements that can be purchased as capsules without a prescription.

How the intervention might work

Studies of the human microbiome have revealed a complex relationship between the microbiome and an individual's overall health and wellbeing. The microbiome is altered by a variety of factors including diet and various health conditions (David 2014), and in turn, the microbiome may influence host metabolism and contribute to the development of obesity and diabetes (Musso 2011). Many studies of the gut microbiome in obese people have revealed an increase in the proportion of bacteria in the *Firmicutes* phylum and a decrease in bacteria belonging to the *Bacteroidetes* phylum (John 2016). Similarly, studies in people with type 2 diabetes and glucose intolerance have revealed a reduction in *Akkermansia muciniphila* and butyrate-producing bacteria such as *Lactobacillus* and *Bifidobacterium* in their microbiome (Brunkwall 2017). These changes in the gut microbiome may be linked to obesity and diabetes through the role bacteria play in host glucose and lipid metabolism (Musso 2011).

Increases in body fat and decreases in insulin sensitivity are normal changes in pregnancy, and these changes appear to be linked to changes in the microbiome as well. Koren 2012 found that the gut microbiome became less diverse as pregnancy progressed, and the microbiome in the third trimester was associated with increased adiposity and insulin insensitivity when transplanted into mice (Koren 2012). In women with GDM, insulin sensitivity is impaired beyond normal levels, leading to hyperglycaemia. Crusell 2018 have looked at the microbiome in women with GDM, and found that the microbiome in GDM differed slightly from that in normal pregnancy and may have resembled that of non-pregnant women with type 2 diabetes (Crusell 2018).

Given the role the microbiome plays in host glucose and lipid metabolism (Musso 2011), probiotics have been suggested as an intervention for improving glycaemic control in diabetes by helping to restore balance among species of bacteria in the microbiome (Tiderencel 2020). Many randomised controlled trials have examined the use of probiotics in people with type 2 diabetes, and one meta-analysis of these trials revealed that probiotics were helpful in improving glycaemic control and may have improved glucose metabolism (Tiderencel 2020). GDM is like type 2 diabetes in that there are similar changes in insulin resistance and possibly in the microbiome (Crusell 2018), and, therefore, probiotics may have similar effects for prevention or treatment of GDM. A Cochrane Review evaluating the use of probiotics to treat GDM was published in 2020 (Okesene-Gafa 2020).

Why it is important to do this review

The incidence of GDM is increasing (Noh 2021; López-de-Andrés 2020; Abouzeid 2014; Dabelea 2005), and GDM is associated with

significant health implications for both mother and infant (HAPO 2008). Therefore, prevention of GDM is ideal. One study evaluating the use of probiotics in pregnancy suggested that probiotics may reduce the incidence of GDM (Laitinen 2009). Since this initial study, other studies have tried to address this question with mixed results (Asgharian 2020; Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). Therefore, a systematic review is necessary to synthesise the available evidence for or against the use of probiotics for preventing GDM in pregnancy.

OBJECTIVES

To systematically assess the effects of probiotic supplements used either alone or in combination with pharmacological and non-pharmacological interventions on the prevention of GDM.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised and cluster-randomised trials; however, we found no cluster-randomised trials. Quasi-randomised and cross-over design studies were not eligible for inclusion in this review. Studies presented only as abstracts with no subsequent full report of study results were only included if we received confirmation from the study authors that the data in the abstract were final. Otherwise, they were listed as awaiting classification.

Types of participants

Studies that included pregnant women not previously diagnosed with diabetes mellitus. Studies of women with GDM in a previous pregnancy but no evidence of diabetes mellitus or GDM in the current pregnancy before entering the trial were eligible for inclusion.

Types of interventions

Probiotic supplementation for prevention of GDM, either alone or in combination with pharmacological (e.g. metformin) or non-pharmacological (e.g. diet/lifestyle) interventions.

Probiotic supplementation (administered by any method) should have been commenced prior to the diagnosis of GDM and continued for any duration.

Comparison interventions of placebo or diet were eligible.

Trials may have used other interventions in a comparison arm or in combination with the probiotic. These other interventions may have included pharmaceutical probiotic supplements as well as food items supplemented with probiotics.

Types of outcome measures

The outcomes for this review are from the Cochrane Core Outcome Set for GDM prevention.

Primary outcomes

Maternal

- Diagnosis of GDM
- Hypertensive disorders of pregnancy (including pre-eclampsia, pregnancy-induced hypertension and eclampsia)
- Caesarean section

Infant

- Large-for-gestational age
- Perinatal mortality (including stillbirth and neonatal mortality)
- Mortality or morbidity composite

Secondary outcomes

Maternal

- Induction of labour
- Perineal trauma
- Placental abruption
- Postpartum haemorrhage
- Postpartum infection
- Weight gain during pregnancy
- Adherence to the intervention
- Behaviour changes associated with the intervention
- Relevant biomarker changes associated with the intervention (including adiponectin, free fatty acids, triglycerides, high-density lipoproteins, low-density lipoproteins, insulin, etc.)
- Sense of wellbeing and quality of life
- Views of the intervention
- Breastfeeding

Long-term maternal

- Postnatal depression
- Postnatal weight retention or return to prepregnancy weight
- Body mass index (BMI)
- GDM in a subsequent pregnancy
- Type 1 diabetes
- Type 2 diabetes
- Impaired glucose tolerance
- Cardiovascular health as defined by trialists (including blood pressure, hypertension, cardiovascular disease and metabolic syndrome)

Infant

- Stillbirth
- Neonatal mortality
- Gestational age at birth
- Preterm birth (less than 37 weeks' gestation and less than 32 weeks' gestation)
- Apgar score (less than seven at five minutes)
- Macrosomia
- Small-for-gestational age (SGA)
- Birthweight and z-score
- Head circumference and z-score
- Length and z-score
- Ponderal index
- Adiposity
- Shoulder dystocia
- Bone fracture
- Nerve palsy
- Respiratory distress syndrome

- Hypoglycaemia as defined by trialists
- Hyperbilirubinaemia

Later infant and childhood

- Weight and z-scores
- Height and z-scores
- Head circumference and z-scores
- Adiposity (including BMI and skinfold thickness)
- Blood pressure
- Type 1 diabetes
- Type 2 diabetes
- Impaired glucose tolerance
- Dyslipidaemia or metabolic syndrome
- Neurodisability
- Educational achievement

Child as an adult

- Weight
- Height
- Adiposity (including BMI and skinfold thickness)
- Cardiovascular health as defined by trialists (including blood pressure, hypertension, cardiovascular disease and metabolic syndrome)
- Type 1 diabetes
- Type 2 diabetes
- Impaired glucose tolerance
- Dyslipidaemia or metabolic syndrome
- Employment, education and social status/achievement

Health service use

- Number of hospital or health professional visits (including midwife, obstetrician, physician, dietician and diabetic nurse)
- Number of antenatal visits or admissions
- Length of antenatal stay
- Neonatal intensive care unit admission
- Length of postnatal stay (mother)
- Length of postnatal stay (baby)
- Costs to families associated with the management provided
- Costs associated with the intervention
- Cost of maternal care
- Cost of offspring care

Search methods for identification of studies

The following methods section of this review is based on a standard template used by Cochrane Pregnancy and Childbirth.

Electronic searches

For this update, we searched Cochrane Pregnancy and Childbirth's Trials Register by contacting their Information Specialist (20 March 2020).

The Register is a database containing over 25,000 reports of controlled trials in the field of pregnancy and childbirth. It represents over 30 years of searching. For full current search methods used to populate Pregnancy and Childbirth's Trials Register including the detailed search strategies for CENTRAL, MEDLINE, Embase and CINAHL; the list of handsearched journals and conference proceedings; and the list of journals reviewed via the current awareness service, see the Cochrane Pregnancy and Childbirth's Trials Register (pregnancy.cochrane.org/pregnancy-and-childbirth-groups-trials-register).

Briefly, Cochrane Pregnancy and Childbirth's Trials Register is maintained by their Information Specialist and contains trials identified from:

- monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
- weekly searches of MEDLINE (Ovid);
- weekly searches of Embase (Ovid);
- monthly searches of CINAHL (EBSCO);
- handsearches of 30 journals and the proceedings of major conferences;
- weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Two people screen search results and review the full text of all relevant trial reports identified through the searching activities. Based on the intervention described, each trial report is assigned a number that corresponds to a specific Pregnancy and Childbirth review topic (or topics), and is then added to the Register. The Information Specialist searches the Register for each review using this topic number rather than keywords. This results in a more specific search set that has been fully accounted for in the relevant review sections (Included studies; Excluded studies; Studies awaiting classification; Ongoing studies).

In addition, we searched ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch/) for unpublished, planned and ongoing trial reports (20 March 2020) using the search methods detailed in Appendix 1.

Searching other resources

We searched the reference lists of all retrieved studies.

We applied no language or date restrictions.

Data collection and analysis

For methods used in the previous version of this review, see Barrett 2014.

For this update, we used the following methods for assessing the 43 reports that were identified as a result of the updated search.

The following methods section is based on a standard template used by Cochrane Pregnancy and Childbirth.

Selection of studies

Two review authors (SJD and MDN) independently assessed for inclusion all the potential studies we identified as a result of the search strategy. We resolved any disagreement through discussion or, if required, we consulted another review author (LC). Since three review authors were also authors on one of the identified studies (Callaway 2019), the two review authors not involved with this study assessed it for inclusion (SJD and SAP).

We created a study flow diagram to map out the number of records identified, included, excluded or awaiting classification (Figure 1).

Figure 1. Study flow diagram.

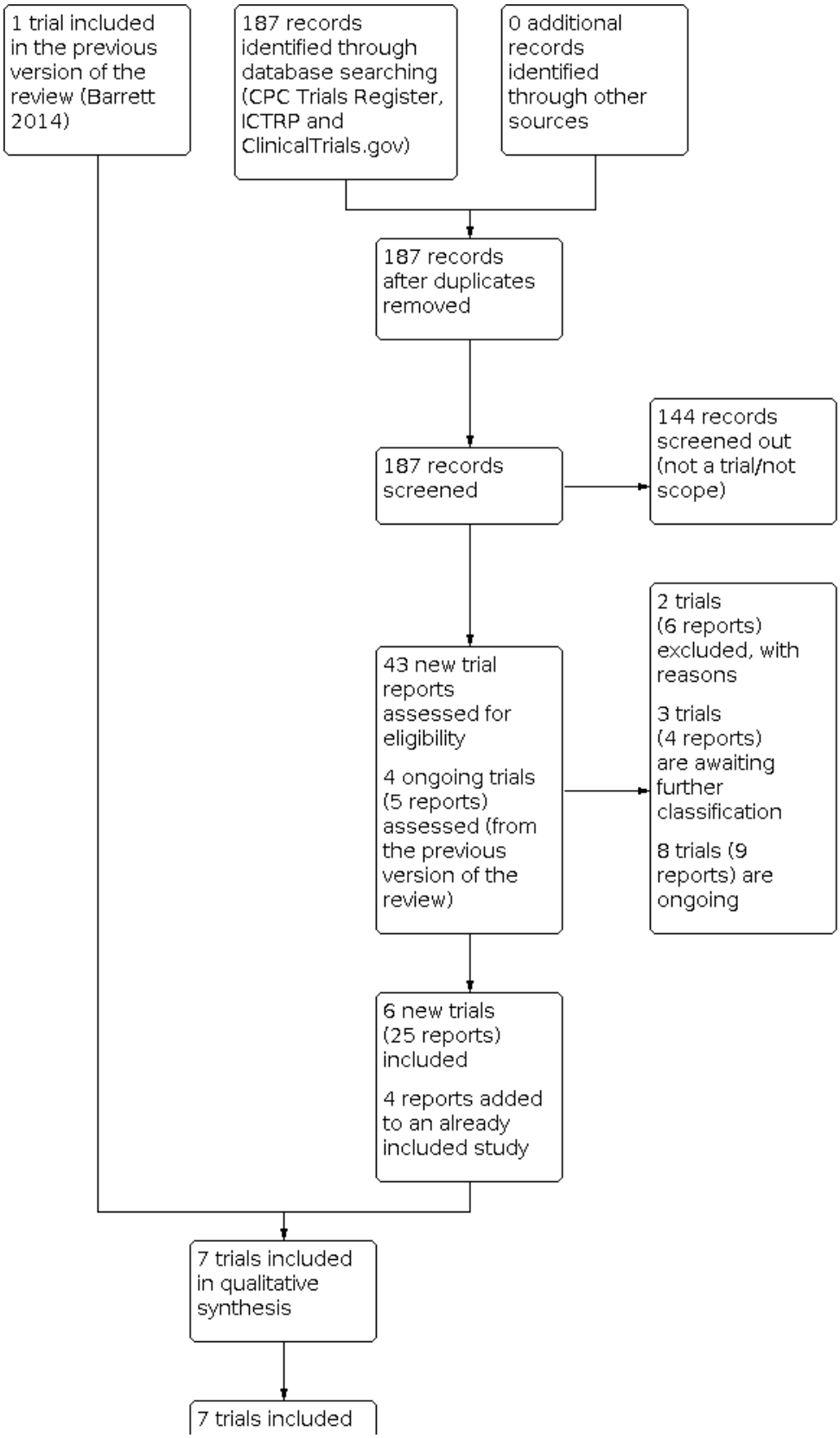
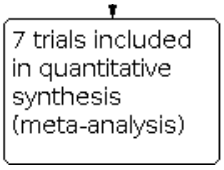


Figure 1. (Continued)



Screening eligible studies for scientific integrity/trustworthiness

Two review authors evaluated all studies meeting our inclusion criteria against predefined criteria to select studies that, based on available information, were deemed to be sufficiently trustworthy to be included in the analysis. The criteria are as follows.

Research governance

- No prospective trial registration for studies published after 2010 without plausible explanation.
- When requested, trial authors refuse to provide/share the protocol or ethics approval letter (or both).
- Trial authors refuse to engage in communication with the Cochrane Review authors.
- Trial authors refuse to provide individual participant data (IPD) data upon request with no justifiable reason.

Baseline characteristics

- Characteristics of the study participants being too similar (distribution of mean (standard deviation (SD)) excessively narrow or excessively wide, as noted by Carlisle 2017.

Feasibility

- Implausible numbers (e.g. 500 women with severe cholestasis of pregnancy recruited in 12 months).
- (Close to) zero losses to follow-up without plausible explanation.

Results

- Implausible results (e.g. massive risk reduction for main outcomes with small sample size).
- Unexpectedly even numbers of women 'randomised' including a mismatch between the numbers and the methods (e.g. if they say no blocking was used but still end up with equal numbers, or they say they used blocks of four but the final numbers differ by six).

Studies assessed as being potentially 'high risk' were not included in the review. Where a study was classified as 'high risk' for one or more of the above criteria, we attempted to contact the study authors to address any possible lack of information/concerns. If adequate information remained unavailable, the study remained in 'awaiting classification' and the reasons and communications with the author (or lack of) described in detail.

The process is described fully in Figure 3.

Abstracts

Data from abstracts were only included if, in addition to the trustworthiness assessment, the study authors confirmed in writing that the data to be included in the review had come from the final analysis and will not change. If such information was not available/provided, the study remained 'awaiting classification' (as above).

Data extraction and management

We designed a form to extract data. For eligible studies, at least two review authors (SJD and MDN for most studies, SJD and SAP for Callaway 2019) extracted data using the agreed form. We resolved discrepancies through discussion, or, if required, through consultation with a third review author. We entered data into Review Manager 5 and checked for accuracy (Review Manager 2014). When information regarding any of the above was unclear, we attempted to contact authors of the original reports to request further details.

Assessment of risk of bias in included studies

Two review authors (SJD and MDN) independently assessed risk of bias for the included studies using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We resolved any disagreement by discussion or by involving a third review author. Different review authors (SJD and SAP) independently assessed risk of bias for Callaway 2019 to limit the effect of conflict of interest.

1. Random sequence generation (checking for possible selection bias)

We described the method used to generate the allocation sequence in sufficient detail to assess whether it produced comparable groups.

We assessed the method as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear risk of bias.

2. Allocation concealment (checking for possible selection bias)

We described the methods used to conceal allocation to interventions prior to assignment and assessed whether the intervention allocation could have been foreseen in advance of, or during, recruitment or changed after assignment.

We assessed the methods as:

- low risk of bias (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk of bias.

3.1. Blinding of participants and personnel (checking for possible performance bias)

We described the methods used to conceal the allocation sequence and determine whether intervention allocation could have been foreseen in advance of, or during, recruitment or changed after assignment.
We assessed the methods as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel.

3.2. Blinding of outcome assessment (checking for possible detection bias)

We described the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies would be at low risk of bias if they were blinded, or if we judged that the lack of blinding would be unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed the methods as:

- low, high or unclear risk of bias.

4. Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We described the completeness of data including attrition and exclusions from the analysis. We stated whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported, or could be supplied by the trial authors, we re-included missing data in the analyses that we undertook.

We assessed methods as:

- low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation);
- unclear risk of bias.

5. Selective reporting (checking for reporting bias)

We described how we investigated the possibility of selective outcome reporting bias and what we found.

We assessed the methods as:

- low risk of bias (where it was clear that all the study's prespecified outcomes and all expected outcomes of interest to the review were reported);
- high risk of bias (where not all the study's prespecified outcomes were reported; one or more reported primary outcomes were not prespecified; outcomes of interest were reported incompletely and so could not be used; study failed to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

6. Other bias (checking for bias due to problems not covered by 1. to 5. above)

We described any important concerns we had about other possible sources of bias.

We assessed whether the included study was free of other problems that could have put it at risk of bias:

- low risk of other bias;
- high risk of other bias;
- unclear whether there is risk of other bias.

7. Overall risk of bias

We made explicit judgements about whether the included study was at high risk of bias, according to the criteria given in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). With reference to 1. to 6. above, we assessed the likely magnitude and direction of the bias and whether we considered it was likely to impact on the findings. We planned to explore the impact of the level of bias through undertaking sensitivity analyses (see Sensitivity analysis).

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results as summary risk ratio (RR) with 95% confidence intervals (CI).

Continuous data

For continuous data, we used the mean difference (MD) with 95% CIs if outcomes were measured in the same way between trials. We planned to use the standardised mean difference with 95% CIs to combine trials that measured the same outcome, but used different scales.

Unit of analysis issues

Cluster-randomised trials

We identified no cluster-randomised trials for inclusion. However, if we identify cluster-randomised trials in updates of this review, we will include them in the analyses along with individually randomised trials. We will adjust their effect measure using the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* using an estimate of the intracluster correlation coefficient (ICC) derived from the trial (if possible), from a similar trial or from a study of a similar population. Where the cluster-randomised trial properly accounts for the cluster design, we will extract an estimate of the effect measure directly. Where the cluster-randomised trial does not properly account for the clustering, we will calculate the effective sample size of the intervention and placebo groups by dividing the sample size by the design effect. The design effect is $1 + (m - 1) \times ICC$ where m is the mean cluster size. We will assess the cluster-randomised trials and the calculation of the effective sample size will be performed with the assistance of a statistician. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised

trials and individually randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs and the interaction between the effect of intervention and the choice of randomisation unit is unlikely.

We will also acknowledge heterogeneity in the randomisation unit and perform a subgroup analysis to investigate the effects of the randomisation unit.

Studies with more than two intervention groups

In studies with more than two groups, only the two groups that best fit the comparisons used in this review were chosen. When there were more than two groups due to a secondary intervention, the groups without the secondary intervention were chosen if possible to minimise the effect of the secondary intervention on the comparison. If this was not possible, the groups were chosen so that the secondary intervention was the same in both groups. For 2×2 factorial trials, groups were combined where appropriate given the participants were independently randomised to the intervention of interest.

Dealing with missing data

For included studies, we noted levels of attrition. We explored the impact of including studies with high levels of missing data in the overall assessment of treatment effect by using sensitivity analysis.

For all outcomes, we carried out analyses, as far as possible, on an intention-to-treat basis (i.e. we attempted to include all participants randomised to each group in the analyses, and analysed all participants in the group to which they were allocated, regardless of whether or not they received the allocated intervention). The denominator for each outcome in each trial was the number randomised minus any participants whose outcomes were known to be missing.

Assessment of heterogeneity

We assessed statistical heterogeneity in each meta-analysis using the I^2 and Chi^2 statistics. For random-effects meta-analyses we also considered the Tau^2 statistic. We regarded heterogeneity as substantial if the I^2 statistic was greater than 30% and either the Tau^2 was greater than zero, or there was a low P value (less than 0.10) in the Chi^2 test for heterogeneity.

Assessment of reporting biases

Given we included only seven studies, reporting bias analysis was not undertaken. In updates of this review, if there are 10 or more studies in the meta-analysis, we will investigate reporting biases (such as publication bias) using funnel plots. We will assess funnel plot asymmetry visually. If asymmetry is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

Data synthesis

We carried out statistical analysis using the Review Manager 5 (Review Manager 2014). We used fixed-effect meta-analysis for combining data where it was reasonable to assume that studies were estimating the same underlying treatment effect (i.e. where trials examined the same intervention, and the trials' populations and methods were judged sufficiently similar). If there was clinical heterogeneity sufficient to expect that the underlying treatment effects differed between trials, or if there was substantial statistical heterogeneity, we used random-effects meta-analysis to produce an overall summary, if a mean treatment effect across trials was considered clinically meaningful. The random-effects summary was treated as the mean range of possible treatment effects and we discussed the clinical implications of treatment effects differing between trials. If the mean treatment effect was not clinically meaningful, we did not combine trials.

If we used random-effects analyses, we presented the results as the mean treatment effect with 95% CIs, and the estimates of the Tau^2 and I^2 statistics.

Subgroup analysis and investigation of heterogeneity

Where we identified substantial heterogeneity, we investigated it using subgroup analyses and sensitivity analyses. We considered whether an overall summary was meaningful, and if it was, used random-effects analysis to produce it.

We planned to carry out the following subgroup analyses.

- History of GDM or family history of type 2 diabetes (yes versus no).
- Probiotic dose (more than five billion colony-forming units (CFU) versus less than five billion CFU).
- Probiotic bacterial species (one species versus another species).
- Probiotic treatment starting in early pregnancy versus starting at more than 20 weeks' gestation.
- Probiotic mode of delivery (capsule versus other).
- Probiotic frequency of administration (daily versus other).

In this update of the review, subgroup analysis by history of GDM or family history of type 2 diabetes was not conducted as outcome data were not available in these subgroups. The subgroup analysis by probiotic mode of delivery and frequency of administration were also not conducted since all included studies administered the intervention daily as a capsule. These subgroups will be included in future updates of the review if possible.

Subgroup analysis was restricted to the review's primary outcomes. We assessed subgroup differences by interaction tests available within Review Manager 5 (Review Manager 2014). We reported the results of subgroup analyses quoting the Chi^2 statistic and P value, and the interaction test I^2 statistic.

Sensitivity analysis

Sensitivity analysis was carried out, where necessary, to explore the influence of diagnostic criteria for GDM. Sensitivity analysis was restricted to the review's primary outcomes.

We planned to conduct a sensitivity analysis to explore the influence of high dropout rates (more than 20%); however, we identified no such studies. This may be possible in updates of this review.

Summary of findings and assessment of the certainty of the evidence

For this update, we used the GRADE approach to assess the certainty of the evidence as outlined in the GRADE Handbook to assess the quality of the body of evidence relating to the following outcomes for the main comparison probiotics versus placebo.

If studies comparing probiotics and diet are identified in future updates, we will evaluate this comparison.

Maternal

- Diagnosis of GDM
- Hypertensive disorders of pregnancy (pre-eclampsia)
- Caesarean section
- Perineal trauma
- Weight gain during pregnancy
- Postnatal depression
- Development of subsequent diabetes

Infant

- Large-for-gestational age
- Perinatal mortality
- Mortality or morbidity composite
- Hypoglycaemia as defined by trialists
- Adiposity
- Diabetes
- Neurodisability

We used GRADEpro GDT to import data from Review Manager 5 (Review Manager 2014) in order to create 'Summary of findings' tables. We produced a summary of the intervention effect and a measure of certainty for each of the above outcomes was produced using the GRADE approach. The GRADE approach uses five considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the certainty of the body of evidence for each outcome. The evidence can be downgraded from 'high certainty' by one level for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, indirectness of evidence, serious inconsistency, imprecision of effect estimates or potential publication bias.

RESULTS

Description of studies

See Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification; and Characteristics of ongoing studies tables.

Results of the search

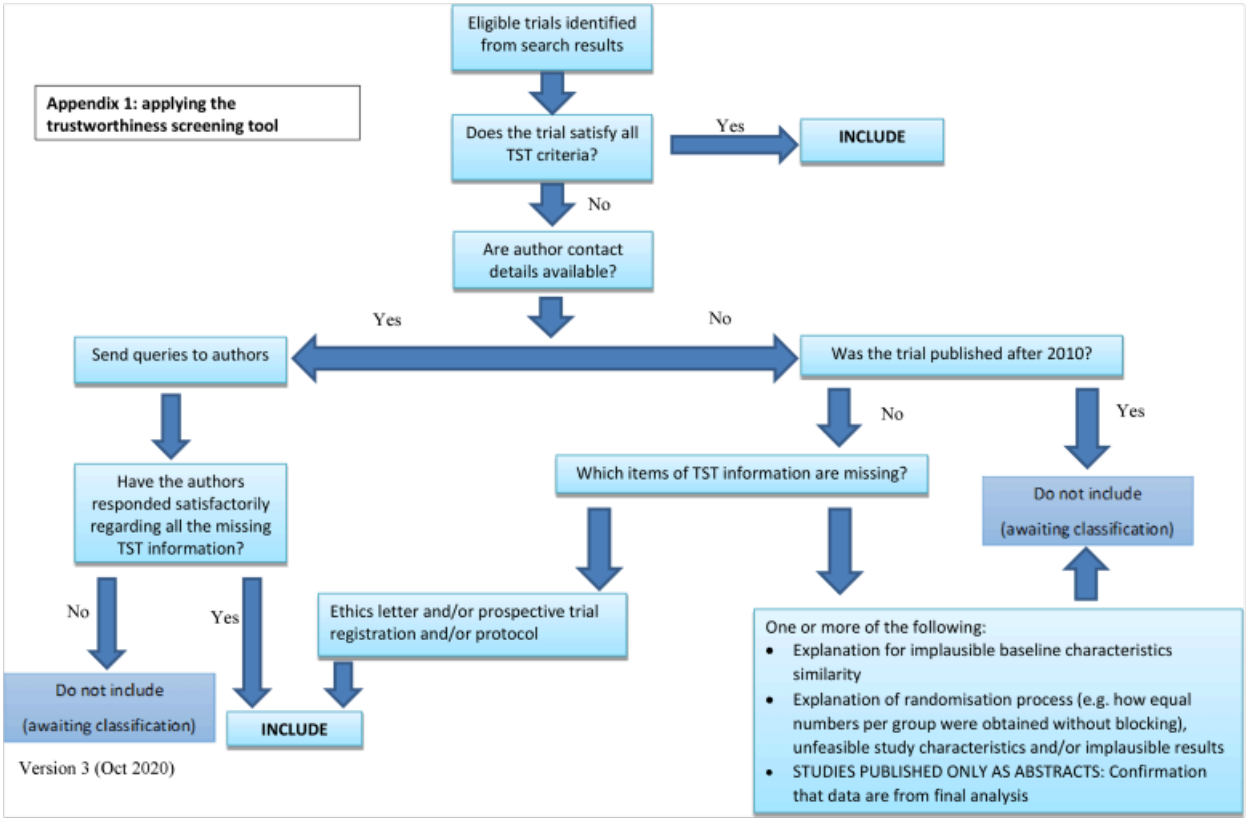
See: Figure 1

We assessed 43 new trial reports from an updated search in March 2020. We also reassessed the four studies (five reports) that were ongoing in the previous version of the review. We included six new trials (25 reports), added four new reports to the previously included study (Laitinen 2009), and excluded two trials (six reports). Three studies (four reports) are awaiting further classification and we added eight studies (nine reports) to the Ongoing studies section.

Screening eligible studies for scientific integrity/trustworthiness

See: Figure 2

Figure 2. Applying the trustworthiness screening tool



One study is awaiting classification since it was only available in abstract form and confirmation that the presented data came from the final analysis was not received (Charles 2018). Another study is awaiting classification because it was unclear whether the study met our inclusion criteria; we sought clarification from the authors but received no response (Si 2019).

Two studies were at high risk according to the prespecified trustworthiness criteria. One study had almost no losses to follow-up (Asgharian 2020), and there was insufficient information provided by the study authors for us to make a definitive classification. Therefore, this study remains in studies awaiting classification. The other study had no losses to follow-up and had limited information regarding their randomisation methods (Jamilian 2016). However, the study authors provided more detail regarding these concerns, and the study was included since it was determined to be at low risk. See Characteristics of included studies; Characteristics of studies awaiting classification for further information.

Included studies

Design

All included studies were parallel randomised controlled trials. Four studies had one intervention arm and one control arm (Callaway 2019; Jamilian 2016; Lindsay 2014; Wickens 2017). Laitinen 2009 had three arms for two interventions. Pellonpera 2019 had four arms for two interventions, and Okesene-Gafa 2019 was designed as a 2×2 factorial with two interventions.

Sample sizes

The number of women recruited in the included studies ranged from 60 (Jamilian 2016) to 438 women (Pellonpera 2019). The other studies recruited 175 (Lindsay 2014), 230 (Okesene-Gafa 2019), 256 (Laitinen 2009), 423 (Wickens 2017), and 433 women (Callaway 2019).

Setting

The included studies in this review were conducted in Iran (Jamilian 2016), Australia (Callaway 2019), Finland (Laitinen 2009; Pellonpera 2019), Ireland (Lindsay 2014), and New Zealand (Okesene-Gafa 2019; Wickens 2017).

Participants

All included studies were conducted in pregnant women with singleton pregnancies without pre-existing diabetes or other significant health conditions, although two studies included women with a history of atopic disease (Laitinen 2009; Wickens 2017). Two studies were conducted in overweight and obese pregnant women (Callaway 2019; Pellonpera 2019), two in obese pregnant women only (Lindsay 2014; Okesene-Gafa 2019), and three did not exclude women based on their body mass index (Jamilian 2016; Laitinen 2009; Wickens 2017).

Interventions and comparisons

All seven trials compared probiotics versus placebo. In four trials, women were only randomised to either probiotics or placebo (Callaway 2019; Jamilian 2016; Lindsay 2014; Wickens 2017). Three studies included a second intervention, two of which included a dietary intervention (Laitinen 2009; Okesene-Gafa 2019), and one included a fish oil capsule (Pellonpera 2019). Laitinen 2009 randomised women to probiotics plus dietary intervention, placebo plus dietary intervention, or placebo plus routine dietary advice. Okesene-Gafa 2019 first randomised all women to the dietary intervention or routine dietary advice, then randomised all women again to either probiotics or placebo. Pellonpera 2019 randomised women to one of four study arms (probiotics plus fish oil, probiotics plus placebo, placebo plus fish oil or placebo plus placebo). Although two trials included diet as a secondary intervention, the trials did not directly compare probiotics versus diet (Laitinen 2009; Okesene-Gafa 2019). Therefore, no conclusions could be drawn about this comparison.

Six trials started the intervention prior to 20 weeks' gestation (Callaway 2019; Jamilian 2016; Laitinen 2009; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017), and one trial started the intervention at 20 weeks' gestation or later (Lindsay 2014). One study initially started the intervention before 16 weeks' gestation, but it was later changed to before 20 weeks' gestation due to a change in hospital policy (Callaway 2019). Women received the intervention daily in all studies.

All included studies delivered the intervention as a capsule. The dose of probiotic used in the studies varied, with three studies reporting a dose of less than five billion CFUs per species (Callaway 2019; Jamilian 2016; Lindsay 2014), and four studies reporting a dose of greater than five billion CFUs per species (Laitinen 2009; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). However, it is important to note that given decay in probiotics over time from the date of manufacturing, the dose can change. Therefore, studies reported either the minimum or mean dose, so individual participants in each study may have received doses that varied from the reported study dose.

Studies used a variety of different bacterial species and strains, and most used a combination of species for their probiotics. The species were *Lactobacillus rhamnosus* GG (Callaway 2019; Laitinen 2009; Okesene-Gafa 2019), *Lactobacillus rhamnosus* HN001 (Pellonpera 2019; Wickens 2017), *Lactobacillus acidophilus* LA5 (Jamilian 2016), *Lactobacillus casei* (Jamilian 2016), *Lactobacillus salivarius* UCC118 (Lindsay 2014), *Bifidobacterium animalis* subspecies *lactis* BB12 (Callaway 2019; Laitinen 2009; Okesene-Gafa 2019), *Bifidobacterium animalis* subspecies *lactis* 420 (Pellonpera 2019), and *Bifidobacterium bifidum* (Jamilian 2016).

Outcomes

Studies were required to have either the diagnosis of GDM or a marker of glucose metabolism in the third trimester of pregnancy as a reported outcome to be eligible for inclusion. Six studies reported the incidence of GDM (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017), while one reported laboratory measures of glucose metabolism such as fasting plasma glucose and insulin levels (Jamilian 2016). The studies that reported the incidence of GDM used several different diagnostic criteria, and some studies reported results according to more than one set of diagnostic criteria. Four studies used the IADPSG criteria (Callaway 2019; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017; criteria: IADPSG 2010); one study used the Carpenter and Coustan criteria (Lindsay 2014; criteria: Carpenter 1982), one used the modified Fourth International Workshop-Conference on GDM criteria (Laitinen 2009; criteria: Metzger 1998), one used the local New Zealand criteria (Australasian Diabetes in Pregnancy Society) (Wickens 2017; criteria: Ministry of Health 2014), and one used the local Finnish criteria (Pellonpera 2019; criteria: The Finnish Medical Society Duodecim 2013). The details for each set of diagnostic criteria can be found in Table 1.

At least one study reported the other primary outcomes in this review. Four studies reported hypertensive disorders of pregnancy (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019), six studies reported caesarean sections (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017), four studies reported large-for-gestational-age infants (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019), three studies reported perinatal mortality including stillbirth and neonatal mortality (Callaway 2019; Laitinen 2009; Lindsay 2014), and two studies reported a neonatal mortality or morbidity composite measure (Callaway 2019; Okesene-Gafa 2019).

Further details on the primary and secondary outcomes of each study can be found in the Characteristics of included studies table.

Dates of study

The studies were all conduced between 2002 and 2017, with the following trial dates: April 2002 to November 2005 (Laitinen 2009), March 2012 to March 2013 (Lindsay 2014), commencement in November 2012 with no end date provided (Callaway 2019), December 2012 to November 2014 (Wickens 2017), October 2013 to July 2017 (Pellonpera 2019), March 2015 to July 2015 (Jamilian 2016), and April 2015 to June 2017 (Okesene-Gafa 2019).

Funding sources

Study authors reported the following sources of funding: National Health and Medical Research Council (Callaway 2019), the Royal Brisbane and Women's Hospital Foundation (Callaway 2019), Vice-Chancellor for Research, AUMS, Iran (Jamilian 2016), Academy of Finland (Laitinen 2009; Pellonpera 2019), Sigrid-Juselius Foundation (Laitinen 2009), Juho Vainio Foundation (Laitinen 2009; Pellonpera 2019), Social Insurance Institution of Finland (Laitinen 2009), Raisio (Laitinen 2009), Chr. Hansen A/S (Laitinen 2009; Okesene-Gafa 2019), Valio Ltd (Laitinen 2009), National Maternity Hospital Medical Fund Ivo Drury Award (Lindsay 2014), Alimentary Health Ltd (Lindsay 2014), Counties Manukau Health (Okesene-Gafa 2019), Cure Kids Grant (Okesene-Gafa 2019), Lottery Health Research (Okesene-Gafa 2019), RANZCOG Two Mercia Barnes Trust (Okesene-Gafa 2019), Gravida National Centre for Growth and Development (Okesene-Gafa 2019), University of Auckland Faculty Development Research Fund and Reinvestment Fund (Okesene-Gafa 2019), Nurture Foundation (Okesene-Gafa 2019), Heart Foundation of New Zealand (Okesene-Gafa 2019), Roche Diagnostics International Ltd (Okesene-Gafa 2019), Turku University Hospital Expert Responsibility Area (Pellonpera 2019), Diabetes Research Foundation (Pellonpera 2019), Business Finland (Pellonpera 2019), the Finnish Medical Foundation (Pellonpera 2019), the University of Turku (Pellonpera 2019), DuPont (Pellonpera 2019), Croda Europe Ltd (Pellonpera 2019), the Health

Research Council of New Zealand (Wickens 2017), and Fonterra (Wickens 2017).

Declarations of interest

All included studies stated they had no declarations of interest.

Further details on each study can be found in the Characteristics of included studies table.

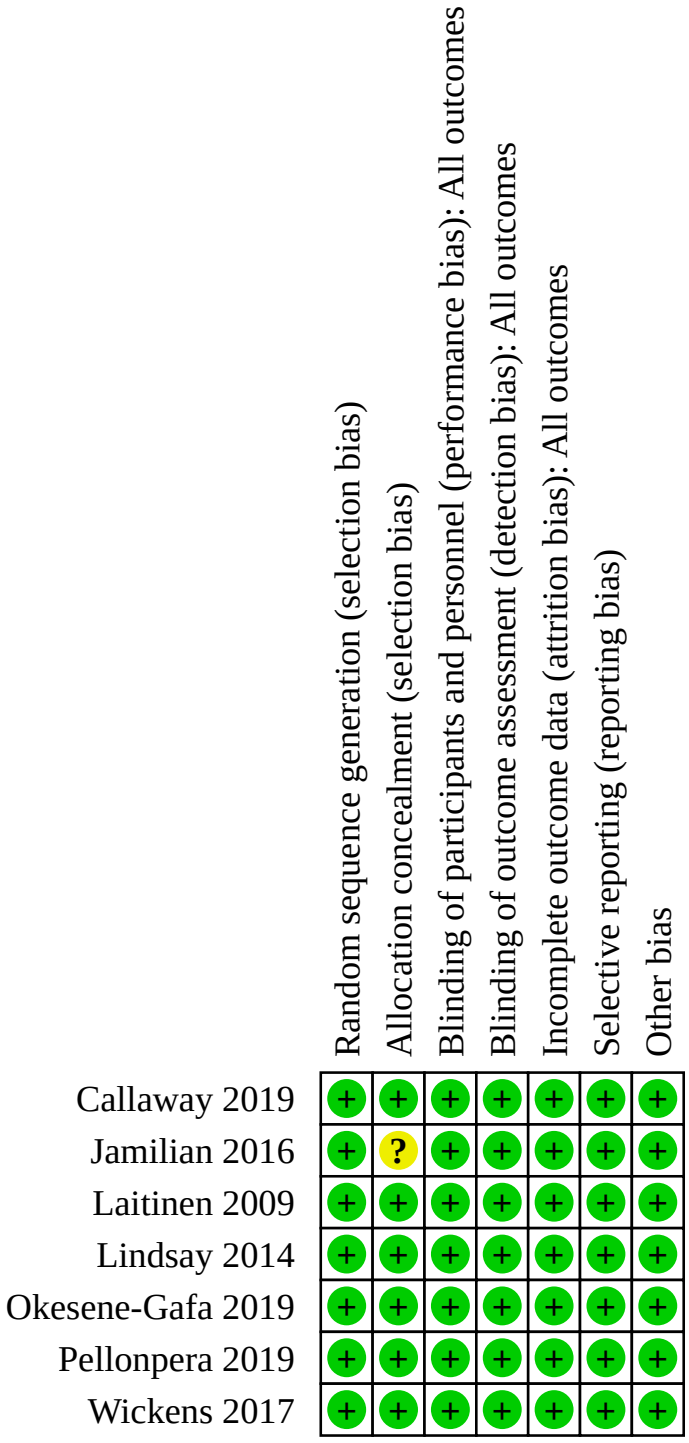
Excluded studies

We excluded two studies from this review since the intervention was not started until the third trimester of pregnancy, after GDM would have been diagnosed (Asemi 2013; Taghizadeh 2014). See Characteristics of excluded studies table.

Risk of bias in included studies

Our risk of bias assessment is summarised in Figure 3. The included studies were at low risk of bias in all domains except for Jamilian 2016, which was at unclear risk of selection bias. Three review authors were authors on one of the included studies (HB, MDN and LC) (Callaway 2019). Therefore, the other two review authors (SJD and SAP) assessed risk of bias in Callaway 2019 to minimise any effects from conflicts of interest.

Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Random sequence generation

All included studies used computer-generated randomisation procedures. Some studies used block randomisation with blocks of four (Pellonpera 2019), six (Laitinen 2009), or random block sizes (Okesene-Gafa 2019; Wickens 2017). Other studies used simple 1:1 randomisation (Lindsay 2014), or did not state whether blocking methods were used (Callaway 2019; Jamilian 2016). All studies were classified at low risk of bias for random sequence generation.

Allocation concealment

All studies stated that allocation concealment was used. Three studies used sealed, opaque envelopes to conceal the allocation sequence prior to recruitment (Callaway 2019; Laitinen 2009; Lindsay 2014), and three studies assigned participants sequentially using a randomisation sequence generated by a third party and unknown to the study staff responsible for enrolment (Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). One study claimed to use allocation concealment but provided no further information, so this study was classified at unclear risk of bias (Jamilian 2016). All other studies were at low risk of bias (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017).

Blinding

All studies blinded both participants and personnel to probiotic/placebo allocation. While Laitinen 2009 had one group where the probiotic/placebo intervention was not blinded to study staff (placebo plus routine dietary advice), this group was not used for comparison in this review. In addition, in the two studies that used a secondary dietary intervention, participants and personnel were not blinded to the dietary intervention (Laitinen 2009; Okesene-Gafa 2019). However, the dietary intervention was the same in both the probiotic and placebo groups, and, therefore, the lack of blinding for this intervention did not affect the probiotics versus placebo comparison. Therefore, all included studies were at low risk for bias for both performance and detection bias.

Incomplete outcome data

There was minimal loss to follow-up at the time of testing for GDM or third trimester measurements of glucose metabolism in all studies. Loss to follow-up rates ranged from 0% to 13.9% and were similar between groups. All studies were classified as low risk for attrition bias. Attrition rates for each study are shown in the Characteristics of included studies table.

Selective reporting

All studies reported their prespecified or mentioned outcomes. All studies were at low risk for reporting bias.

Other potential sources of bias

The studies had no other sources of bias.

Effects of interventions

See: **Summary of findings 1** Probiotics compared to placebo for preventing gestational diabetes (maternal outcomes); **Summary of findings 2** Probiotics compared to placebo for preventing gestational diabetes (infant outcomes)

All seven included studies compared probiotics versus placebo. While two studies included a secondary dietary intervention (Laitinen 2009; Okesene-Gafa 2019), the studies were not conducted in a way that facilitated our second comparison of probiotics versus diet. This comparison will be included in review updates.

Probiotics versus placebo

Four studies had one intervention with the comparison probiotics versus placebo (Callaway 2019; Jamilian 2016; Lindsay 2014; Wickens 2017). In the three studies that had two simultaneous interventions, the study groups used in this review were chosen to balance the effect of the secondary intervention between the probiotic and placebo groups (Laitinen 2009; Okesene-Gafa 2019; Pellonpera 2019). Laitinen 2009 had three arms, and for this comparison we used two of these arms to isolate the effect of the probiotic intervention (probiotics plus dietary intervention for probiotics, placebo plus dietary intervention for placebo). Okesene-Gafa 2019 conducted a 2×2 factorial study where all participants were separately randomised to the dietary intervention and probiotics, so we used all groups for this comparison (probiotics with or without dietary intervention for probiotics, placebo with or without dietary intervention for placebo). Pellonpera 2019 was a four-arm study of two interventions, so we only used the groups without the fish oil intervention for this comparison to isolate the effect of the probiotics (probiotics plus placebo for probiotics, placebo plus placebo for placebo).

Primary outcomes

Maternal

Diagnosis of gestational diabetes mellitus

Six studies reported GDM (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). We used a random-effects model given the substantial heterogeneity present (I^2 = 64%). It is uncertain if probiotics have any effect on the risk for GDM compared to placebo (average RR 0.80, 95% CI 0.54 to 1.20; 1440 women; I^2 = 64%; Tau^2 = 0.15; Analysis 1.1). Given the substantial heterogeneity and the wide CI including both appreciable benefit and appreciable harm, this evidence was low certainty (Summary of findings 1).

The studies that reported GDM used different criteria for the diagnosis. Four studies used IADPSG criteria (Callaway 2019; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017), one study used local criteria based on the Fourth International Workshop-Conference on Gestational Diabetes criteria (Laitinen 2009), and one study used Carpenter and Coustan criteria (Lindsay 2014). Sensitivity analysis was performed based on these criteria, and the results were largely unchanged. There was a reduced risk of GDM when using the Fourth International Workshop-Conference on Gestational Diabetes criteria, but this is only based on one study and, therefore, should be interpreted with caution.

Subgroup analyses based on whether the reported dose of probiotics was less than five billion CFU (2 studies, 547 participants) or greater than five billion CFU (4 studies, 911 participants) found a difference between the subgroups (Chi^2 = 6.92, P = 0.009, I^2 = 85.5%; Analysis 1.2). However, there was still substantial heterogeneity in the subgroup with a dose greater than five billion CFU (Tau^2 = 0.07, I^2 = 51%), and the subgroup with a

dose less than five billion CFU had only two studies. In addition, given the decay in probiotics over time, this subgroup analysis was conducted based on reported minimum or mean dose and there was no guarantee all participants received the reported dose. Therefore, this subgroup analysis should be interpreted with caution. The subgroup analysis based on bacterial species and duration of treatment revealed no clear differences, although both had subgroups with only one trial (Analysis 1.3; Analysis 1.4).

Hypertensive disorders of pregnancy (including pre-eclampsia, pregnancy-induced hypertension and eclampsia)

Four studies reported hypertensive disorders of pregnancy (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). Probiotics may increase the risk of hypertensive disorders of pregnancy compared to placebo (RR 1.39, 95% CI 0.96 to 2.01; 955 women; I^2 = 0%; Analysis 1.5). Subgroup analyses found no differences in the results, although most subgroups only included one or two studies (Analysis 1.6; Analysis 1.7; Analysis 1.8).

Four studies reported pre-eclampsia (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). Probiotics increase the risk of pre-eclampsia compared to placebo (RR 1.85, 95% CI 1.04 to 3.29; 955 women; I^2 = 0%; Analysis 1.9; high-certainty evidence; Summary of findings 1).

Caesarean section

Six studies reported caesarean section (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). Probiotics make little to no difference in the rate of caesarean sections compared to placebo (RR 1.00, 95% CI 0.86 to 1.17; 1520 women; I^2 = 0%; Analysis 1.10; high-certainty evidence; Summary of findings 1). Subgroup analyses revealed no differences in the results, although most subgroups only included one or two studies (Analysis 1.11; Analysis 1.12; Analysis 1.13).

Infant

Large-for-gestational age

Four studies reported large-for-gestational age (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). One study defined large-for-gestational age as greater than 90th percentile on customised percentile charts (Okesene-Gafa 2019), while the other three studies also defined large-for-gestational age as greater than 90th percentile but did not specify what charts were used (Callaway 2019; Lindsay 2014; Pellonpera 2019). Probiotics probably make little to no difference in the risk of being large-for-gestational age compared to placebo (RR 0.99, 95% CI 0.72 to 1.36; 919 infants; I^2 = 0%; Analysis 1.14; moderate-certainty evidence; Summary of findings 2). Subgroup analyses revealed no differences in the results, although most subgroups only included one or two studies (Analysis 1.15; Analysis 1.16; Analysis 1.17).

Perinatal mortality (including stillbirth and neonatal death)

Three studies reported perinatal mortality (Callaway 2019; Laitinen 2009; Lindsay 2014). However, two of these studies had no stillbirths or neonatal deaths in either group (Laitinen 2009; Lindsay 2014), and the other study had only one perinatal death across groups (Callaway 2019). We do not know if probiotics have an effect on perinatal mortality compared to placebo because the wide CI crossed the line of no effect (RR 0.33, 95% CI 0.01 to 8.02; 3 studies, 709 infants; I^2 = 0%; Analysis 1.18; low-certainty evidence; Summary of findings 2). This evidence was of low certainty due to the small number of events and very wide CIs. Given the lack of data on this outcome, subgroup analyses would not be meaningful and were not performed.

Mortality or morbidity composite

Two studies reported a composite measure of neonatal morbidity (Callaway 2019; Okesene-Gafa 2019). Callaway 2019 used a composite measure of birth injury including nerve injury, bone fracture and intracranial haemorrhage. Okesene-Gafa 2019 used a composite measure of morbidity including birth trauma, hypoxic-ischaemic encephalopathy, sepsis, respiratory distress requiring continuous positive airway pressure and hypoglycaemia requiring intravenous therapy. It is uncertain if probiotics have any effect on neonatal morbidity compared to placebo because the CIs were consistent with appreciable harm and appreciable benefit (RR 0.69, 95% CI 0.36 to 1.35; 623 infants; I^2 = 0%; Analysis 1.19; low-certainty evidence; Summary of findings 2). Subgroup analyses were not performed because only two studies reported this outcome.

Secondary outcomes

Maternal

Induction of labour

Two studies reported induction of labour (Callaway 2019; Lindsay 2014). Probiotics may make little to no difference in induction of labour rates compared to placebo (RR 1.08, 95% CI 0.85 to 1.39; 544 women; I^2 = 23%; Analysis 1.20).

Perineal trauma

No studies reported perineal trauma.

Placental abruption

No studies reported placental abruption.

Postpartum haemorrhage

Two studies reported postpartum haemorrhage (Lindsay 2014; Pellonpera 2019). One study defined postpartum haemorrhage as greater than 1000 mL (Pellonpera 2019), while the other study provided no definition (Lindsay 2014). It is uncertain if probiotics have any effect on the risk of postpartum haemorrhage compared to placebo (RR 1.05, 95% CI 0.60 to 1.85; 324 women; I^2 = 0%; Analysis 1.21).

Postpartum infection

No studies reported postpartum infection.

Weight gain during pregnancy

Four studies reported weight gain during pregnancy (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019). Two studies specified the reported weight gain was from baseline to 36 weeks' gestation (Callaway 2019; Okesene-Gafa 2019), while the other two studies stated "total" weight gain over the course of the pregnancy (Laitinen 2009; Lindsay 2014). There was substantial heterogeneity using a random-effects model (I^2 = 40%). Probiotics probably make little to no difference in weight gain during pregnancy compared to placebo (MD 0.30 kg, 95% CI –0.67 to 1.26; 853 women; Analysis 1.22).

Adherence to the intervention

Six studies reported intervention adherence (Jamilian 2016; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). However, it was reported differently by each study, and, therefore, was not amenable to meta-analysis. Jamilian 2016 reported that all participants received all capsules throughout the intervention (probiotics 30/30 women, placebo 30/30 women). Laitinen 2009 reported that participants reported 99.5% of capsules taken at visit two, 99% at visit three and 95% at visit four; these data were not separated out by group. Lindsay 2014 reported that the number of missed capsules was similar between groups, with 9/63 participants missing three or more capsules in the probiotics group and 12/75 participants missing three or more capsules in the placebo group. Okesene-Gafa 2019 reported that over 75% of capsules were taken by 87/115 participants, but these data were not separated by group. Pellonpera 2019 reported good compliance by 88.4% of the entire study cohort, and stated that adherence was similar between groups. Wickens 2017 reported median adherence rates with interquartile ranges (IQR), and found no clear difference between groups (probiotics: median 94.9%, IQR 85.7 to 98.8; placebo: median 94.0%, IQR 85.9 to 98.8). Overall, there may be little to no difference in adherence rates between probiotics and placebo.

Behaviour changes associated with the intervention

No studies reported behaviour changes associated with the intervention.

Relevant biomarker changes associated with the intervention (e.g. adiponectin, free fatty acids, triglycerides, high-density lipoproteins, low-density lipoproteins, insulin)

All included studies reported at least one relevant biomarker.

Seven studies reported fasting plasma glucose in the third trimester. Given the substantial heterogeneity, we used a random-effects model (I^2 = 69%). Probiotics may make little to no difference in fasting plasma glucose levels in the third trimester compared to placebo (MD −0.04 mmol/L, 95% CI −0.12 to 0.05; 1519 women; I^2 = 69%; Analysis 1.23).

Four studies reported plasma glucose at one hour of a 75 g oral glucose tolerance test (OGTT) (Callaway 2019; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). Probiotics may make little to no difference in one-hour OGTT results compared to placebo (MD −0.07 mmol/L, 95% CI −0.27 to 0.13; 1110 women; I^2 = 0%; Analysis 1.24).

Four studies reported plasma glucose at two hours of a 75 g OGTT (Callaway 2019; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). Probiotics may make little to no difference in two-hour OGTT results compared to placebo (0.02 mmol/L, 95% −0.13 to 0.18; 1186 women; I^2 = 0%; Analysis 1.25).

Two studies reported triglycerides at the end of the intervention period (Jamilian 2016; Lindsay 2014). Probiotics may slightly reduce triglyceride levels compared to placebo (MD −0.21 mmol/L, 95% CI −0.40 to −0.02; 198 women; I^2 = 0%; Analysis 1.26). However, given this is based on two small studies with a wide CI, the certainty of this evidence was low.

Two studies reported high-density lipoproteins at the end of the intervention period (Jamilian 2016; Lindsay 2014). Probiotics may make little to no difference in high-density lipoprotein levels compared to placebo (MD 0.02 mmol/L, 95% CI −0.08 to 0.11; 198 women; I^2 = 0%; Analysis 1.27).

Two studies reported low-density lipoproteins at the end of the intervention period (Jamilian 2016; Lindsay 2014). Probiotics may slightly reduce low-density lipoprotein levels compared to placebo, but the CIs indicates probiotics may make little or no difference (MD −0.22 mmol/L, 95% CI −0.48 to 0.04; 198 women; I^2 = 0%; Analysis 1.28).

Two studies reported total cholesterol at the end of the intervention period (Jamilian 2016; Lindsay 2014). Probiotics may slightly reduce total cholesterol levels compared to placebo, but the 95% CI included zero indicating the possibility that probiotics may make little or no difference (MD −0.31 mmol/L, 95% CI −0.62 to −0.00; 198 women; I^2 = 0%; Analysis 1.29).

Four studies reported insulin levels in the third trimester (Jamilian 2016; Laitinen 2009; Lindsay 2014; Pellonpera 2019). Probiotics may reduce insulin levels slightly compared to placebo (MD −1.95 mU/L, 95% CI −3.01 to −0.88; 538 women; I^2 = 0%; Analysis 1.30).

Sense of wellbeing and quality of life

One study reported sense of wellbeing and quality of life (Okesene-Gafa 2019). This study reported several different measures of wellbeing and quality of life at 36 weeks' gestation. It is uncertain if probiotics have any effect compared to placebo in Edinburgh Postnatal Depression scores at 36 weeks (MD 0.42, 95% CI −0.89 to 1.73; 164 women; Analysis 1.31.1), Spielberger State-Trait Anxiety Inventory Short Form scores at 36 weeks (MD −0.94, 95% CI −4.09 to 2.21; 164 women; Analysis 1.31.2), Short-Form Health Survey scores Mental Component Score (MD 0.31, 95% CI −2.54 to 3.16; 164 women; Analysis 1.31.3) or Physical Component Score (MD 0.87, 95% CI −1.94 to 3.68; 164 women; Analysis 1.31.4).

Views of the intervention

One study reported views of the intervention (Lindsay 2014). Specifically, Lindsay 2014 reported if participants thought the "intervention was an inconvenience" (probiotics: 11/56 women, placebo: 12/63 women), "capsules were difficult to swallow" (probiotics: 1/56 women, placebo: 5/64 women), and if they "would consider taking a probiotic in a future pregnancy" (probiotics: 55/55 women, placebo: 60/64 women). Overall, Lindsay 2014 reported no differences in views of the intervention between groups.

Breastfeeding (e.g. at discharge, six weeks postpartum)

Two studies reported women who were breastfeeding any amount at six months (Laitinen 2009; Wickens 2017). It is uncertain if probiotics have any effect on numbers of women breastfeeding at six months compared to placebo (non-event RR 1.08, 95% CI 0.77 to 1.50; 552 women; I^2 = 0%; Analysis 1.32).

Long-term maternal

Postnatal depression

No studies reported postnatal depression.

Postnatal weight retention or return to prepregnancy weight

One study reported weight at four to seven days postpartum (Wickens 2017). Probiotics may make little to no difference in postpartum weight compared with placebo (MD −0.10 kg, 95% CI −0.91 to 0.71; 391 women; Analysis 1.33). Laitinen 2009 also reported that weight decreased similarly between groups but provided no data.

Body mass index

One study reported body mass index at four to seven days postpartum (Wickens 2017), one at one-year postpartum (Laitinen 2009), and one at four years postpartum (Laitinen 2009). At all time points, there was no clear difference between groups (4 to 7 days: MD −0.10 kg/m^2, 95% CI −0.38 to 0.18; 391 women; 12 months: MD −0.10 kg/m^2, 95% CI −0.65 to 0.45; 128 women; 4 years: MD 0.70 kg/m^2, 95% CI −0.18 to 1.58; 80 women; Analysis 1.34).

Gestational diabetes mellitus in a subsequent pregnancy

No studies reported GDM in a subsequent pregnancy.

Type 1 diabetes

No studies reported type 1 diabetes.

Type 2 diabetes

No studies reported type 2 diabetes.

Impaired glucose tolerance

No studies reported glucose tolerance.

Cardiovascular health as defined by trialists (including blood pressure, hypertension, cardiovascular disease and metabolic syndrome)

No studies reported cardiovascular health.

Infant

Stillbirth

Five studies reported stillbirth (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). Of the 1128 participants, there were six stillbirths. Therefore, we do not know if probiotics reduce stillbirth compared to placebo because there were so few events and the CIs were very wide (RR 0.59, 95% CI 0.14 to 2.46; 1128 women; I^2 = 0%; Analysis 1.35).

Neonatal mortality

Three studies reported neonatal mortality (Callaway 2019; Laitinen 2009; Lindsay 2014). However, there were no neonatal mortalities in any study, so the effect of probiotics could not be estimated (Analysis 1.36). Therefore, we do not know if probiotics reduce neonatal mortality compared to placebo.

Gestational age at birth

Six studies reported gestational age at birth (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). One study reported gestational age at birth as median and IQR, so this study was not included in the meta-analysis (Wickens 2017). However, Wickens 2017 found no difference between groups (probiotics: 39.7 weeks, IQR 38.7 to 40.7; placebo: 39.6 weeks, IQR 38.7 to 40.4). We included the other five studies in the meta-analysis (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). Probiotics may make little to no difference in gestational age at birth compared to placebo (MD 0.01 weeks, 95% CI −0.19 to 0.21; 5 studies, 1073 infants; I^2 = 26%; Analysis 1.37).

Preterm birth (less than 37 weeks' gestation and less than 32 weeks' gestation)

Six studies reported preterm birth (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). All studies defined preterm birth as prior to 37 weeks' gestation. It is uncertain if probiotics have an effect on the risk of preterm birth compared to placebo (RR 1.32, 95% CI 0.86 to 2.01; 1484 participants; I^2 = 0%; Analysis 1.38).

Apgar score (less than seven at five minutes)

Three studies reported Apgar score (Laitinen 2009; Pellonpera 2019; Wickens 2017). However, all studies reported this outcome differently, so the data were not conducive to a meta-analysis. Laitinen 2009 reported the median and range of five-minute Apgar scores, which showed no difference between groups (probiotics: median 9, range 6 to 10; placebo: median 9, range 3 to 10). Pellonpera 2019 reported the mean and SD of five-minute Apgar scores, which showed no difference between groups (probiotics: mean 9.0, SD 0.7; placebo: 9.0, SD 0.8). Finally, Wickens 2017 reported the proportion of infants who had an Apgar score of seven or greater at five minutes and found no difference between groups (probiotics: 200/203; placebo: 198/202). Overall, probiotics may make little to no difference in five-minute Apgar scores compared to placebo.

Macrosomia

Three studies reported macrosomia defined as a birthweight greater than 4000 g (Callaway 2019; Lindsay 2014; Wickens 2017). Probiotics may make little to no difference in the risk of macrosomia compared to placebo (RR 1.13, 95% CI 0.86 to 1.48; 952 infants; I^2 = 20%; Analysis 1.39).

Small-for-gestational age

Three studies reported SGA (Callaway 2019; Okesene-Gafa 2019; Pellonpera 2019). One study defined SGA as less than 10th percentile using customised percentile charts (Okesene-Gafa 2019), while the other two studies also used less than 10th percentile but did not state which charts they used (Callaway 2019; Pellonpera 2019). Probiotics may reduce the incidence of SGA infants compared to placebo (RR 0.51, 95% CI 0.30 to 0.85; 814 infants; Analysis 1.40).

Birthweight and z-score

Six studies reported birthweight and z-score (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). We used a random-effects model due to substantial heterogeneity (I^2 = 42%). It is uncertain if probiotics have an effect on birthweight compared to placebo (MD 26.87 g, 95% CI −49.52 to 103.26; 1524 infants; Analysis 1.41). Two studies also reported birthweight z-scores (Okesene-Gafa 2019; Pellonpera 2019), and one study reported birthweight percentiles (Lindsay 2014). Two of these studies reported no difference between groups (Lindsay 2014; Pellonpera 2019), while one study reported a slight increase in birthweight z-score in the probiotics group compared to placebo (Okesene-Gafa 2019). We do not know if probiotics affect birthweight z-score compared to placebo.

Head circumference and z-score

Three studies reported head circumference and z-score (Laitinen 2009; Okesene-Gafa 2019; Wickens 2017). Probiotics may make little to no difference in head circumference compared to placebo (MD −0.04 cm, 95% CI −0.27 to 0.18; 789 infants; I^2 = 21%; Analysis 1.42). One study reported head circumference z-scores and also found no difference between groups (Okesene-Gafa 2019).

Length and z-score

Three studies reported length and z-score (Laitinen 2009; Okesene-Gafa 2019; Wickens 2017). We used a random-effects model due to substantial heterogeneity (I^2 = 59%). Probiotics may make little to no difference in length compared to placebo (MD 0.02 cm, 95% CI −0.54 to 0.59; 786 infants; Analysis 1.43). One study reported length z-scores and also found no difference between groups (Okesene-Gafa 2019).

Ponderal index

Two studies reported ponderal index (Lindsay 2014; Wickens 2017). Probiotics may make little to no difference in ponderal index compared to placebo (MD 0.25 kg/m^3, 95% CI −0.21 to 0.70; 539 infants; I^2 = 0%; Analysis 1.44).

Adiposity

Two studies reported adiposity (Callaway 2019; Okesene-Gafa 2019). Okesene-Gafa 2019 reported adiposity as fat mass (MD −0.04 kg, 95% CI −0.12 to 0.04; 110 infants; Analysis 1.45), and Callaway 2019 reported adiposity as percentage fat (MD −0.10%, 95% CI −1.19 to 0.99; 210 infants; Analysis 1.46), which both showed no difference between probiotics and placebo.

Shoulder dystocia

No studies reported shoulder dystocia.

Bone fracture

No studies reported bone fracture.

Nerve palsy

No studies reported nerve palsy.

Respiratory distress syndrome

No studies reported respiratory distress syndrome.

Hypoglycaemia as defined by trialists

Two studies reported hypoglycaemia (Callaway 2019; Pellonpera 2019). One study defined hypoglycaemia as a fasting blood sugar level of less than 2.2 mmol/L (Callaway 2019), and one study defined hypoglycaemia as a fasting blood sugar level of less than 2.4 mmol/L (Pellonpera 2019). We used a random-effects model due to substantial heterogeneity (I^2 = 37%). Given the heterogeneity and the wide CIs, we do not know if probiotics reduce the risk of hypoglycaemia compared to placebo (mean RR 1.15, 95% CI 0.69 to 1.92; 586 infants; Analysis 1.47).

Hyperbilirubinaemia

Two studies reported hyperbilirubinaemia, both as hyperbilirubinaemia requiring phototherapy (Callaway 2019; Pellonpera 2019). It is uncertain if probiotics have any effect on the risk of hyperbilirubinaemia compared to placebo (RR 0.95, 95% CI 0.66 to 1.38; 593 infants; I^2 = 10%; Analysis 1.48).

Later infant and childhood

Weight and z-scores

One study reported weight gain in grams per month from 0 to 6, 6 to 12 and 12 to 24 months (Laitinen 2009). Another study reported that weights and z-scores were similar between groups at five months (Okesene-Gafa 2019). Probiotics may make little to no difference in weight compared to placebo (0 to 6 months: MD −3 g/month, 95% CI −53.07 to 47.07; 6 to 12 months: MD 27 g/month, 95% CI −0.76 to 54.76; 12 to 24 months: MD −19 g/month, 95% CI −42.62 to 4.62; Analysis 1.49).

Height and z-scores

One study reported growth in centimetres per month from 0 to 6, 6 to 12 and 12 to 24 months (Laitinen 2009). Another study reported that heights and z-scores were similar between groups at five months (Okesene-Gafa 2019). Probiotics may make little to no difference in height compared to placebo (0 to 6 months: MD −0.05 cm/month, 95% CI −0.15 to 0.05; 6 to 12 months: MD 0.02 cm/month, 95% CI −0.04 to 0.08; 12 to 24 months: MD 0.01 cm/month, 95% CI −0.04 to 0.06; Analysis 1.50).

Head circumference and z-scores

One study reported head circumference with 119 participants at six months of age (Laitinen 2009). Another study reported head circumferences and z-scores were similar between groups at five months (Okesene-Gafa 2019). Probiotics may make little to no difference in head circumference compared to placebo (MD 0.30 cm, 95% CI −0.26 to 0.86; Analysis 1.51).

Adiposity (including body mass index and skinfold thickness)

One study reported that infant body mass indexes and skinfold thicknesses were similar between groups at five months (Okesene-Gafa 2019). There were no data for meta-analysis. Probiotics may have no effect on long-term adiposity in children compared with placebo.

Blood pressure

One study reported mean blood pressures in infants aged six months (Laitinen 2009). Probiotics may make little to no difference in mean blood pressure compared to placebo (MD −1.00 mmHg, 95% CI −4.19 to 2.19; 114 participants; Analysis 1.52).

Type 1 diabetes

No studies reported type 1 diabetes.

Type 2 diabetes

No studies reported type 2 diabetes.

Impaired glucose tolerance

One study reported impaired glucose tolerance (Laitinen 2009). This study used 32–33 split proinsulin as a measure of glucose tolerance at six months, and levels were defined as abnormal if they were above the 85th percentile. We do not know if probiotics affect impair glucose tolerance in children (RR 0.95, 95% CI 0.34 to 2.69; Analysis 1.53).

Dyslipidaemia or metabolic syndrome

One study reported dyslipidaemia or metabolic syndrome (Laitinen 2009). Laitinen 2009 stated there were no differences in lipid levels with probiotics in children at one, two and four years of age compared to placebo. There were no data for meta-analysis.

Neurodisability

No studies reported neurodisability.

Educational achievement

No studies reported educational achievement.

Child as an adult

Weight

No studies reported weight.

Height

No studies reported height.

Adiposity (including body mass index and skinfold thickness)

No studies reported adiposity.

Cardiovascular health as defined by trialists (including blood pressure, hypertension, cardiovascular disease and metabolic syndrome)

No studies reported cardiovascular health.

Type 1 diabetes

No studies reported type 1 diabetes.

Type 2 diabetes

No studies reported type 2 diabetes.

Impaired glucose tolerance

No studies reported impaired glucose tolerance.

Dyslipidaemia or metabolic syndrome

No studies reported dyslipidaemia or metabolic syndrome.

Employment, education and social status/achievement

No studies reported employment, education and social status/achievement.

Health service use

Number of hospital or health professional visits (including midwife, obstetrician, physician, dietician and diabetic nurse)

No studies reported health service use outcomes.

Number of antenatal visits or admissions

No studies reported number of antenatal visits or admissions.

Length of antenatal stay

No studies reported length of antenatal stay.

Neonatal intensive care unit admission

Five studies reported neonatal intensive care unit admission (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017). All studies reported neonatal intensive care unit admission alone except for Callaway 2019, which reported special care unit admission. Probiotics may make little to no difference in neonatal intensive care unit admissions compared to placebo (RR 0.97, 95% CI 0.75 to 1.26; 1354 infants; I^2 = 0%; Analysis 1.54).

Length of postnatal stay (mother)

No studies reported length of postnatal stay (mother).

Length of postnatal stay (baby)

No studies reported length of postnatal stay (baby).

Costs to families associated with the management provided

No studies reported costs to families associated with the management provided.

Costs associated with the intervention

No studies reported costs associated with the intervention.

Cost of maternal care

No studies reported cost of maternal care.

Cost of offspring care

No studies reported cost of offspring care.

Probiotics versus diet

We found no trials comparing probiotics versus diet.

DISCUSSION

Summary of main results

The aim of this review was to determine the effect of probiotic supplementation during pregnancy on the risk of developing gestational diabetes. Seven trials met our inclusion criteria, and all included studies compared probiotics with placebo.

Six included studies in this review reported the incidence of GDM in 1440 participants. It is uncertain if probiotics have any effect on the risk of GDM compared to placebo because there was substantial heterogeneity between studies and wide CIs that included both appreciable benefit and harm (low-certainty evidence). Two of these studies reported a reduction in the risk of GDM with probiotics, while the other four studies reported no difference. This heterogeneity was explored through subgroup analysis, and identified no clear causes for the heterogeneity.

Among the other primary outcomes for this review, we found probiotics increase the risk of pre-eclampsia (high-certainty evidence) and may increase the risk of hypertensive disorders of pregnancy, although the CIs for hypertensive disorders of pregnancy included the possibility of no effect.

There were few differences between groups for this review's other main outcomes. Probiotics make little to no difference in the risk of caesarean section (high-certainty evidence), and probably make little to no difference in maternal weight gain during pregnancy (moderate-certainty evidence). Probiotics probably make little to no difference in the incidence of large-for-gestational age infants (moderate-certainty evidence) and may make little to no difference in neonatal adiposity (low-certainty evidence, data from two studies not pooled). We do not know

the effect of probiotics on perinatal mortality (low-certainty evidence), a composite measure of neonatal morbidity (low-certainty evidence) or neonatal hypoglycaemia (low-certainty evidence) because of serious imprecision in the effect estimates and CIs, which are consistent with possible benefit and possible harm. No included studies reported on perineal trauma, postnatal depression, maternal and infant development of diabetes, and neurosensory disability.

There were few differences between groups for most of the secondary outcomes reported in this review. Among the markers for glucose tolerance, there was no difference for fasting plasma glucose and OGTT results. While there was a reduction in insulin levels with probiotics, this finding was of minimal importance given there were no differences in glucose levels or the diagnosis of GDM. The only other notable finding was that probiotics may reduce the risk of SGA infants compared to placebo. However, SGA was not one of our selected outcomes for inclusion in the 'Summary of findings' tables, therefore, we have not assessed the certainty of the evidence contributing to this outcome. The SGA data were based on relatively few events and it is not certain if the effect estimate is due to chance or a real difference between the intervention and control groups.

Overall completeness and applicability of evidence

This review included seven studies with 1647 participants. Given the incidence of GDM in the study population, interpretation of the results was limited by wide CIs and substantial heterogeneity between studies. We identified eight ongoing or unpublished studies as part of our search, which will add to the body of evidence when published and will hopefully help to overcome some of the present limitations.

The studies that reported the incidence of GDM were conducted in women at high risk of developing GDM due to being overweight or obese, or women with a history of atopic disease of any body mass index. Although this was not formally explored through subgroup analysis, the difference in study populations may explain some of the heterogeneity observed between studies. In particular, the authors would like to note that the two studies conducted in women of any weight with a history of atopic disease were the only two studies to detect a reduction in the risk of GDM with probiotics. Therefore, women with atopic disease may be a population where probiotics are effective. Another possibility is that probiotics are more effective in normal-weight women compared to the higher-risk overweight and obese women. Genetic differences or differences in diet between populations may also be responsible, although both Finland and New Zealand have each had one trial that showed benefit and one trial that showed no effect. More data are required before any conclusions can be made, but we plan to explore the effect of probiotics in these populations through subgroup analyses in updates of this review.

While multiple studies reported all our primary outcomes, many of our secondary outcomes were not reported or were reported by only one study. In particular, data were lacking in most of the long-term outcomes since only one included study had significant long-term follow-up. There were also almost no data on health service use apart from neonatal intensive care unit admission. More studies will need to include long-term follow-up and data on health service use before any conclusions can be drawn about these outcomes.

Quality of the evidence

Overall, risk of bias among the included studies was low across all domains, apart from one study (Jamilian 2016), which had an unclear risk of bias in relation to allocation concealment. While some studies lacked blinding for secondary interventions, all studies were double blind for the probiotics versus placebo comparison.

We assessed certainty of evidence using GRADE methodology, and this assessment is given in Summary of findings 1 and Summary of findings 2. Overall, the certainty of evidence ranged from low to high, with downgrading due to concerns around inconsistency and imprecision. Certainty varied widely across the assessed outcomes, with evidence of high certainty for caesarean section, pre-eclampsia; moderate certainty for weight gain during pregnancy, large-for-gestational age and composite neonatal morbidity; and low certainty for GDM, perinatal mortality, neonatal hypoglycaemia and adiposity.

Potential biases in the review process

There are possible sources of bias with any review, and we minimised these sources of bias. We performed the search for studies in this area using the Cochrane Pregnancy and Childbirth Group's Trials Register, which is updated weekly to monthly with information from CENTRAL, MEDLINE, Embase, handsearches from 30 journals and conference proceeding of major conferences, and alerts for a further 44 journals to minimise the risk of missing eligible studies. Two review authors independently assessed studies for inclusion and risk of bias, and resolved any discrepancies through discussion or with the entire author team if necessary.

This review was also uniquely susceptible to bias given three of the review authors (MDN, LC and HB) were also authors on a study that was identified for possible inclusion. For this Cochrane Review, the two review authors who were not involved with the study (SJD and SAP) independently assessed the relevant trial reports for inclusion, determined risk of bias and collected all data to minimise any conflict of interest.

Agreements and disagreements with other studies or reviews

Many reviews have evaluated the impact of probiotics on GDM, either as part of a specific review or a general review on prevention strategies for GDM or the use of probiotics during pregnancy. Since many of the studies included in this review were only published in the past few years, many older reviews on the topic include only one or two studies (Agha-Jaffar 2016; Barrett 2012; Facchinetti 2014; Gomez Arango 2015; Griffin 2015; Jarde 2018; Lindsay 2013; Plows 2019; Rogozinska 2015; Simmons 2015; van de Vusse 2013). Depending on inclusion criteria and publication date, some combinations of Laitinen 2009, Lindsay 2014, and Wickens 2017, and one study we excluded (Asemi 2013) were included in each review. Overall, these reviews concluded that probiotics may reduce the risk of GDM, but agreed that more data are needed to make firm conclusions.

Two recent systematic reviews have been published that include the newer trials. Masulli 2020 published a systematic review and meta-analysis including 17 trials evaluating the use of probiotics during pregnancy on various metabolic outcomes in both women

with and without GDM. For the diagnosis of GDM outcome, this review included seven studies: the six included in this review (Callaway 2019; Laitinen 2009; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019; Wickens 2017) and one awaiting classification (Asgharian 2020). In agreement with our review, Masulli 2020 reported no benefit with probiotics on the incidence of GDM, although they also demonstrated substantial heterogeneity between studies (RR 0.77, 95% CI 0.51 to 1.16; I^2 = 62%).

The other recently published systematic review on the topic was a network meta-analysis evaluating a variety of interventions to prevent GDM specifically in overweight and obese pregnant women (Chatzakis 2019). In total, the review included 23 studies, five of which evaluated the use of probiotics. Four of these studies were included in our review (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019), while the other study is awaiting classification (Asgharian 2020). Overall, Chatzakis 2019 found no interventions to be superior to placebo. Their direct comparison of probiotics and placebo revealed no clear differences between groups in agreement with our review (RR 1.02, 95% CI 0.78 to 1.32). However, there was no substantial heterogeneity between the studies as seen in our review, which is likely reflective of the fact that Chatzakis 2019 limited their review to overweight and obese women. The effect of body mass index was not evaluated through subgroup analysis in our review, but further supports our observation that probiotics may not be effective for preventing GDM in overweight and obese women.

AUTHORS' CONCLUSIONS

Implications for practice

Probiotics may increase, decrease or make little to no difference in the risk of gestational diabetes mellitus (GDM), although the current evidence is of low certainty due to concerns regarding imprecision and inconsistency. While analysis revealed a small reduction in insulin levels with probiotics, this is unlikely to be clinically meaningful. Given the substantial heterogeneity observed between studies in the risk of GDM, there may be certain populations in which probiotics are effective, but there is currently insufficient evidence to identify these populations.

High-certainty evidence suggests that probiotics probably increase the risk of pre-eclampsia and could increase hypertensive disorders of pregnancy but the 95% confidence intervals for hypertensive disorders of pregnancy includes the possibility of no effect. While further research is needed to explore the underlying potential physiology of this relationship, given the potential risk of harm and little observed benefit, we urge caution in using probiotics during pregnancy at this time.

Implications for research

This review identified high-certainty evidence that probiotics increase the risk of pre-eclampsia, so great care needs to be taken in any future study of probiotics in pregnancy. Safety needs to be carefully monitored, and women in these studies need to be made aware of this outcome when informed consent is obtained. In the eight studies that are currently ongoing, particular care should be taken in participant follow-up and analysis of the effect of probiotics on pre-eclampsia. Further research is needed to elucidate the underlying potential physiology of the relationship between probiotics and pre-eclampsia. The effect of probiotics on risk of small-for-gestational age infants should be explored further.

More data are required to fully determine the effect of probiotics on the risk for GDM. While future studies should be conducted with caution, the eight ongoing studies will hopefully explore sources of the substantial heterogeneity in the current data. In the studies where women were recruited for a personal or family history of atopic disease (Laitinen 2009; Wickens 2017), there appears to be a differential impact on GDM diagnosis compared to studies where women were recruited specifically for being at high risk for GDM (Callaway 2019; Lindsay 2014; Okesene-Gafa 2019; Pellonpera 2019). If probiotics are deemed to be safe to use in pregnancy, this difference should be explored further.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Callaway 2019

Study characteristics	
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: Royal Brisbane & Women's Hospital, Redcliffe Hospital, and Mater Mothers Hospital in Brisbane, Australia
Participants	Inclusion criteria: < 16 weeks' gestation (changed to < 20 weeks' gestation during the study), singleton pregnancy, BMI > 25 kg/m^2, > 18 years of age, able to read and understand English, and ability to provide informed consent Exclusion criteria: > 16 weeks' gestation (changed to > 20 weeks' gestation during the study), multiple pregnancy, known pre-existing diabetes, impaired fasting glucose or impaired glucose tolerance, GDM prior to recruitment, taking medications likely to influence glucose metabolism, medical conditions associated with altered glucose metabolism, known major fetal abnormality noted on 12-week ultrasound examination and known ingestion of probiotics

Callaway 2019 *(Continued)*

Interventions	Probiotic (n = 219): capsule containing *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12 at 10^9 CFU taken daily from enrolment until birth Placebo (n = 214): capsule containing microcrystalline cellulose and dextrose anhydrate taken daily from enrolment until birth
Outcomes	Primary: diagnosis of GDM Secondary: gestational weight gain, pre-eclampsia, induction of labour, caesarean delivery, change in prevalence of *L rhamnosus* and *B lactis* in gut microbiome, change in lipids and inflammatory profile, change in dietary indices and physical activity levels between baseline and 28 weeks; gestation, neonatal body composition, preterm delivery, shoulder dystocia, hypoglycaemia, neonatal treatment with supplementary fluids/feeds, nerve palsy, admission to NICU, jaundice requiring phototherapy, bone fracture, perinatal death, visit attendance, adherence to probiotic/placebo regimen, birthweight and congenital anomaly
Notes	Sources of funding: National Health and Medical Research Council grant APP1028575, Royal Brisbane and Women's Hospital Foundation Study dates: commenced November 2012 Declarations of interest: none declared

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation conducted using computer-generated random number codes stratified by centre and BMI category.
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes used for randomisation, and all medical staff, research assistants, nursing staff and participants were blinded to the randomised allocation.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All medical staff, research assistants, nursing staff and participants were blinded to the randomised allocation. Placebo and probiotic supplements were identically packaged.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All study staff and participants were blinded to the randomised allocation.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Loss to follow-up was minimal and similar in both groups (10/214 participants in the placebo group, 12/219 participants in the probiotic group).
Selective reporting (reporting bias)	Low risk	Prespecified outcomes reported.
Other bias	Low risk	No other sources of bias identified.

Jamilian 2016

Study characteristics

Methods	Study design: parallel randomised controlled trial

Jamilian 2016 *(Continued)*

	Blinding: double blind Location: Arak University of Medical Sciences, Arak, Iran
Participants	Inclusion criteria: first half of pregnancy (≤ 20 weeks' gestation), aged 18–37 years Exclusion criteria: recognised cause of recurrent miscarriages or a structural uterine abnormality distorting the cavity, history of rheumatoid arthritis, thyroid, parathyroid, or adrenal diseases, hepatic or renal failure
Interventions	Probiotic (n = 30): capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* at 2×10^9 taken once per day for 12 weeks Placebo (n = 30): capsule containing starch taken once per day for 12 weeks
Outcomes	Primary: insulin levels after intervention Secondary: fasting blood sugar, glutathione, HDL-cholesterol, hs-CRP, LDL, malondialdehyde, nitric oxide, total antioxidant capacity, total cholesterol, triglycerides, and VLDL after the intervention period.
Notes	Sources of funding: grant from Vice-Chancellor for Research, AUMS, Iran Study dates: March 2015 to July 2015 Declarations of interest: none declared This study was initially deemed high risk for scientific integrity/trustworthiness given no participants were lost to follow-up over the course of the study and the numbers were equal in both groups with no mention of what type of randomisation method was used. Clarification from the study authors was sought on 11 May 2020 and 28 May 2020. The authors confirmed that all participants completed the study given their methods for ensuring patient follow-up and high levels of adherence to specialist care in the study population. The authors also confirmed that randomisation was conducted using computer-generated random numbers to select equal groups of 30 participants. We deemed the authors responses sufficient to change the study classification to low risk.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation performed using computer-generated numbers.
Allocation concealment (selection bias)	Unclear risk	While the authors stated allocation was concealed, no details were provided as to how allocation was concealed.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The group assignments were concealed from the researchers and participants until the final analyses were completed.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Group assignments were not revealed to the researchers until after the final analyses were completed.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No participants were lost to follow-up during the study.
Selective reporting (reporting bias)	Low risk	All outcomes listed in the trial registry were reported.

Jamilian 2016 *(Continued)*

Other bias	Low risk	No other sources of bias identified.

Laitinen 2009

Study characteristics	
Methods	Study design: randomised controlled trial Blinding: double blind for probiotics/placebo, single blind for dietary intervention Location: Turku University Hospital, Turku, Finland
Participants	Inclusion criteria: < 17 weeks' gestation Exclusion criteria: metabolic or chronic diseases such as diabetes apart from atopic eczema, allergic rhinitis or asthma
Interventions	Probiotic + dietary intervention (n = 85): capsule containing *Lactobacillus rhamnosus* GG, ATCC 53 103 and *Bifidobacterium lactis* BB12 at dose of 10^{10} CFU taken daily from early pregnancy until the end of exclusive breastfeeding + intensive dietary counselling aiming to conform to currently recommended pregnancy diet Placebo + dietary intervention (n = 86): capsule containing microcrystalline cellulose and dextrose anhydrate taken daily from early pregnancy until the end of exclusive breastfeeding + intensive dietary counselling aiming to conform to currently recommended pregnancy diet Placebo + routine diet (n = 85): capsule containing microcrystalline cellulose and dextrose anhydrate taken daily from early pregnancy until the end of exclusive breastfeeding
Outcomes	Primary: maternal glucose metabolism as measured by plasma glucose, blood HbA1c, serum insulin and HOMA and QUICKI indices at baseline, third trimester of pregnancy, 1, 6 and 12 months postpartum.
Notes	Sources of funding: Academy of Finland, Sigrid-Juselius Foundation, Juho Vainio Foundation, Social Insurance Institution of Finland, Raisio, Chr. Hansen, Valio Ltd. Study dates: April 2002 to November 2005 Declarations of interest: none declared

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated block randomisation of 6 women. The use of only 1 block size could make it possible to guess the randomisation of the dietary intervention of the last participant of each block. However, since the probiotic/placebo randomisation was double blind and we only included the 2 groups who received the dietary intervention, the selection bias risk for probiotics vs placebo is still considered low.
Allocation concealment (selection bias)	Low risk	Randomisation list generated by a non-investigator statistician, sealed envelopes.
Blinding of participants and personnel (performance bias)	Low risk	Placebo/probiotic allocation was blind to both participants and personnel, dietary therapy was not blinded to personnel.

Laitinen 2009 *(Continued)*

All outcomes		
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All personnel who handled or analysed blood samples were blind to the intervention.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Minimal loss to follow-up by assessment of glucose tolerance. Total loss to follow-up was 18.75% by 1-year postpartum.
Selective reporting (reporting bias)	Low risk	Reported all outcomes they intended to report.
Other bias	Low risk	No other biases detected.

Lindsay 2014

Study characteristics	
Methods	Study design: parallel randomised controlled trial Blinding: double blind for probiotics/placebo Location: National Maternity Hospital, Dublin, Ireland
Participants	Inclusion criteria: < 20 weeks' gestation, BMI 30–39.9 kg/m^2 at first pregnancy visit, singleton pregnancy, and aged > 18 years Exclusion criteria: history of gestational or non-gestational diabetes (type 1 or 2), presence of fetal anomaly, multiple pregnancy, and inability to give full informed consent
Interventions	Probiotic (n = 83): capsule containing 10^9 CFU of *Lactobacillus salivarius* UCC118 taken once daily from 24 to 28 weeks' gestation Placebo (n = 92): placebo capsule taken once daily from 24 to 28 weeks' gestation
Outcomes	Primary: change in maternal fasting glucose Secondary: incidence of gestational diabetes and impaired glucose tolerance, neonatal anthropometric measures, metabolic variables, gestational weight gain, pre-eclampsia, delivery complications, cord blood metabolic variables, fetal growth at 34 weeks' gestation, 5-minute Apgar score and NICU admission.
Notes	Sources of funding: National Maternity Hospital Medical Fund with support from an Ivo Drury Award, Alimentary Health Ltd Study dates: March 2012 to March 2013 Declarations of interest: none declared

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation conducted by an independent researcher using a computer-generated, simple randomisation process in a 1:1 ratio.

Lindsay 2014 *(Continued)*

Allocation concealment (selection bias)	Low risk	Randomisation sequence was concealed in sequentially numbered, sealed, opaque envelopes that were not opened until after enrolment.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All capsules were identical in appearance, and all participants and researchers were blinded.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All researchers were blind to the intervention.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Of the participants who ever received capsules, only 1 participant from each group stopped taking the capsules (1/64 in the probiotic group, 1/76 in the placebo group). No participants were lost to follow-up, and intention-to-treat analyses were performed.
Selective reporting (reporting bias)	Low risk	Results for all prespecified outcomes were reported.
Other bias	Low risk	No other bias identified.

Okesene-Gafa 2019

Study characteristics	
Methods	Study design: parallel 2×2 factorial randomised controlled trial Blinding: double blind for probiotic intervention, no blinding for dietary intervention Location: Counties Manukau Health region, South Auckland, New Zealand
Participants	Inclusion criteria: singleton pregnancy, BMI ≥ 30 kg/m^2, 12–17.6 weeks' gestation and able to provide informed consent Exclusion criteria: pre-existing diabetes or HbA1c ≥ 50 mmol/mol at booking in, taking probiotic supplements, known congenital abnormality, medications or medical conditions that alter glucose metabolism, multiple pregnancy, bariatric surgery and severe hyperemesis
Interventions	First randomisation: dietary intervention Dietary intervention (n = 116): multifaceted intervention including encounters with nutrition advisor, behaviour change techniques, physical activity advice and motivational texting Routine diet (n = 114): routine dietary advice including a pamphlet about diet, healthy weight gain and physical activity in pregnancy Second randomisation: probiotic intervention Probiotic (n = 115): capsule containing *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12 at a dose of 7 × 10^9 CFU taken daily from enrolment to delivery Placebo (n = 115): capsule containing microcrystalline cellulose and dextrose anhydrate taken daily from enrolment to delivery
Outcomes	Primary: proportion of women with excessive gestational weight gain and infant birthweight

Okesene-Gafa 2019 *(Continued)*

	Secondary: maternal pregnancy glucose metabolism, changes in diet quality and dietary patterns, functional health and well-being, depression and anxiety scores, maternal adiposity postpartum, gestational diabetes, pregnancy-induced hypertension, mode of birth, blood lipid concentrations, maternal feedback about study participation, neonatal anthropometry, gestational age at birth, LGA, small-for-gestational age, NICU admission, neonatal composite morbidity, breastfeeding, infant anthropometry, infant feeding, infant nutritional intake, attendance at study visits, adherence to probiotic/placebo regimen and cost effectiveness of the intervention
Notes	Sources of funding: Counties Manukau Health, Cure Kids Grant 2556, Lottery Health Research 353084, RANZCOG Two Mercia Barnes Trust, Gravida National Centre for Growth and Development, University of Auckland Faculty Development Research Fund and Reinvestment Fund, Nurture Foundation, Heart Foundation of New Zealand, Roche Diagnostics International Ltd, Chr. Hansen A/S Study dates: April 2015 to June 2017 Declarations of interest: none declared

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation conducted using a web-based protocol (randomize.net) using random block sizes, stratified by clinical site and BMI category.
Allocation concealment (selection bias)	Low risk	The research midwife responsible for enrolment did not have access to the probiotic/placebo allocation.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The capsules were identically packaged and were labelled by a third party using a pre-allocated random list. Only the project manager had access to the probiotic/placebo allocation, and all participants and other staff were blinded to the intervention.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	The project manager was the only study staff member with access to the probiotic/placebo allocation, and the protocol stated that the primary outcomes were not subject to bias.
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were minimal losses to follow-up, and losses were similar between groups (7/115 participants in the probiotics group, 6/115 participants in the placebo group).
Selective reporting (reporting bias)	Low risk	Prespecified outcomes were reported.
Other bias	Low risk	No other sources of bias identified.

Pellonpera 2019

Study characteristics

Methods	Study design: parallel 4-arm randomised controlled trial of 2 interventions Blinding: double blind for both interventions Location: Turku University Hospital/University of Turku, Finland
Participants	Inclusion criteria: self-reported prepregnancy BMI ≥ 25 kg/m^2, < 18 weeks' gestation, and absence of chronic disease (except for asthma and allergies)

Pellonpera 2019 *(Continued)*

	Exclusion criteria: diabetes before pregnancy (including HbA1c ≥ 6.5% or fasting glucose ≥ 7.0 mmol/L at randomisation), multifetal pregnancy, chronic diseases impacting metabolic or gastrointestinal health, refusal to terminate the intake of other probiotic or fish oil supplements, diagnosis or history of coagulopathy, and use of anticoagulants
Interventions	Intervention 1: probiotics Probiotic: capsules containing *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* ssp *lactis* 420 at dose of 10^{10} CFU taken daily from enrolment until 6 months postpartum Placebo: capsules containing microcrystalline cellulose taken once daily from enrolment until 6 months postpartum Intervention 2: fish oil Fish oil: capsules containing 2.4 g n-3 fatty acids (79% docosahexaenoic acid, 9.4% eicosapentaenoic acid) taken daily from enrolment until 6 months postpartum Placebo: capsules containing 2.4 g medium-chain fatty acids (54.6% capric acid C8, 40.3% caprylic acid C10) taken daily from enrolment until 6 months postpartum Study arms: Arm 1: probiotics + fish oil (n = 109) Arm 2: probiotics + placebo (n = 110) Arm 3: placebo + fish oil (n = 109) Arm 4: placebo + placebo (n = 110)
Outcomes	Primary: prevalence of GDM and fasting glucose levels Secondary: change in insulin and HOMA values, need for medication in GDM management, gestational hypertensive disorders, mode of delivery, postpartum haemorrhage, birthweight and macrosomia
Notes	Sources of funding: Academy of Finland, state research funding for university-level health research of the Turku University Hospital Expert Responsibility Area, the Diabetes Research Foundation, the Juho Vainio Foundation, Business Finland, the Finish Medical Foundation, the University of Turku, DuPont, and Croda Europe Ltd. Study dates: October 2013 to July 2017 Declarations of interest: none reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation performed by a statistician not involved in the study in permutated blocks of 4 and stratified by parity and history of GDM.
Allocation concealment (selection bias)	Low risk	Participants were assigned from the randomisation list in order of recruitment, and the staff responsible for enrolment were blinded to the intervention.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All staff responsible for enrolment, study visits and outcome assessment and all participants were blinded to the intervention.
Blinding of outcome assessment (detection bias)	Low risk	All staff responsible for outcome assessment were blinded to the intervention.

Pellonpera 2019 *(Continued)*

All outcomes		
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were minimal losses to follow-up, and losses were similar between groups (18/109 participants in the probiotics + fish oil group, 11/110 participants in the probiotics + placebo group, 13/109 participants in the placebo + fish oil group, and 19/110 participants in the placebo + placebo group for the primary outcome of GDM).
Selective reporting (reporting bias)	Low risk	All prespecified outcomes were reported.
Other bias	Low risk	No other sources of bias identified.

Wickens 2017

Study characteristics	
Methods	Study design: randomised controlled trial Blinding: double blind for probiotics/placebo Location: Wellington and Auckland, New Zealand
Participants	Inclusion criteria: 14–16 weeks' gestation, English speaking, intend to breastfeed, and personal history or child's biological father's history of asthma, eczema or allergic rhinitis treated by a doctor or pharmacist Exclusion criteria: aged < 16 years, did not intend to stay in either of the study centres for the 18 months following enrolment, serious immunological disorder that suppresses immune function or taking immunosuppressant drugs, known cardiac valve disease for which antibiotic prophylaxis was required when undergoing dental procedures, history of transplant or HIV, long-term continuous antibiotic therapy, IVF pregnancy, pre-enrolment scan showing major fetal abnormality, using or intend to use probiotic drinks or supplements themselves or in their child, participation in another RCT, severe allergy to cow's milk, previously participated in the study with an older child, deemed unsuitable for participation due to medical reason, or pre-existing type 1 or 2 diabetes (only for OGTT and GDM outcomes)
Interventions	Probiotic (n = 212): capsules containing *Lactobacillus rhamnosus* HN001 at 6×10^9 CFU taken once daily from 16 weeks' gestation until 6 months after birth or until no longer breastfeeding Placebo (n = 211): capsules containing corn-derived maltodextrin taken once daily until 6 months after birth or no longer breastfeeding
Outcomes	Primary: infant eczema and atopic sensitisation at age 12 months Secondary: GDM (OGTT 75 g using ADIPS criteria), bacterial vaginosis, group B strep colonisation, and maternal postpartum depression and anxiety.
Notes	Sources of funding: Health Research Council of New Zealand, Fonterra Study dates: December 2012 to November 2014 Declarations of interest: none reported

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Wickens 2017 *(Continued)*

Random sequence generation (selection bias)	Low risk	Randomisation stratified by study centre and performed in blocks of random-lengths according to a computer-generated random list.
Allocation concealment (selection bias)	Low risk	Research staff assigned women to consecutive study numbers, and the randomisation list was managed by an external group (Fonterra Co-operative Group Ltd) who concealed the list from all study staff and participants.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The randomisation sequence was concealed from all study staff and participants. Placebo capsules had the same look and smell as the probiotic capsules, and both were provided in opaque bottles by an external company (Alaron Products Ltd).
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Randomisation sequence concealed from all study staff.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Loss to follow-up minimal and similar between groups (5/212 participants in the probiotic group and 4/211 participants in the placebo group at gestational diabetes testing).
Selective reporting (reporting bias)	Low risk	All predetermined gestational diabetes outcomes were reported. Subgroup analysis was conducted that was not prespecified, but this was clearly stated.
Other bias	Low risk	No other sources of bias identified.

ADIPS: Australasian Diabetes in Pregnancy Society; BMI: body mass index; CFU: colony-forming units; GDM: gestational diabetes mellitus; HbA1c: haemoglobin A1c; HDL: high-density lipoprotein; HOMA: Homeostatic Model Assessment; hs-CRP: high-sensitivity C-reactive protein; IUFD: intrauterine fetal demise; IVF: in vitro fertilisation; LDL: low-density lipoprotein; LGA: large-for-gestational age; n: number of participants; NICU: neonatal intensive care unit; OGTT: oral glucose tolerance test; QUICKI: quantitative insulin sensitivity check index; RCT: randomised controlled trial; VLDL: very low-density lipoprotein.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Asemi 2013	Probiotics were started in the third trimester, after gestational diabetes would have been diagnosed. In addition, diagnosis of gestational diabetes was not a study outcome.
Taghizadeh 2014	Women did not start the intervention until 27 weeks' gestation, which was too late to prevent gestational diabetes. In addition, diagnosis of gestational diabetes was not a study outcome.

Characteristics of studies awaiting classification *[ordered by study ID]*

Asgharian 2020

Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: 5 public health centres northwest of Tabriz, Iran
Participants	Inclusion criteria: 20–22 weeks' gestation, pre- or early-pregnancy BMI ≥ 25 kg/m^2, aged ≥ 18 years, fasting plasma glucose < 92 mg/dL

Asgharian 2020 *(Continued)*

	Exclusion criteria: multiple pregnancy, history of GDM, taking any medication likely to influence glucose metabolism (metformin, corticosteroids, immunosuppressants, etc.), medical conditions associated with altered glucose metabolism (Cushing's syndrome, hepatic cirrhosis), regular consumption of probiotics for any reason, smoking, regular use of alcohol or illegal drugs, any antibiotic intake during current pregnancy, illiteracy/low literacy, established major fetal anomaly
Interventions	Probiotic (n = 65): yoghurt containing 5×10^8 CFU *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* BB12 with starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies b*ulgaricus* at 10^7 CFU/g) with 100 g taken daily from 24 weeks' gestation until delivery Placebo (n = 65): yoghurt containing only starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies b*ulgaricus* at 10^7 CFU/g) with 100 g taken daily from 24 weeks' gestation until delivery
Outcomes	Primary: fasting plasma glucose and 1-and 2-hour plasma glucose after 75 g OGTT. Secondary: GDM, weight gain over pregnancy, pre-eclampsia, preterm delivery, delivery mode, satisfaction with the yoghurts, total serum bilirubin (3–5 days after birth), infant weight, infant length, infant head circumference, macrosomia, LGA, neonatal jaundice, jaundice treatments and neonatal death (within 30 days after birth).
Notes	Sources of funding: Research Vice-Chancellor of Tabriz University of Medical Sciences Study dates: April 2016 to September 2017 Declarations of interest: none declared Only 1 participant in each group did not complete the study due to intrauterine fetal death. Since there were almost 0 losses to follow-up, this study was considered high risk according to our scientific integrity/trustworthiness criteria. We contacted study authors for clarification on 11 May 2020, and the study authors provided information about how participants were followed up, but did not confirm that all other participants completed the study. We determined this was insufficient information to make a final risk assessment. Further information was requested on 20 May 2020 and 11 June 2020, but we received no response.

Charles 2018

Methods	Study design: parallel, randomised controlled trial Blinding: double blind Location: Barts Health NHS Trust and Homerton University Hospital, London, UK
Participants	Inclusion criteria: aged ≥ 16 years, 9–14 weeks' gestation Exclusion criteria: lack of informed consent
Interventions	Probiotic (n unknown): capsule containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-1 at 2.5×10^9 CFU and excipients given daily from early pregnancy until delivery Placebo (n unknown): capsule containing excipients only given daily from early pregnancy until delivery
Outcomes	Primary: vaginal flora during pregnancy, recruitment of eligible women, and intervention adherence Secondary: reasons for participation and adherence, core outcomes from studies on preterm birth prevention

Charles 2018 *(Continued)*

Notes	Sources of funding: Queen Mary University of London, UK Study dates: May 2016 to June 2017 Declarations of interest: not reported Study was only published in abstract form at the time of publication. We attempted to contact the study authors on 11 May 2020 and 28 May 2020 to confirm if the presented data came from the final analysis, but we did not receive a response.

Si 2019

Methods	Study design: parallel, randomised controlled trial Blinding: double blind Location: The Second Hospital of Jilin University, Changchun, Jilin, China
Participants	Inclusion criteria: first antenatal visit before 12 weeks' gestation, singleton pregnancy, meeting diagnostic criteria for GDM, and fasting blood glucose > 5.1 mmol/L, 2-hour OGTT result > 8.5 mmol/L, or both Exclusion criteria: hypertension, kidney disease, cardiovascular disease, medications that may interfere with sugar or lipid metabolism, taking antioxidants, placenta previa, threatened abortion, artificial infertility and history of previous adverse pregnancy
Interventions	Probiotic (n = 113): 5 g black garlic fermented by *Lactobacillus bulgaricus* daily for 40 weeks Placebo (n = 113): 5 g black garlic without fermentation daily for 40 weeks
Outcomes	Primary: proportion of participants who were screened for GDM with an OGTT within 4 weeks after the study start date Secondary: gestational age at delivery, weight gain during pregnancy, presence of induced labour, caesarean section, pre-eclampsia, stillbirth, neonatal death, low-birthweight infants, macrosomia, preterm infants, respiratory distress syndrome, hyperbilirubinaemia and neonatal intensive care unit admission
Notes	Sources of funding: Projects of Science and Technology Development Plan of Jilin Province (grant number 20160101054JC) Study dates: September 2015 to June 2016 Declarations of interest: none declared It was unclear whether this study met our inclusion criteria as we were unable to determine whether participants were diagnosed with gestational diabetes before or after enrolment in the study. We sought clarification from the study authors on 22 April 2020 and 6 May 2020, but received no response.

BMI: body mass index; CFU: colony-forming unit; GDM: gestational diabetes mellitus; LGA: large-for-gestational age; n: number of participants; OGTT: oral glucose tolerance test.

Characteristics of ongoing studies *[ordered by study ID]*

ACTRN12620000359932

Study name	Probiotics in the prevention of gestational diabetes mellitus in women at increased risk: a prospective randomised controlled trial
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: North West Regional Hospital, Burnie, Tasmania, Australia
Participants	Inclusion criteria: aged 18–40 years, > 10 weeks' gestation, singleton pregnancy, increased risk of gestational diabetes as per the Australasian Diabetes in Pregnancy Society criteria (previous GDM, family history of diabetes (first-degree relative with diabetes or a sister with GDM), BMI > 35 kg/m^2, previous macrosomia (baby birthweight > 4500 g or > 90th centile), or polycystic ovarian syndrome) Exclusion criteria: unable to read and understand English, unable to provide informed consent, aged < 18 years, pregnancy > 16 weeks' gestation at recruitment, known pre-existing diabetes, impaired fasting glucose or impaired glucose tolerance, GDM prior to recruitment as diagnosed by early pregnancy glucose testing, medications likely to influence glucose metabolism (e.g. metformin, glucocorticoids, immunosuppressants), medical conditions with altered glucose metabolism (e.g. Cushing's syndrome, hepatic cirrhosis), major fetal anomaly on ultrasound, current ingestion of probiotics via capsules or sachets and antibiotic use during the study period
Interventions	Probiotic: capsule taken once daily from enrolment until results are received from their glucose tolerance test (26–28 weeks' gestation) containing a combination of strains *Lactobacillus rhamnosus* and *Bifidobacterium animalis* ssp *lactis* with or without *Bifidobacterium breve* and *Bifidobacterium longum* Placebo: capsule taken once daily from enrolment until the results are received from their glucose tolerance test (26–28 weeks' gestation) containing maize-derived maltodextrin
Outcomes	Primary: incidence of gestational diabetes Secondary: change in weight from booking-in appointment (approximately 12 weeks' gestation) until routine antenatal care appointment at 26–28 weeks' gestation
Starting date	1 April 2020
Contact information	Anushika Samarage, North West Regional Hospital, anushika.samarage@ths.tas.gov.au
Notes	Sources of funding: North West Regional Hospital Declarations of interest: not reported

Godfrey 2017

Study name	Nutritional intervention preconception and during pregnancy to maintain healthy glucose metabolism and offspring health (NiPPeR)
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: research and hospital facilities in Auckland (University of Auckland, Auckland, Waitemata and Counties Manukau District Health Boards and Clinics, New Zealand), Singapore (National University Hospital and National University Health System Investigational Medicine Unit), and Southampton (National Institute for Health Research Wellcome Trust Southampton Clinical Research Facility and Princess Anne Hospital, University Hospital Southampton, UK)

Godfrey 2017 *(Continued)*

Participants	Inclusion criteria: aged 18–38 years; living in Southampton, Singapore or Auckland; planning to have maternity care in Southampton and Auckland if in Southampton or Auckland; willing to deliver at the National University Hospital if in Singapore; planning to conceive within 6 months (but conception up to 12 months after phenotyping will be included); Chinese, Malay, Indian or mixed Chinese/Malay/Indian ethnicity if in Singapore and able to provide written informed consent Exclusion criteria: pregnant or lactating at recruitment; assisted fertility apart from clomifene or letrozole alone; pre-existing type 1 or 2 diabetes; oral or implanted contraception currently or in the last month or with an intrauterine contraceptive device in situ; metformin or systemic steroids currently or in the last month; anticonvulsant medication currently or in the last month; treatment for HIV, hepatitis B or hepatitis C currently or in the last month; and known serious food allergy
Interventions	Probiotic: nutritional drink containing myo-inositol, vitamin D, riboflavin, vitamin B_6, vitamin B_{12} and zinc with standard folic acid, iodine, calcium, beta-carotene and iron with probiotics containing *Lactobacillus rhamnosus* NCC 4007 and *Bifidobacterium animalis species lactis* NCC 2818 taken twice daily starting before conception Placebo: control drink containing standard amounts of folic acid, beta-carotene, iron, calcium and iodine taken twice daily starting before conception
Outcomes	Primary: maternal glucose metabolism at 28 weeks' gestation Secondary: maintenance of a healthy pregnancy, reduction in maternal micronutrient insufficiency, alteration in gut microbiota, alteration in maternal metabolomic and epigenetic biomarkers, enhancement of breast milk micronutrient content, altered immunological factors, epigenetic and metabolomic profiles, and maintenance of healthy lactogenesis, neonatal adiposity, birthweight and size for gestational age, reduced adiposity gain during infancy, reduction in cord blood C-peptide, promotion of offspring wellbeing and healthy cardiometabolic risk factors, alteration in offspring metabolomic and epigenetic biomarkers and alteration in offspring gut microbiota
Starting date	3 August 2015
Contact information	Keith M Godfrey, NIHR Southampton Biomedical Research Centre and MRC Lifecourse Epidemiology Unit, UK, kmg@mrc.soton.ac.uk
Notes	Sources of funding: UK Medical Research Council (as part of an MRC award to the MRC Lifecourse Epidemiology Unit), the Singapore government (as part of the Growth, Development, and Metabolism Programme of the Singapore Institute for Clinical Sciences), the New Zealand government (as part of the Gravida, Centre of Research Excellence: Growth and Development) and Nestec SA Declarations of interest: KMG has received reimbursement for speaking at conferences sponsored by companies selling nutritional products. KMG, WSC, CYS, SYC, SJB and GCB are part of an academic consortium that has received research funding from Abbott Nutrition, Nestle and Danone. GCB is member of the Scientific Advisory Board and of the Asia-Pacific grant panel of BASF. LE, TMS, ISZ, KM and SKT are employees of Nestec SA working at the Nestle Research Centre.

Halkjaer 2016

Study name	Effect of probiotics (Vivomixx) on weight, microbiota and glucose tolerance in obese pregnant women and their newborn
Methods	Study design: parallel randomised controlled trial Blinding: double bind Location: Copenhagen University Hospital Hvidovre, Copenhagen, Denmark

Halkjaer 2016 *(Continued)*

Participants	Inclusion criteria: aged > 18 years, prepregnancy BMI 30–34.9 kg/m^2, nulliparous, singleton pregnancy, ability to read and speak Danish, and normal ultrasound scan of the fetus at gestational age 12–14 weeks Exclusion criteria: pregnancy > 20 weeks' gestation at recruitment, pregestational diabetes or other serious diseases, multiple pregnancy, previous bariatric surgery, ingestion of probiotics for > 1 month before inclusion or ingestion of probiotics other than the study probiotics and alcohol or drug abuse
Interventions	Probiotic: Vivomixx capsule containing *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *Bifidobacterium infantis* DSM 24737, *Lactobacillus acidophilus* DSM 24735, *Lactobacillus plantarum* DSM 24730, *Lactobacillus paracasei* DSM 24733, and *Lactobacillus delbrueckii subspecies bulgaricus* DSM 24734 taken twice daily from 14–20 weeks' gestation to delivery Placebo: capsule containing microcrystalline cellulose, magnesium stearate and silicon dioxide taken twice daily from 14–20 weeks' gestation until delivery
Outcomes	Primary: gestational weight gain and change in maternal fasting glucose from 14–20 weeks' to 27–30 weeks' gestation Secondary: changes in microbiota and inflammatory markers in mother and child, changes in vaginal microbiological profile and frequency of urinary tract infections, changes in concentrations of lipids and inflammatory markers, incidence of GDM, pre-eclampsia, and gestational hypertension, change in mode of delivery, gestational age at birth, macrosomia, large- and small-for-gestational age, diet, physical activity levels, breastfeeding, birthweight and z-score, Apgar scores, umbilical cord pH, neonatal intensive care unit admission, and child's weight gain and body composition at 9 months
Starting date	March 2015
Contact information	Andreas Munk Petersen, Copenhagen University Hospital Hvidovre and University of Copenhagen, Denmark, andreas.munk.petersen@regionh.dk
Notes	Sources of funding: grants from Jeppe Juhls og hustru Ovita Juhls Mindelegat, Else og Mogens Wedell-Wedellborgs Fond, Aase og Ejnar Danielsens Fond, Knud og Edith Eriksens Mindefond, Toyota-Fonden Denmark, Next Gen Pharma India Pvt. Ltd., and Faculty of Health and Medical Sciences University of Copenhagen Declarations of interest: probiotics, placebo capsules and a 6-month salary for SIH donated by Next Gen Pharma India Pvt. Ltd.

IRCT20161025030502N2

Study name	Effect of oral probiotic lactofem on metabolic parameters in overweight pregnant women referred to prenatal clinics of the Shiraz hospitals in 2016
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: not stated
Participants	Inclusion criteria: aged 18–35 years, willing to participate in research, prepregnancy BMI 25–30 kg/m^2, 20–24 weeks' gestation, no medical comorbidities (including diabetes; hypertension; liver disease; kidney, adrenal or thyroid conditions; hypercholesterolaemia or bleeding), no medication use affecting glucose, fat metabolism, or blood pressure, normal diet and non-smoker

IRCT20161025030502N2 *(Continued)*

	Exclusion criteria: prior use of probiotics, allergies to medication or placebo, the occurrence of acute bleeding and pre-eclampsia
Interventions	Probiotic: capsule taken every 12 hours from 20 to 36 weeks' gestation Placebo: capsule taken every 12 hours from 20 to 36 weeks' gestation
Outcomes	Primary: fasting and 2-hour post breakfast blood glucose levels, plasma lipids and blood pressure
Starting date	21 November 2016
Contact information	Sara Azima, Shiraz University of Medical Sciences, Iran
Notes	Sources of funding: Shiraz University of Medical Sciences Declarations of interest: not reported

IRCT20180509039595N1

Study name	The effect of probiotic capsule on prevention of gestational diabetes in high-risk pre-diabetic pregnant women
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: not stated
Participants	Inclusion criteria: aged 16–49 years, willing to participate, literate, minimum score 2/20 on flow-chart to assess GDM risk factors, have a telephone number, prediabetic, 14–16 weeks' gestation, normal screening tests for fetal abnormalities, single pregnancy and fasting blood glucose < 92 mg/dL and < 120 mg/dL 2 hours after food Exclusion criteria: allergy to cow's milk, severe mental problems, stressful incidence in the last 3 months, smoking, intention to terminate pregnancy, immune disorders, medical conditions, pregnancy with IVF and taking antibiotics continuously over the past 3 months
Interventions	Probiotic: capsule daily and routine care Placebo: capsule daily and routine care
Outcomes	Primary: frequency of abnormal OGTT after 70 days of intervention
Starting date	22 May 2018
Contact information	Mahdieh Ebrahimzadeh, Mashhad University of Medical Sciences, Iran
Notes	Sources of funding: Mashhad University of Medical Sciences Declarations of interest: not reported

NCT01436448

Study name	Probiotics (*Lactobacillus rhamnosus*) in reducing glucose intolerance during and after pregnancy (GRIP)

NCT01436448 *(Continued)*

Methods	Study design: randomised controlled trial Blinding: double blind for probiotics/placebo Location: Karachi, Pakistan
Participants	Inclusion criteria: high-risk pregnancy (defined as ≥ 1 of maternal age ≥ 35 years, family history of diabetes among a first-degree relative or BMI > 23 kg/m^2), attending antenatal clinic at 12–14 weeks' gestation, singleton pregnancy and planning delivery at the study hospital Exclusion criteria: history of GDM; known diabetes mellitus; known chronic diseases; medication such as corticosteroids, azathioprine or antiepileptic drugs; known polycystic ovarian syndrome- and not a resident of Karachi
Interventions	Probiotic: capsule containing *Lactobacillus rhamnosus* at 10^{10} CFU taken daily until delivery. Placebo: capsule containing microcrystalline cellulose taken daily until delivery
Outcomes	Primary: glucose tolerance by OGTT using ADA guidelines between 24–28 weeks' gestation and at 6–8 weeks' postpartum. Secondary: feasibility, intervention compliance, maternal safety and fetal/neonatal safety
Starting date	October 2011
Contact information	Principal Investigator: Bilal Ahmed, MSc, Aga Khan University
Notes	Sources of funding: not stated Declarations of interest: not reported

NCT03240419

Study name	Probiotic supplementation in obese pregnant women. a feasibility study
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: Little Rock, Arkansas, US
Participants	Inclusion criteria: BMI ≥ 30 kg/m^2, aged ≥ 18 years, singleton pregnancy, < 12 weeks' gestation, consuming < 1 serving of yoghurt with live cultures or cultured milk per week, and conceived without assisted fertility treatments Exclusion criteria: women with pre-existing medical conditions (e.g. diabetes, hypertension, thyroid disorders, heart disease or immune disorders); immunosuppressed women; women taking medications during pregnancy known to affect fetal growth; women using recreational drugs, tobacco or alcohol during pregnancy; milk intolerance/allergy and consuming probiotic supplements
Interventions	Probiotic: capsule containing *Bifidobacterium* BB-12 and *Lactobacillus rhamnosus* LGG with minimum of 6.5 × 10^9 CFU per capsule taken once daily from recruitment until delivery Placebo: capsule containing microcrystalline cellulose, maltodextrin, silicon dioxide, and magnesium stearate taken once daily from recruitment until delivery
Outcomes	Primary: change in acceptance of intervention throughout pregnancy and change in compliance with intervention throughout pregnancy

NCT03240419 *(Continued)*

Starting date	23 August 2017
Contact information	Eva C Diaz Fuentes, University of Arkansas, US, ecdiazfuentes@uams.edu
Notes	Sources of funding: Arkansas Children's Hospital Research Institute Declarations of interest: not reported

NCT04009889

Study name	Double blind, randomised, controlled trial on impact of oral probiotic blend (*Lactobacillus rhamnosus* GG, *L. crispatus* LBV88, *L. rhamnosus* LBV96, *L. jensenii* LBV116 and *L. gasseri* LBV150) on pregnancy outcome
Methods	Study design: parallel randomised controlled trial Blinding: double blind Location: Clinical Research Center Kiel GmbH, Kiel, Schleswig-Holstein, Germany
Participants	Inclusion criteria: pregnant women aged > 18 years, < 14 weeks' gestation, willing to consume the study product, willing to abstain from probiotic food and supplements containing probiotics, and able to provide written informed consent Exclusion criteria: enrolment in another clinical study or having finished another clinical study within the last 4 weeks before inclusion; diabetes mellitus; acute metabolic disorder interfering with glucose metabolism; known cancer < 5 years ago; any ano-rectal infection; disease or surgery in the medical history or current that may impact microbiota; anus praeter; hypersensitivity allergy or reaction to any component of the test product; any disease or condition that might significantly compromise the hematopoietic, renal, endocrine, pulmonary, hepatic, gastrointestinal, cardiovascular, immunological, central nervous, dermatological or any other body system; history of active hepatitis B or C; history of HIV; regular medical treatments including non-prescription medications that may impact study aims; major cognitive or psychiatric disorders; present drug abuse or alcoholism; reformed alcoholic and legal incapacity
Interventions	Probiotic: capsule containing a probiotic blend of 5 different *Lactobacilli* (*L rhamnosus* GG, *L. crispatus* LBV88, *L rhamnosus* LBV96, *L jensenii* LBV116 and *L gasseri* LBV150) taken from before 14 weeks' gestation until delivery Placebo: capsule containing microcrystalline cellulose, magnesium stearate and silicon dioxide from before 14 weeks' gestation until delivery
Outcomes	Primary: HOMA-IR values in weeks 24–28 and weeks 36–40
Starting date	27 March 2018
Contact information	Christiane Laue, Clinical Research Center Kiel GmbH, Germany, c.laue@crc-kiel.de
Notes	Funding sources: i-Health, Inc. Declarations of interest: not reported

ADA: American Diabetes Association; BMI: body mass index; CFU: colony-forming unit; GDM: gestational diabetes mellitus; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; IVF: in vitro fertilisation; OGTT: oral glucose tolerance test.

DATA AND ANALYSES

Comparison 1. Probiotics versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Gestational diabetes mellitus	6	1440	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.54, 1.20]
1.2 Gestational diabetes mellitus (by dose)	6	1440	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.54, 1.20]
1.2.1 < 5 billion CFU	2	547	Risk Ratio (M-H, Random, 95% CI)	1.47 [0.94, 2.30]
1.2.2 > 5 billion CFU	4	893	Risk Ratio (M-H, Random, 95% CI)	0.67 [0.46, 0.98]
1.3 Gestational diabetes mellitus (by bacterial species)	6	1440	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.54, 1.20]
1.3.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*	4	931	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.50, 1.37]
1.3.2 *Lactobacillus rhamnosus*	1	373	Risk Ratio (M-H, Random, 95% CI)	0.59 [0.32, 1.08]
1.3.3 *Lactobacillus salivarius*	1	136	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.25, 5.70]
1.4 Gestational diabetes mellitus (by duration of treatment)	6	1440	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.54, 1.20]
1.4.1 Started early pregnancy	5	1304	Risk Ratio (M-H, Random, 95% CI)	0.78 [0.51, 1.20]
1.4.2 Started ≥ 20 weeks' gestation	1	136	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.25, 5.70]
1.5 Hypertensive disorders of pregnancy	4	955	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.96, 2.01]
1.6 Hypertensive disorders of pregnancy (by dose)	4	955	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.96, 2.01]
1.6.1 < 5 billion CFU	2	545	Risk Ratio (M-H, Fixed, 95% CI)	1.35 [0.87, 2.12]
1.6.2 > 5 billion CFU	2	410	Risk Ratio (M-H, Fixed, 95% CI)	1.47 [0.77, 2.81]
1.7 Hypertensive disorders of pregnancy (by bacterial species)	4	955	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.96, 2.01]
1.7.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*	3	819	Risk Ratio (M-H, Fixed, 95% CI)	1.35 [0.92, 1.98]
1.7.2 *Lactobacillus salivarius*	1	136	Risk Ratio (M-H, Fixed, 95% CI)	1.99 [0.49, 7.99]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.8 Hypertensive disorders of pregnancy (by duration of treatment)	4	955	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.96, 2.01]
1.8.1 Started early pregnancy	3	819	Risk Ratio (M-H, Fixed, 95% CI)	1.35 [0.92, 1.98]
1.8.2 Started ≥ 20 weeks' gestation	1	136	Risk Ratio (M-H, Fixed, 95% CI)	1.99 [0.49, 7.99]
1.9 Pre-eclampsia	4	955	Risk Ratio (M-H, Fixed, 95% CI)	1.85 [1.04, 3.29]
1.10 Caesarean section	6	1520	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.86, 1.17]
1.11 Caesarean section (by dose)	6	1520	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.86, 1.17]
1.11.1 < 5 billion CFU	2	547	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.73, 1.14]
1.11.2 > 5 billion CFU	4	973	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.87, 1.36]
1.12 Caesarean section (by bacterial species)	6	1520	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.86, 1.17]
1.12.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*	4	977	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.81, 1.19]
1.12.2 *Lactobacillus rhamnosus*	1	407	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.79, 1.51]
1.12.3 *Lactobacillus salivarius*	1	136	Risk Ratio (M-H, Fixed, 95% CI)	0.95 [0.59, 1.55]
1.13 Caesarean section (by duration of treatment)	6	1520	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.86, 1.17]
1.13.1 Started early pregnancy	5	1384	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.86, 1.19]
1.13.2 Started ≥ 20 weeks' gestation	1	136	Risk Ratio (M-H, Fixed, 95% CI)	0.95 [0.59, 1.55]
1.14 Large-for-gestational age	4	919	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.72, 1.36]
1.15 Large-for-gestational age (by dose)	4	919	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.72, 1.36]
1.15.1 < 5 billion CFU	2	509	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.72, 1.62]
1.15.2 > 5 billion CFU	2	410	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.53, 1.46]
1.16 Large-for-gestational age (by bacterial species)	4	919	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.72, 1.36]
1.16.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*	3	783	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.71, 1.38]
1.16.2 *Lactobacillus salivarius*	1	136	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.36, 2.89]
1.17 Large-for-gestational age (by duration of treatment)	4	919	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.72, 1.36]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.17.1 Started early pregnancy	3	783	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.71, 1.38]
1.17.2 Started ≥ 20 weeks' gestation	1	136	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.36, 2.89]
1.18 Perinatal mortality (stillbirth and neonatal mortality)	3	709	Risk Ratio (IV, Fixed, 95% CI)	0.33 [0.01, 8.02]
1.19 Mortality or morbidity composite	2	623	Risk Ratio (M-H, Fixed, 95% CI)	0.69 [0.36, 1.35]
1.20 Induction of labour	2	544	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.85, 1.39]
1.21 Postpartum haemorrhage	2	324	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.60, 1.85]
1.22 Weight gain during pregnancy (kg)	4	853	Mean Difference (IV, Random, 95% CI)	0.30 [-0.67, 1.26]
1.23 Fasting plasma glucose (mmol/L)	7	1519	Mean Difference (IV, Random, 95% CI)	-0.04 [-0.12, 0.05]
1.24 1-hour oral glucose tolerance test (OGTT) plasma glucose (mmol/L)	4	1110	Mean Difference (IV, Fixed, 95% CI)	-0.07 [-0.27, 0.13]
1.25 2-hour OGTT plasma glucose (mmol/L)	4	1186	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.13, 0.18]
1.26 Triglycerides (mmol/L)	2	198	Mean Difference (IV, Fixed, 95% CI)	-0.21 [-0.40, -0.02]
1.27 High-density lipoprotein (mmol/L)	2	198	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.08, 0.11]
1.28 Low-density lipoprotein (mmol/L)	2	198	Mean Difference (IV, Fixed, 95% CI)	-0.22 [-0.48, 0.04]
1.29 Total cholesterol (mmol/L)	2	198	Mean Difference (IV, Fixed, 95% CI)	-0.31 [-0.62, -0.00]
1.30 Insulin (mU/L)	4	538	Mean Difference (IV, Fixed, 95% CI)	-1.95 [-3.01, -0.88]
1.31 Sense of wellbeing and quality of life	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.31.1 Edinburgh Postnatal Depression Score – 36 weeks	1	164	Mean Difference (IV, Fixed, 95% CI)	0.42 [-0.89, 1.73]
1.31.2 Spielberger State-Trait Anxiety Inventory Short Form Score – 36 weeks	1	164	Mean Difference (IV, Fixed, 95% CI)	-0.94 [-4.09, 2.21]

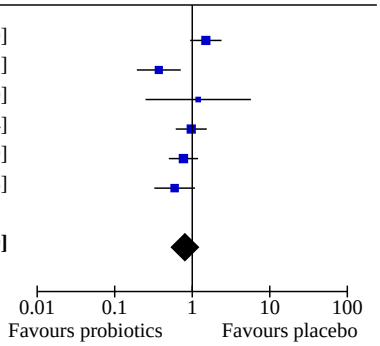
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.31.3 12-Item Short-Form Health Survey – Mental Component Score, 36 weeks	1	164	Mean Difference (IV, Fixed, 95% CI)	0.31 [-2.54, 3.16]
1.31.4 12-Item Short-Form Health Survey – Physical Component Score, 36 weeks	1	164	Mean Difference (IV, Fixed, 95% CI)	0.87 [-1.94, 3.68]
1.32 Breastfeeding at 6 months	2	552	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.77, 1.50]
1.33 Postnatal weight retention (kg)	1	391	Mean Difference (IV, Fixed, 95% CI)	-0.10 [-0.91, 0.71]
1.34 Body mass index (kg/m^2)	2		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.34.1 4–7 days postpartum	1	391	Mean Difference (IV, Fixed, 95% CI)	-0.10 [-0.38, 0.18]
1.34.2 12 months postpartum	1	128	Mean Difference (IV, Fixed, 95% CI)	-0.10 [-0.65, 0.45]
1.34.3 4 years postpartum	1	80	Mean Difference (IV, Fixed, 95% CI)	0.70 [-0.18, 1.58]
1.35 Stillbirth	5	1128	Risk Ratio (M-H, Fixed, 95% CI)	0.59 [0.14, 2.46]
1.36 Neonatal mortality	3	709	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
1.37 Gestational age at birth (weeks)	5	1073	Mean Difference (IV, Fixed, 95% CI)	0.01 [-0.19, 0.21]
1.38 Preterm birth	6	1484	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.86, 2.01]
1.39 Macrosomia	3	952	Risk Ratio (M-H, Fixed, 95% CI)	1.13 [0.86, 1.48]
1.40 Small-for-gestational age	3	814	Risk Ratio (M-H, Fixed, 95% CI)	0.51 [0.30, 0.85]
1.41 Birthweight (g)	6	1524	Mean Difference (IV, Random, 95% CI)	26.87 [-49.52, 103.26]
1.42 Head circumference (cm)	3	789	Mean Difference (IV, Fixed, 95% CI)	-0.04 [-0.27, 0.18]
1.43 Length (cm)	3	786	Mean Difference (IV, Random, 95% CI)	0.02 [-0.54, 0.59]
1.44 Ponderal index (kg/m^3)	2	539	Mean Difference (IV, Fixed, 95% CI)	0.25 [-0.21, 0.70]
1.45 Adiposity – fat mass (kg)	1	110	Mean Difference (IV, Fixed, 95% CI)	-0.04 [-0.12, 0.04]
1.46 Adiposity – % fat	1	210	Mean Difference (IV, Fixed, 95% CI)	-0.10 [-1.19, 0.99]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.47 Hypoglycaemia	2	586	Risk Ratio (M-H, Random, 95% CI)	1.15 [0.69, 1.92]
1.48 Hyperbilirubinaemia	2	593	Risk Ratio (M-H, Fixed, 95% CI)	0.95 [0.66, 1.38]
1.49 Infant weight gain (g/month)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.49.1 at 0–6 months	1	162	Mean Difference (IV, Fixed, 95% CI)	-3.00 [-53.07, 47.07]
1.49.2 at 6–12 months	1	162	Mean Difference (IV, Fixed, 95% CI)	27.00 [-0.76, 54.76]
1.49.3 at 12–24 months	1	130	Mean Difference (IV, Fixed, 95% CI)	-19.00 [-42.62, 4.62]
1.50 Infant height (cm/month)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.50.1 at 0–6 months	1	156	Mean Difference (IV, Fixed, 95% CI)	-0.05 [-0.15, 0.05]
1.50.2 at 6–12 months	1	156	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.04, 0.08]
1.50.3 at 12–24 months	1	130	Mean Difference (IV, Fixed, 95% CI)	0.01 [-0.04, 0.06]
1.51 Infant head circumference – 6 months (cm)	1	119	Mean Difference (IV, Fixed, 95% CI)	0.30 [-0.26, 0.86]
1.52 Infant mean blood pressure – 6 months (mmHg)	1	114	Mean Difference (IV, Fixed, 95% CI)	-1.00 [-4.19, 2.19]
1.53 32–33 split proinsulin > 85th percentile – 6 months	1	131	Risk Ratio (M-H, Fixed, 95% CI)	0.95 [0.34, 2.69]
1.54 Neonatal intensive care unit admission	5	1354	Risk Ratio (IV, Fixed, 95% CI)	0.97 [0.75, 1.26]

Analysis 1.1. Comparison 1: Probiotics versus placebo, Outcome 1: Gestational diabetes mellitus

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Callaway 2019	38	207	25	204	20.2%	1.50 [0.94 , 2.39]
Laitinen 2009	10	76	27	76	16.0%	0.37 [0.19 , 0.71]
Lindsay 2014	3	62	3	74	5.3%	1.19 [0.25 , 5.70]
Okesene-Gafa 2019	28	105	25	91	20.4%	0.97 [0.61 , 1.54]
Pellonpera 2019	25	88	31	84	21.0%	0.77 [0.50 , 1.19]
Wickens 2017	15	184	26	189	17.1%	0.59 [0.32 , 1.08]
Total (95% CI)		**722**		**718**	**100.0%**	**0.80 [0.54 , 1.20]**
Total events:	119		137			

Heterogeneity: Tau² = 0.15; Chi² = 13.99, df = 5 (P = 0.02); I² = 64%
Test for overall effect: Z = 1.07 (P = 0.28)
Test for subgroup differences: Not applicable



Analysis 1.2. Comparison 1: Probiotics versus placebo, Outcome 2: Gestational diabetes mellitus (by dose)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
1.2.1 < 5 billion CFU						
Callaway 2019	38	207	25	204	20.2%	1.50 [0.94 , 2.39]
Lindsay 2014	3	62	3	74	5.3%	1.19 [0.25 , 5.70]
Subtotal (95% CI)		**269**		**278**	**25.5%**	**1.47 [0.94 , 2.30]**
Total events:	41		28			

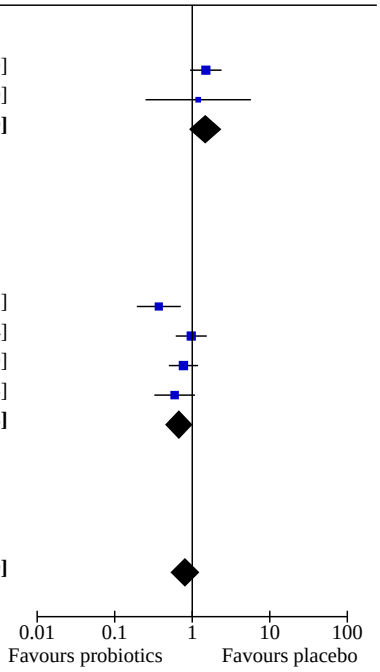
Heterogeneity: Tau² = 0.00; Chi² = 0.07, df = 1 (P = 0.78); I² = 0%
Test for overall effect: Z = 1.69 (P = 0.09)

1.2.2 > 5 billion CFU						
Laitinen 2009	10	76	27	76	16.0%	0.37 [0.19 , 0.71]
Okesene-Gafa 2019	28	105	25	91	20.4%	0.97 [0.61 , 1.54]
Pellonpera 2019	25	88	31	84	21.0%	0.77 [0.50 , 1.19]
Wickens 2017	15	184	26	189	17.1%	0.59 [0.32 , 1.08]
Subtotal (95% CI)		**453**		**440**	**74.5%**	**0.67 [0.46 , 0.98]**
Total events:	78		109			

Heterogeneity: Tau² = 0.07; Chi² = 6.14, df = 3 (P = 0.10); I² = 51%
Test for overall effect: Z = 2.07 (P = 0.04)

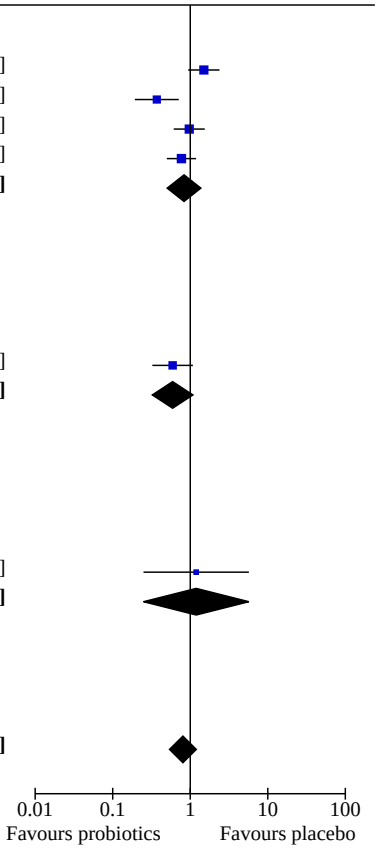
Total (95% CI)		**722**		**718**	**100.0%**	**0.80 [0.54 , 1.20]**
Total events:	119		137			

Heterogeneity: Tau² = 0.15; Chi² = 13.99, df = 5 (P = 0.02); I² = 64%
Test for overall effect: Z = 1.07 (P = 0.28)
Test for subgroup differences: Chi² = 6.92, df = 1 (P = 0.009), I² = 85.5%



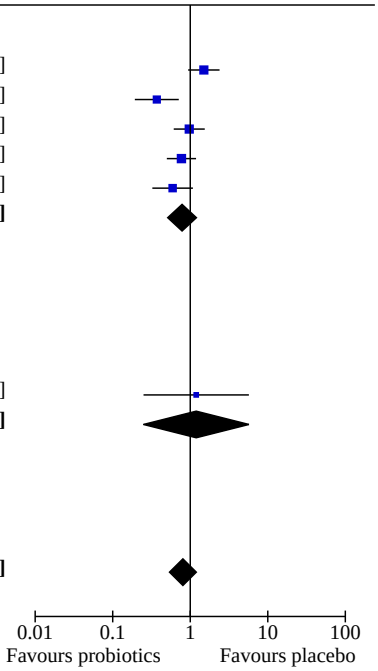
Analysis 1.3. Comparison 1: Probiotics versus placebo, Outcome 3: Gestational diabetes mellitus (by bacterial species)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
1.3.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*						
Callaway 2019	38	207	25	204	20.2%	1.50 [0.94 , 2.39]
Laitinen 2009	10	76	27	76	16.0%	0.37 [0.19 , 0.71]
Okesene-Gafa 2019	28	105	25	91	20.4%	0.97 [0.61 , 1.54]
Pellonpera 2019	25	88	31	84	21.0%	0.77 [0.50 , 1.19]
Subtotal (95% CI)		**476**		**455**	**77.7%**	**0.83 [0.50 , 1.37]**
Total events:	101		108			
Heterogeneity: Tau² = 0.19; Chi² = 12.30, df = 3 (P = 0.006); I² = 76%						
Test for overall effect: Z = 0.72 (P = 0.47)						
1.3.2 *Lactobacillus rhamnosus*						
Wickens 2017	15	184	26	189	17.1%	0.59 [0.32 , 1.08]
Subtotal (95% CI)		**184**		**189**	**17.1%**	**0.59 [0.32 , 1.08]**
Total events:	15		26			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.70 (P = 0.09)						
1.3.3 *Lactobacillus salivarius*						
Lindsay 2014	3	62	3	74	5.3%	1.19 [0.25 , 5.70]
Subtotal (95% CI)		**62**		**74**	**5.3%**	**1.19 [0.25 , 5.70]**
Total events:	3		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.22 (P = 0.82)						
Total (95% CI)		**722**		**718**	**100.0%**	**0.80 [0.54 , 1.20]**
Total events:	119		137			
Heterogeneity: Tau² = 0.15; Chi² = 13.99, df = 5 (P = 0.02); I² = 64%						
Test for overall effect: Z = 1.07 (P = 0.28)						
Test for subgroup differences: Chi² = 1.09, df = 2 (P = 0.58), I² = 0%						



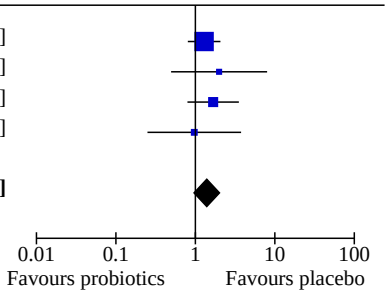
Analysis 1.4. Comparison 1: Probiotics versus placebo, Outcome 4: Gestational diabetes mellitus (by duration of treatment)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
1.4.1 Started early pregnancy						
Callaway 2019	38	207	25	204	20.2%	1.50 [0.94 , 2.39]
Laitinen 2009	10	76	27	76	16.0%	0.37 [0.19 , 0.71]
Okesene-Gafa 2019	28	105	25	91	20.4%	0.97 [0.61 , 1.54]
Pellonpera 2019	25	88	31	84	21.0%	0.77 [0.50 , 1.19]
Wickens 2017	15	184	26	189	17.1%	0.59 [0.32 , 1.08]
Subtotal (95% CI)		**660**		**644**	**94.7%**	**0.78 [0.51 , 1.20]**
Total events:	116		134			
Heterogeneity: Tau² = 0.17; Chi² = 13.80, df = 4 (P = 0.008); I² = 71%						
Test for overall effect: Z = 1.12 (P = 0.26)						
1.4.2 Started ≥ 20 weeks' gestation						
Lindsay 2014	3	62	3	74	5.3%	1.19 [0.25 , 5.70]
Subtotal (95% CI)		**62**		**74**	**5.3%**	**1.19 [0.25 , 5.70]**
Total events:	3		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.22 (P = 0.82)						
Total (95% CI)		**722**		**718**	**100.0%**	**0.80 [0.54 , 1.20]**
Total events:	119		137			
Heterogeneity: Tau² = 0.15; Chi² = 13.99, df = 5 (P = 0.02); I² = 64%						
Test for overall effect: Z = 1.07 (P = 0.28)						
Test for subgroup differences: Chi² = 0.26, df = 1 (P = 0.61), I² = 0%						



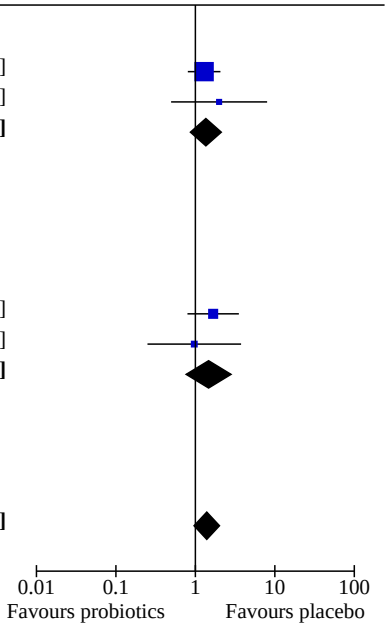
Analysis 1.5. Comparison 1: Probiotics versus placebo, Outcome 5: Hypertensive disorders of pregnancy

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	34	206	26	203	61.2%	1.29 [0.80 , 2.07]
Lindsay 2014	5	62	3	74	6.4%	1.99 [0.49 , 7.99]
Okesene-Gafa 2019	16	108	10	113	22.9%	1.67 [0.79 , 3.53]
Pellonpera 2019	4	96	4	93	9.5%	0.97 [0.25 , 3.76]
Total (95% CI)		**472**		**483**	**100.0%**	**1.39 [0.96 , 2.01]**
Total events:	59		43			
Heterogeneity: Chi² = 0.87, df = 3 (P = 0.83); I² = 0%						
Test for overall effect: Z = 1.76 (P = 0.08)						
Test for subgroup differences: Not applicable						



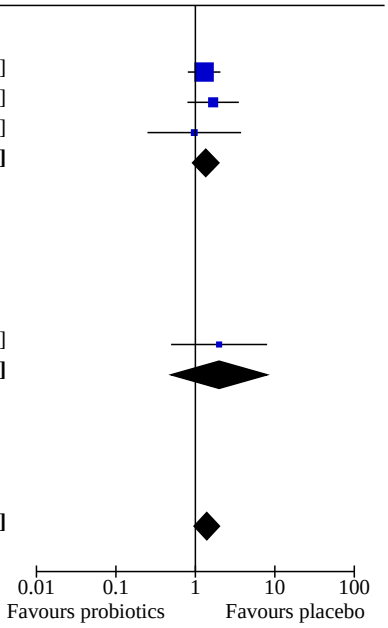
Analysis 1.6. Comparison 1: Probiotics versus placebo, Outcome 6: Hypertensive disorders of pregnancy (by dose)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.6.1 < 5 billion CFU						
Callaway 2019	34	206	26	203	61.2%	1.29 [0.80 , 2.07]
Lindsay 2014	5	62	3	74	6.4%	1.99 [0.49 , 7.99]
Subtotal (95% CI)		**268**		**277**	**67.6%**	**1.35 [0.87 , 2.12]**
Total events:	39		29			
Heterogeneity: Chi² = 0.34, df = 1 (P = 0.56); I² = 0%						
Test for overall effect: Z = 1.33 (P = 0.18)						
1.6.2 > 5 billion CFU						
Okesene-Gafa 2019	16	108	10	113	22.9%	1.67 [0.79 , 3.53]
Pellonpera 2019	4	96	4	93	9.5%	0.97 [0.25 , 3.76]
Subtotal (95% CI)		**204**		**206**	**32.4%**	**1.47 [0.77 , 2.81]**
Total events:	20		14			
Heterogeneity: Chi² = 0.48, df = 1 (P = 0.49); I² = 0%						
Test for overall effect: Z = 1.16 (P = 0.25)						
Total (95% CI)		**472**		**483**	**100.0%**	**1.39 [0.96 , 2.01]**
Total events:	59		43			
Heterogeneity: Chi² = 0.87, df = 3 (P = 0.83); I² = 0%						
Test for overall effect: Z = 1.76 (P = 0.08)						
Test for subgroup differences: Chi² = 0.04, df = 1 (P = 0.84), I² = 0%						



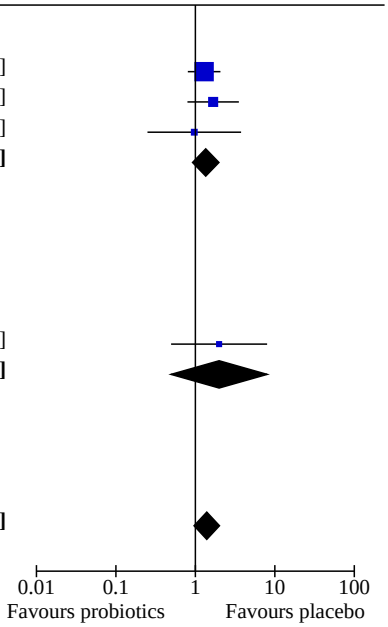
Analysis 1.7. Comparison 1: Probiotics versus placebo, Outcome 7: Hypertensive disorders of pregnancy (by bacterial species)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.7.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*						
Callaway 2019	34	206	26	203	61.2%	1.29 [0.80 , 2.07]
Okesene-Gafa 2019	16	108	10	113	22.9%	1.67 [0.79 , 3.53]
Pellonpera 2019	4	96	4	93	9.5%	0.97 [0.25 , 3.76]
Subtotal (95% CI)		**410**		**409**	**93.6%**	**1.35 [0.92 , 1.98]**
Total events:	54		40			
Heterogeneity: Chi² = 0.59, df = 2 (P = 0.75); I² = 0%						
Test for overall effect: Z = 1.54 (P = 0.12)						
1.7.2 *Lactobacillus salivarius*						
Lindsay 2014	5	62	3	74	6.4%	1.99 [0.49 , 7.99]
Subtotal (95% CI)		**62**		**74**	**6.4%**	**1.99 [0.49 , 7.99]**
Total events:	5		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.97 (P = 0.33)						
Total (95% CI)		**472**		**483**	**100.0%**	**1.39 [0.96 , 2.01]**
Total events:	59		43			
Heterogeneity: Chi² = 0.87, df = 3 (P = 0.83); I² = 0%						
Test for overall effect: Z = 1.76 (P = 0.08)						
Test for subgroup differences: Chi² = 0.28, df = 1 (P = 0.60), I² = 0%						



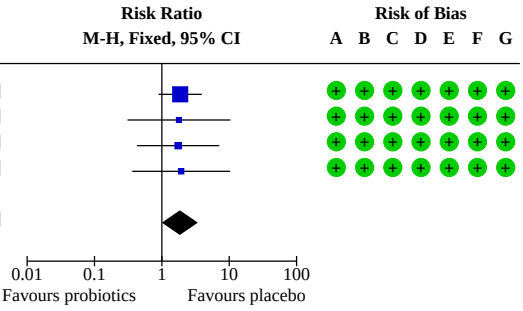
Analysis 1.8. Comparison 1: Probiotics versus placebo, Outcome 8: Hypertensive disorders of pregnancy (by duration of treatment)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.1 Started early pregnancy						
Callaway 2019	34	206	26	203	61.2%	1.29 [0.80 , 2.07]
Okesene-Gafa 2019	16	108	10	113	22.9%	1.67 [0.79 , 3.53]
Pellonpera 2019	4	96	4	93	9.5%	0.97 [0.25 , 3.76]
Subtotal (95% CI)		**410**		**409**	**93.6%**	**1.35 [0.92 , 1.98]**
Total events:	54		40			
Heterogeneity: Chi² = 0.59, df = 2 (P = 0.75); I² = 0%						
Test for overall effect: Z = 1.54 (P = 0.12)						
1.8.2 Started ≥ 20 weeks' gestation						
Lindsay 2014	5	62	3	74	6.4%	1.99 [0.49 , 7.99]
Subtotal (95% CI)		**62**		**74**	**6.4%**	**1.99 [0.49 , 7.99]**
Total events:	5		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.97 (P = 0.33)						
Total (95% CI)		**472**		**483**	**100.0%**	**1.39 [0.96 , 2.01]**
Total events:	59		43			
Heterogeneity: Chi² = 0.87, df = 3 (P = 0.83); I² = 0%						
Test for overall effect: Z = 1.76 (P = 0.08)						
Test for subgroup differences: Chi² = 0.28, df = 1 (P = 0.60), I² = 0%						



Analysis 1.9. Comparison 1: Probiotics versus placebo, Outcome 9: Pre-eclampsia

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	19	206	10	203	59.7%	1.87 [0.89 , 3.93]
Lindsay 2014	3	62	2	74	10.8%	1.79 [0.31 , 10.38]
Okesene-Gafa 2019	5	108	3	113	17.4%	1.74 [0.43 , 7.12]
Pellonpera 2019	4	96	2	93	12.1%	1.94 [0.36 , 10.33]
Total (95% CI)		**472**		**483**	**100.0%**	**1.85 [1.04 , 3.29]**
Total events:	31		17			
Heterogeneity: Chi² = 0.01, df = 3 (P = 1.00); I² = 0%						
Test for overall effect: Z = 2.09 (P = 0.04)						
Test for subgroup differences: Not applicable						

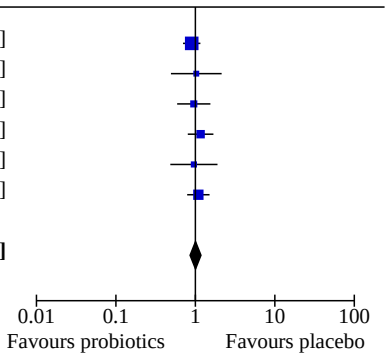


Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Analysis 1.10. Comparison 1: Probiotics versus placebo, Outcome 10: Caesarean section

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	73	207	80	204	37.3%	0.90 [0.70 , 1.16]
Laitinen 2009	12	75	12	77	5.5%	1.03 [0.49 , 2.14]
Lindsay 2014	20	62	25	74	10.6%	0.95 [0.59 , 1.55]
Okesene-Gafa 2019	40	112	35	114	16.1%	1.16 [0.80 , 1.69]
Pellonpera 2019	14	96	14	92	6.6%	0.96 [0.48 , 1.90]
Wickens 2017	57	206	51	201	23.9%	1.09 [0.79 , 1.51]
Total (95% CI)		**758**		**762**	**100.0%**	**1.00 [0.86 , 1.17]**
Total events:	216		217			

Heterogeneity: Chi² = 1.65, df = 5 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.05 (P = 0.96)
Test for subgroup differences: Not applicable



Analysis 1.11. Comparison 1: Probiotics versus placebo, Outcome 11: Caesarean section (by dose)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.11.1 < 5 billion CFU						
Callaway 2019	73	207	80	204	37.3%	0.90 [0.70 , 1.16]
Lindsay 2014	20	62	25	74	10.6%	0.95 [0.59 , 1.55]
Subtotal (95% CI)		**269**		**278**	**47.9%**	**0.91 [0.73 , 1.14]**
Total events:	93		105			

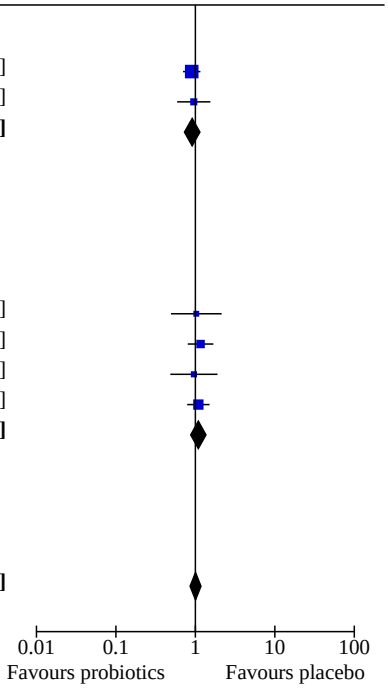
Heterogeneity: Chi² = 0.05, df = 1 (P = 0.83); I² = 0%
Test for overall effect: Z = 0.81 (P = 0.42)

1.11.2 > 5 billion CFU						
Laitinen 2009	12	75	12	77	5.5%	1.03 [0.49 , 2.14]
Okesene-Gafa 2019	40	112	35	114	16.1%	1.16 [0.80 , 1.69]
Pellonpera 2019	14	96	14	92	6.6%	0.96 [0.48 , 1.90]
Wickens 2017	57	206	51	201	23.9%	1.09 [0.79 , 1.51]
Subtotal (95% CI)		**489**		**484**	**52.1%**	**1.09 [0.87 , 1.36]**
Total events:	123		112			

Heterogeneity: Chi² = 0.28, df = 3 (P = 0.96); I² = 0%
Test for overall effect: Z = 0.76 (P = 0.45)

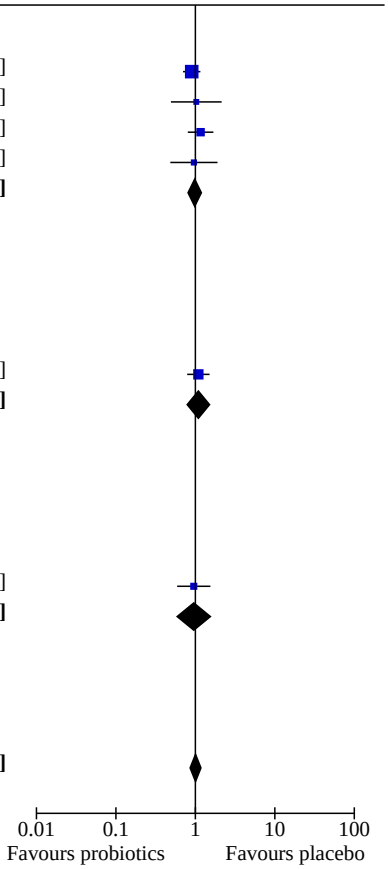
Total (95% CI)		**758**		**762**	**100.0%**	**1.00 [0.86 , 1.17]**
Total events:	216		217			

Heterogeneity: Chi² = 1.65, df = 5 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.05 (P = 0.96)
Test for subgroup differences: Chi² = 1.24, df = 1 (P = 0.26), I² = 19.6%



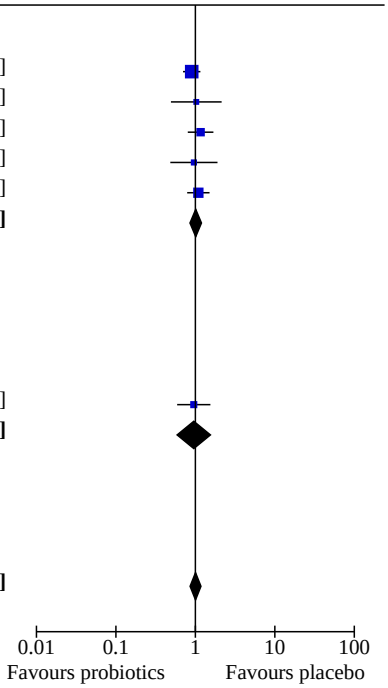
Analysis 1.12. Comparison 1: Probiotics versus placebo, Outcome 12: Caesarean section (by bacterial species)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.12.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*						
Callaway 2019	73	207	80	204	37.3%	0.90 [0.70 , 1.16]
Laitinen 2009	12	75	12	77	5.5%	1.03 [0.49 , 2.14]
Okesene-Gafa 2019	40	112	35	114	16.1%	1.16 [0.80 , 1.69]
Pellonpera 2019	14	96	14	92	6.6%	0.96 [0.48 , 1.90]
Subtotal (95% CI)		**490**		**487**	**65.5%**	**0.98 [0.81 , 1.19]**
Total events:	139		141			
Heterogeneity: Chi² = 1.29, df = 3 (P = 0.73); I² = 0%						
Test for overall effect: Z = 0.20 (P = 0.84)						
1.12.2 *Lactobacillus rhamnosus*						
Wickens 2017	57	206	51	201	23.9%	1.09 [0.79 , 1.51]
Subtotal (95% CI)		**206**		**201**	**23.9%**	**1.09 [0.79 , 1.51]**
Total events:	57		51			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.52 (P = 0.60)						
1.12.3 *Lactobacillus salivarius*						
Lindsay 2014	20	62	25	74	10.6%	0.95 [0.59 , 1.55]
Subtotal (95% CI)		**62**		**74**	**10.6%**	**0.95 [0.59 , 1.55]**
Total events:	20		25			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.19 (P = 0.85)						
Total (95% CI)		**758**		**762**	**100.0%**	**1.00 [0.86 , 1.17]**
Total events:	216		217			
Heterogeneity: Chi² = 1.65, df = 5 (P = 0.89); I² = 0%						
Test for overall effect: Z = 0.05 (P = 0.96)						
Test for subgroup differences: Chi² = 0.35, df = 2 (P = 0.84), I² = 0%						



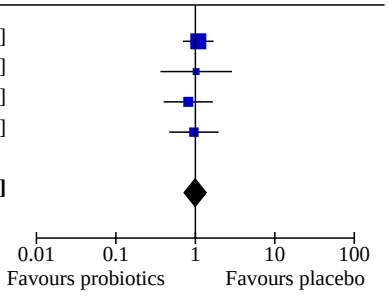
Analysis 1.13. Comparison 1: Probiotics versus placebo, Outcome 13: Caesarean section (by duration of treatment)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.13.1 Started early pregnancy						
Callaway 2019	73	207	80	204	37.3%	0.90 [0.70 , 1.16]
Laitinen 2009	12	75	12	77	5.5%	1.03 [0.49 , 2.14]
Okesene-Gafa 2019	40	112	35	114	16.1%	1.16 [0.80 , 1.69]
Pellonpera 2019	14	96	14	92	6.6%	0.96 [0.48 , 1.90]
Wickens 2017	57	206	51	201	23.9%	1.09 [0.79 , 1.51]
Subtotal (95% CI)		**696**		**688**	**89.4%**	**1.01 [0.86 , 1.19]**
Total events:	196		192			
Heterogeneity: Chi² = 1.62, df = 4 (P = 0.81); I² = 0%						
Test for overall effect: Z = 0.12 (P = 0.91)						
1.13.2 Started ≥ 20 weeks' gestation						
Lindsay 2014	20	62	25	74	10.6%	0.95 [0.59 , 1.55]
Subtotal (95% CI)		**62**		**74**	**10.6%**	**0.95 [0.59 , 1.55]**
Total events:	20		25			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.19 (P = 0.85)						
Total (95% CI)		**758**		**762**	**100.0%**	**1.00 [0.86 , 1.17]**
Total events:	216		217			
Heterogeneity: Chi² = 1.65, df = 5 (P = 0.89); I² = 0%						
Test for overall effect: Z = 0.05 (P = 0.96)						
Test for subgroup differences: Chi² = 0.05, df = 1 (P = 0.83), I² = 0%						



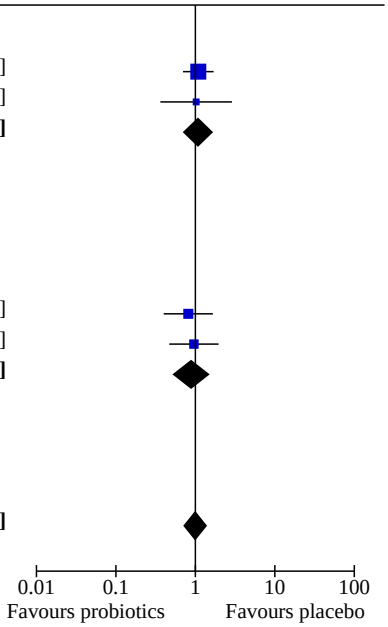
Analysis 1.14. Comparison 1: Probiotics versus placebo, Outcome 14: Large-for-gestational age

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	35	193	30	180	47.3%	1.09 [0.70 , 1.70]
Lindsay 2014	6	62	7	74	9.7%	1.02 [0.36 , 2.89]
Okesene-Gafa 2019	12	110	15	112	22.7%	0.81 [0.40 , 1.66]
Pellonpera 2019	13	96	13	92	20.2%	0.96 [0.47 , 1.96]
Total (95% CI)		**461**		**458**	**100.0%**	**0.99 [0.72 , 1.36]**
Total events:	66		65			
Heterogeneity: Chi² = 0.47, df = 3 (P = 0.92); I² = 0%						
Test for overall effect: Z = 0.04 (P = 0.97)						
Test for subgroup differences: Not applicable						



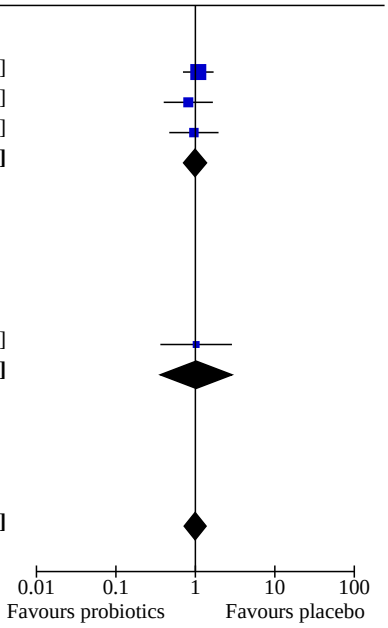
Analysis 1.15. Comparison 1: Probiotics versus placebo, Outcome 15: Large-for-gestational age (by dose)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.15.1 < 5 billion CFU						
Callaway 2019	35	193	30	180	47.3%	1.09 [0.70 , 1.70]
Lindsay 2014	6	62	7	74	9.7%	1.02 [0.36 , 2.89]
Subtotal (95% CI)		**255**		**254**	**57.1%**	**1.08 [0.72 , 1.62]**
Total events:	41		37			
Heterogeneity: Chi² = 0.01, df = 1 (P = 0.91); I² = 0%						
Test for overall effect: Z = 0.36 (P = 0.72)						
1.15.2 > 5 billion CFU						
Okesene-Gafa 2019	12	110	15	112	22.7%	0.81 [0.40 , 1.66]
Pellonpera 2019	13	96	13	92	20.2%	0.96 [0.47 , 1.96]
Subtotal (95% CI)		**206**		**204**	**42.9%**	**0.88 [0.53 , 1.46]**
Total events:	25		28			
Heterogeneity: Chi² = 0.10, df = 1 (P = 0.75); I² = 0%						
Test for overall effect: Z = 0.49 (P = 0.63)						
Total (95% CI)		**461**		**458**	**100.0%**	**0.99 [0.72 , 1.36]**
Total events:	66		65			
Heterogeneity: Chi² = 0.47, df = 3 (P = 0.92); I² = 0%						
Test for overall effect: Z = 0.04 (P = 0.97)						
Test for subgroup differences: Chi² = 0.36, df = 1 (P = 0.55), I² = 0%						



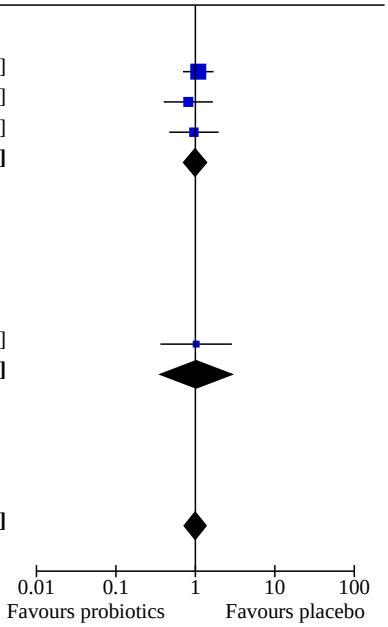
Analysis 1.16. Comparison 1: Probiotics versus placebo, Outcome 16: Large-for-gestational age (by bacterial species)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.16.1 *Lactobacillus rhamnosus* + *Bifidobacterium animalis*						
Callaway 2019	35	193	30	180	47.3%	1.09 [0.70 , 1.70]
Okesene-Gafa 2019	12	110	15	112	22.7%	0.81 [0.40 , 1.66]
Pellonpera 2019	13	96	13	92	20.2%	0.96 [0.47 , 1.96]
Subtotal (95% CI)		**399**		**384**	**90.3%**	**0.99 [0.71 , 1.38]**
Total events:	60		58			
Heterogeneity: Chi² = 0.47, df = 2 (P = 0.79); I² = 0%						
Test for overall effect: Z = 0.06 (P = 0.95)						
1.16.2 *Lactobacillus salivarius*						
Lindsay 2014	6	62	7	74	9.7%	1.02 [0.36 , 2.89]
Subtotal (95% CI)		**62**		**74**	**9.7%**	**1.02 [0.36 , 2.89]**
Total events:	6		7			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.04 (P = 0.97)						
Total (95% CI)		**461**		**458**	**100.0%**	**0.99 [0.72 , 1.36]**
Total events:	66		65			
Heterogeneity: Chi² = 0.47, df = 3 (P = 0.92); I² = 0%						
Test for overall effect: Z = 0.04 (P = 0.97)						
Test for subgroup differences: Chi² = 0.00, df = 1 (P = 0.95), I² = 0%						



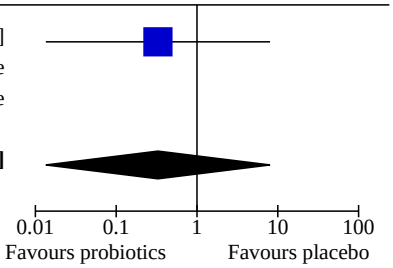
Analysis 1.17. Comparison 1: Probiotics versus placebo, Outcome 17: Large-for-gestational age (by duration of treatment)

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.17.1 Started early pregnancy						
Callaway 2019	35	193	30	180	47.3%	1.09 [0.70 , 1.70]
Okesene-Gafa 2019	12	110	15	112	22.7%	0.81 [0.40 , 1.66]
Pellonpera 2019	13	96	13	92	20.2%	0.96 [0.47 , 1.96]
Subtotal (95% CI)		**399**		**384**	**90.3%**	**0.99 [0.71 , 1.38]**
Total events:	60		58			
Heterogeneity: Chi² = 0.47, df = 2 (P = 0.79); I² = 0%						
Test for overall effect: Z = 0.06 (P = 0.95)						
1.17.2 Started ≥ 20 weeks' gestation						
Lindsay 2014	6	62	7	74	9.7%	1.02 [0.36 , 2.89]
Subtotal (95% CI)		**62**		**74**	**9.7%**	**1.02 [0.36 , 2.89]**
Total events:	6		7			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.04 (P = 0.97)						
Total (95% CI)		**461**		**458**	**100.0%**	**0.99 [0.72 , 1.36]**
Total events:	66		65			
Heterogeneity: Chi² = 0.47, df = 3 (P = 0.92); I² = 0%						
Test for overall effect: Z = 0.04 (P = 0.97)						
Test for subgroup differences: Chi² = 0.00, df = 1 (P = 0.95), I² = 0%						



Analysis 1.18. Comparison 1: Probiotics versus placebo, Outcome 18: Perinatal mortality (stillbirth and neonatal mortality)

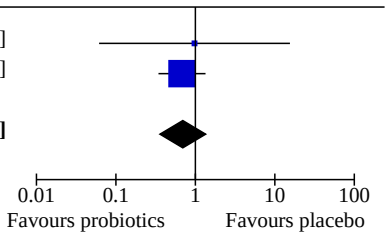
Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio IV, Fixed, 95% CI
Callaway 2019	0	207	1	204	100.0%	0.33 [0.01 , 8.02]
Laitinen 2009	0	82	0	80		Not estimable
Lindsay 2014	0	62	0	74		Not estimable
Total (95% CI)		**351**		**358**	**100.0%**	**0.33 [0.01 , 8.02]**
Total events:	0		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.68 (P = 0.49)						
Test for subgroup differences: Not applicable						



Analysis 1.19. Comparison 1: Probiotics versus placebo, Outcome 19: Mortality or morbidity composite

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	1	203	1	198	5.4%	0.98 [0.06 , 15.49]
Okesene-Gafa 2019	12	110	18	112	94.6%	0.68 [0.34 , 1.34]
Total (95% CI)		**313**		**310**	**100.0%**	**0.69 [0.36 , 1.35]**
Total events:	13		19			

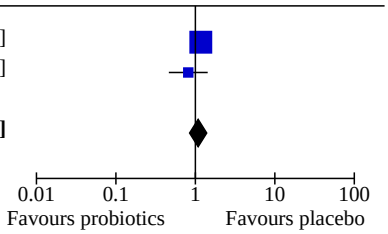
Heterogeneity: Chi² = 0.06, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 1.08 (P = 0.28)
Test for subgroup differences: Not applicable



Analysis 1.20. Comparison 1: Probiotics versus placebo, Outcome 20: Induction of labour

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	74	206	62	202	75.7%	1.17 [0.89 , 1.54]
Lindsay 2014	15	62	22	74	24.3%	0.81 [0.46 , 1.43]
Total (95% CI)		**268**		**276**	**100.0%**	**1.08 [0.85 , 1.39]**
Total events:	89		84			

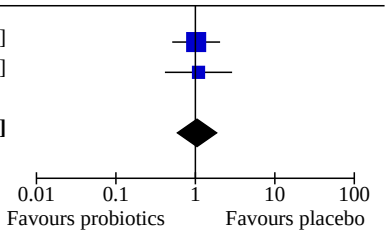
Heterogeneity: Chi² = 1.29, df = 1 (P = 0.26); I² = 23%
Test for overall effect: Z = 0.64 (P = 0.52)
Test for subgroup differences: Not applicable



Analysis 1.21. Comparison 1: Probiotics versus placebo, Outcome 21: Postpartum haemorrhage

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Lindsay 2014	12	62	14	74	64.1%	1.02 [0.51 , 2.05]
Pellonpera 2019	8	96	7	92	35.9%	1.10 [0.41 , 2.90]
Total (95% CI)		**158**		**166**	**100.0%**	**1.05 [0.60 , 1.85]**
Total events:	20		21			

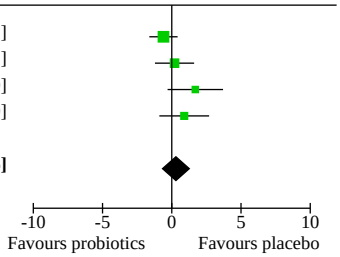
Heterogeneity: Chi² = 0.01, df = 1 (P = 0.91); I² = 0%
Test for overall effect: Z = 0.17 (P = 0.87)
Test for subgroup differences: Not applicable



Analysis 1.22. Comparison 1: Probiotics versus placebo, Outcome 22: Weight gain during pregnancy (kg)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Random, 95% CI
Callaway 2019	8.9	5.3	169	9.5	4.3	176	36.7%	-0.60 [-1.62 , 0.42]
Laitinen 2009	15	4.3	85	14.8	5.1	86	26.7%	0.20 [-1.21 , 1.61]
Lindsay 2014	11.1	6.2	62	9.4	5.6	74	16.9%	1.70 [-0.30 , 3.70]
Okesene-Gafa 2019	11	6.5	100	10.1	6.5	101	19.7%	0.90 [-0.90 , 2.70]
Total (95% CI)			**416**			**437**	**100.0%**	**0.30 [-0.67 , 1.26]**

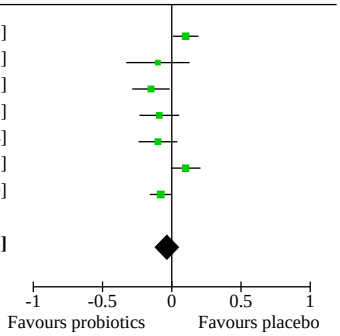
Heterogeneity: Tau² = 0.39; Chi² = 5.04, df = 3 (P = 0.17); I² = 40%
Test for overall effect: Z = 0.61 (P = 0.54)
Test for subgroup differences: Not applicable



Analysis 1.23. Comparison 1: Probiotics versus placebo, Outcome 23: Fasting plasma glucose (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Random, 95% CI
Callaway 2019	4.4	0.5	205	4.3	0.45	202	17.2%	0.10 [0.01 , 0.19]
Jamilian 2016	4.5	0.5	30	4.6	0.4	30	8.2%	-0.10 [-0.33 , 0.13]
Laitinen 2009	4.47	0.3	62	4.62	0.48	69	13.8%	-0.15 [-0.29 , -0.01]
Lindsay 2014	4.6	0.4	63	4.69	0.46	75	13.2%	-0.09 [-0.23 , 0.05]
Okesene-Gafa 2019	4.6	0.5	105	4.7	0.5	91	13.4%	-0.10 [-0.24 , 0.04]
Pellonpera 2019	4.9	0.43	99	4.8	0.32	91	16.0%	0.10 [-0.01 , 0.21]
Wickens 2017	4.32	0.36	195	4.4	0.44	202	18.2%	-0.08 [-0.16 , -0.00]
Total (95% CI)			**759**			**760**	**100.0%**	**-0.04 [-0.12 , 0.05]**

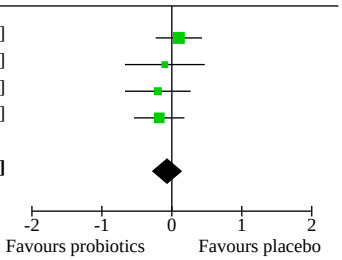
Heterogeneity: Tau² = 0.01; Chi² = 19.66, df = 6 (P = 0.003); I² = 69%
Test for overall effect: Z = 0.84 (P = 0.40)
Test for subgroup differences: Not applicable



Analysis 1.24. Comparison 1: Probiotics versus placebo, Outcome 24: 1-hour oral glucose tolerance test (OGTT) plasma glucose (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Callaway 2019	7.6	1.8	205	7.5	1.6	202	37.3%	0.10 [-0.23 , 0.43]
Okesene-Gafa 2019	8	1.6	75	8.1	1.8	64	12.5%	-0.10 [-0.67 , 0.47]
Pellonpera 2019	7.5	1.7	99	7.7	1.6	91	18.6%	-0.20 [-0.67 , 0.27]
Wickens 2017	6.71	1.73	185	6.89	1.82	189	31.6%	-0.18 [-0.54 , 0.18]
Total (95% CI)			**564**			**546**	**100.0%**	**-0.07 [-0.27 , 0.13]**

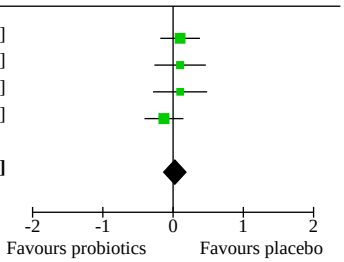
Heterogeneity: Chi² = 1.68, df = 3 (P = 0.64); I² = 0%
Test for overall effect: Z = 0.67 (P = 0.50)
Test for subgroup differences: Not applicable



Analysis 1.25. Comparison 1: Probiotics versus placebo, Outcome 25: 2-hour OGTT plasma glucose (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Callaway 2019	6.4	1.5	205	6.3	1.4	202	31.6%	0.10 [-0.18 , 0.38]
Okesene-Gafa 2019	6.3	1.3	105	6.2	1.3	90	18.7%	0.10 [-0.27 , 0.47]
Pellonpera 2019	6.5	1.3	99	6.4	1.4	91	16.9%	0.10 [-0.29 , 0.49]
Wickens 2017	5.65	1.28	194	5.78	1.52	200	32.7%	-0.13 [-0.41 , 0.15]
Total (95% CI)			**603**			**583**	**100.0%**	**0.02 [-0.13 , 0.18]**

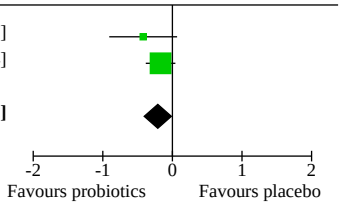
Heterogeneity: Chi² = 1.78, df = 3 (P = 0.62); I² = 0%
Test for overall effect: Z = 0.31 (P = 0.76)
Test for subgroup differences: Not applicable



Analysis 1.26. Comparison 1: Probiotics versus placebo, Outcome 26: Triglycerides (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Jamilian 2016	1.6	0.73	30	2.02	1.15	30	16.0%	-0.42 [-0.91 , 0.07]
Lindsay 2014	1.94	0.67	63	2.11	0.59	75	84.0%	-0.17 [-0.38 , 0.04]
Total (95% CI)			**93**			**105**	**100.0%**	**-0.21 [-0.40 , -0.02]**

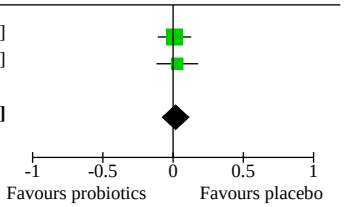
Heterogeneity: Chi² = 0.85, df = 1 (P = 0.36); I² = 0%
Test for overall effect: Z = 2.11 (P = 0.03)
Test for subgroup differences: Not applicable



Analysis 1.27. Comparison 1: Probiotics versus placebo, Outcome 27: High-density lipoprotein (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Jamilian 2016	1.51	0.23	30	1.5	0.24	30	60.9%	0.01 [-0.11 , 0.13]
Lindsay 2014	1.9	0.47	63	1.87	0.41	75	39.1%	0.03 [-0.12 , 0.18]
Total (95% CI)			**93**			**105**	**100.0%**	**0.02 [-0.08 , 0.11]**

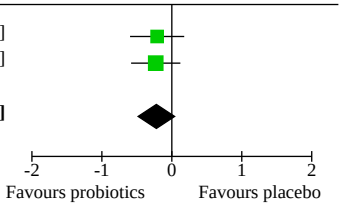
Heterogeneity: Chi² = 0.04, df = 1 (P = 0.84); I² = 0%
Test for overall effect: Z = 0.38 (P = 0.71)
Test for subgroup differences: Not applicable



Analysis 1.28. Comparison 1: Probiotics versus placebo, Outcome 28: Low-density lipoprotein (mmol/L)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Jamilian 2016	2.27	0.76	30	2.48	0.77	30	45.3%	-0.21 [-0.60 , 0.18]
Lindsay 2014	3.55	0.95	63	3.78	1.16	75	54.7%	-0.23 [-0.58 , 0.12]
Total (95% CI)			**93**			**105**	**100.0%**	**-0.22 [-0.48 , 0.04]**

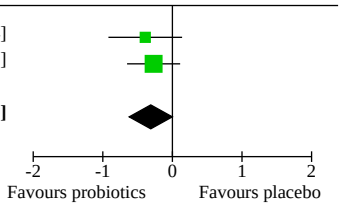
Heterogeneity: Chi² = 0.01, df = 1 (P = 0.94); I² = 0%
Test for overall effect: Z = 1.66 (P = 0.10)
Test for subgroup differences: Not applicable



Analysis 1.29. Comparison 1: Probiotics versus placebo, Outcome 29: Total cholesterol (mmol/L)

Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Jamilian 2016	4.51	0.93	30	4.9	1.15	30	34.2%	-0.39 [-0.92 , 0.14]
Lindsay 2014	6.33	1.12	63	6.6	1.16	75	65.8%	-0.27 [-0.65 , 0.11]
Total (95% CI)			**93**			**105**	**100.0%**	**-0.31 [-0.62 , -0.00]**

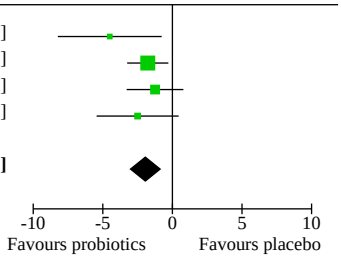
Heterogeneity: Chi² = 0.13, df = 1 (P = 0.72); I² = 0%
Test for overall effect: Z = 1.97 (P = 0.05)
Test for subgroup differences: Not applicable



Analysis 1.30. Comparison 1: Probiotics versus placebo, Outcome 30: Insulin (mU/L)

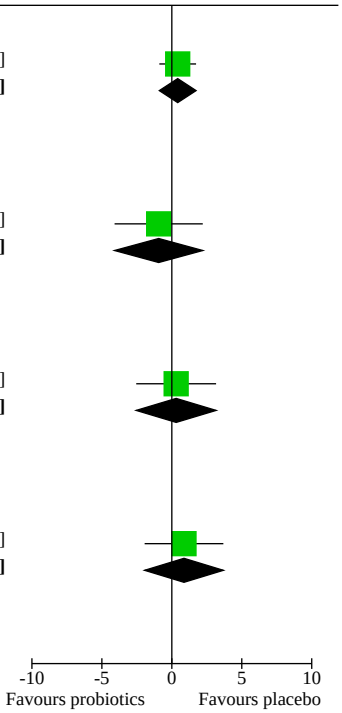
Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Jamilian 2016	9.6	4.7	30	14.1	9.3	30	8.1%	-4.50 [-8.23 , -0.77]
Laitinen 2009	7.55	4.14	76	9.32	5.28	81	51.6%	-1.77 [-3.25 , -0.29]
Lindsay 2014	15.63	6.35	63	16.88	5.75	75	27.2%	-1.25 [-3.29 , 0.79]
Pellonpera 2019	15.6	6.8	93	18.1	12.6	90	13.0%	-2.50 [-5.45 , 0.45]
Total (95% CI)			**262**			**276**	**100.0%**	**-1.95 [-3.01 , -0.88]**

Heterogeneity: Chi² = 2.44, df = 3 (P = 0.49); I² = 0%
Test for overall effect: Z = 3.59 (P = 0.0003)
Test for subgroup differences: Not applicable



Analysis 1.31. Comparison 1: Probiotics versus placebo, Outcome 31: Sense of wellbeing and quality of life

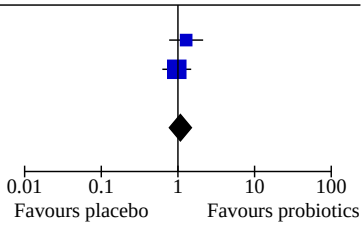
Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
1.31.1 Edinburgh Postnatal Depression Score – 36 weeks								
Okesene-Gafa 2019	7.18	3.8	88	6.76	4.65	76	100.0%	0.42 [-0.89 , 1.73]
Subtotal (95% CI)			**88**			**76**	**100.0%**	**0.42 [-0.89 , 1.73]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.63 (P = 0.53)								
1.31.2 Spielberger State-Trait Anxiety Inventory Short Form Score – 36 weeks								
Okesene-Gafa 2019	31.94	10.22	88	32.88	10.31	76	100.0%	-0.94 [-4.09 , 2.21]
Subtotal (95% CI)			**88**			**76**	**100.0%**	**-0.94 [-4.09 , 2.21]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.58 (P = 0.56)								
1.31.3 12-Item Short-Form Health Survey – Mental Component Score, 36 weeks								
Okesene-Gafa 2019	48.62	8.56	88	48.31	9.89	76	100.0%	0.31 [-2.54 , 3.16]
Subtotal (95% CI)			**88**			**76**	**100.0%**	**0.31 [-2.54 , 3.16]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.21 (P = 0.83)								
1.31.4 12-Item Short-Form Health Survey – Physical Component Score, 36 weeks								
Okesene-Gafa 2019	36.77	9.75	88	35.9	8.63	76	100.0%	0.87 [-1.94 , 3.68]
Subtotal (95% CI)			**88**			**76**	**100.0%**	**0.87 [-1.94 , 3.68]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.61 (P = 0.54)								



Analysis 1.32. Comparison 1: Probiotics versus placebo, Outcome 32: Breastfeeding at 6 months

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio (Non-event) M-H, Fixed, 95% CI
Laitinen 2009	51	75	57	76	35.3%	1.28 [0.77 , 2.13]
Wickens 2017	170	204	163	197	64.7%	0.97 [0.63 , 1.49]
Total (95% CI)		**279**		**273**	**100.0%**	**1.08 [0.77 , 1.50]**
Total events:	221		220			

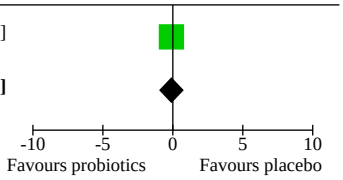
Heterogeneity: Chi² = 0.68, df = 1 (P = 0.41); I² = 0%
Test for overall effect: Z = 0.44 (P = 0.66)
Test for subgroup differences: Not applicable



Analysis 1.33. Comparison 1: Probiotics versus placebo, Outcome 33: Postnatal weight retention (kg)

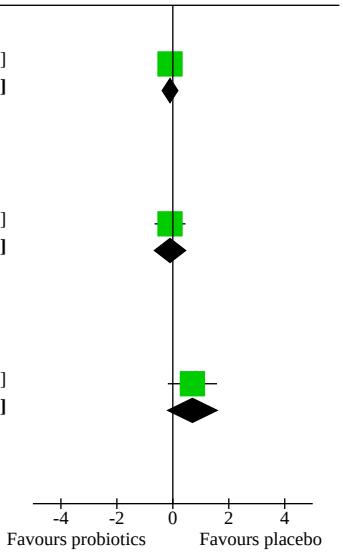
Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Wickens 2017	76.7	3.9	197	76.8	4.3	194	100.0%	-0.10 [-0.91 , 0.71]
Total (95% CI)			**197**			**194**	**100.0%**	**-0.10 [-0.91 , 0.71]**

Heterogeneity: Not applicable
Test for overall effect: Z = 0.24 (P = 0.81)
Test for subgroup differences: Not applicable



Analysis 1.34. Comparison 1: Probiotics versus placebo, Outcome 34: Body mass index (kg/m²)

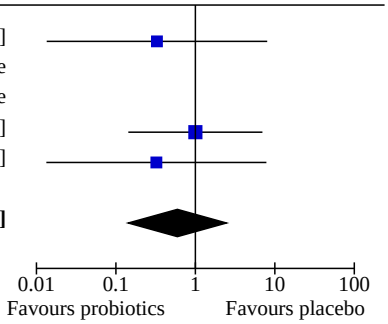
Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
1.34.1 4–7 days postpartum								
Wickens 2017	28	1.4	197	28.1	1.4	194	100.0%	-0.10 [-0.38 , 0.18]
Subtotal (95% CI)			**197**			**194**	**100.0%**	**-0.10 [-0.38 , 0.18]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.71 (P = 0.48)								
1.34.2 12 months postpartum								
Laitinen 2009	24.3	1.6	64	24.4	1.6	64	100.0%	-0.10 [-0.65 , 0.45]
Subtotal (95% CI)			**64**			**64**	**100.0%**	**-0.10 [-0.65 , 0.45]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.35 (P = 0.72)								
1.34.3 4 years postpartum								
Laitinen 2009	24.3	2	43	23.6	2	37	100.0%	0.70 [-0.18 , 1.58]
Subtotal (95% CI)			**43**			**37**	**100.0%**	**0.70 [-0.18 , 1.58]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.56 (P = 0.12)								



Analysis 1.35. Comparison 1: Probiotics versus placebo, Outcome 35: Stillbirth

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	0	207	1	204	30.0%	0.33 [0.01 , 8.02]
Laitinen 2009	0	82	0	80		Not estimable
Lindsay 2014	0	62	0	74		Not estimable
Okesene-Gafa 2019	2	115	2	115	39.7%	1.00 [0.14 , 6.98]
Pellonpera 2019	0	96	1	93	30.3%	0.32 [0.01 , 7.83]
Total (95% CI)		**562**		**566**	**100.0%**	**0.59 [0.14 , 2.46]**
Total events:	2		4			

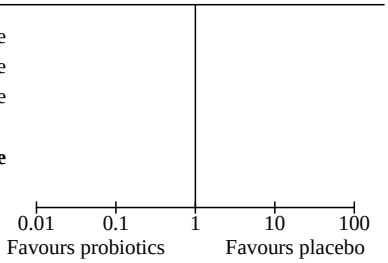
Heterogeneity: Chi² = 0.55, df = 2 (P = 0.76); I² = 0%
Test for overall effect: Z = 0.72 (P = 0.47)
Test for subgroup differences: Not applicable



Analysis 1.36. Comparison 1: Probiotics versus placebo, Outcome 36: Neonatal mortality

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	0	207	0	204		Not estimable
Laitinen 2009	0	82	0	80		Not estimable
Lindsay 2014	0	62	0	74		Not estimable
Total (95% CI)		**351**		**358**		**Not estimable**
Total events:	0		0			

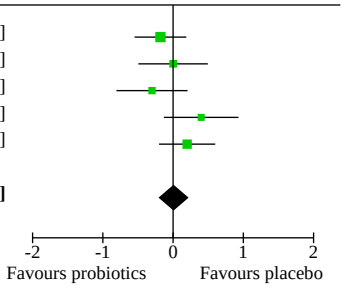
Heterogeneity: Not applicable
Test for overall effect: Not applicable
Test for subgroup differences: Not applicable



Analysis 1.37. Comparison 1: Probiotics versus placebo, Outcome 37: Gestational age at birth (weeks)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Callaway 2019	39.14	1.88	193	39.32	1.75	180	29.3%	-0.18 [-0.55 , 0.19]
Laitinen 2009	39.9	1.3	75	39.9	1.8	79	16.3%	0.00 [-0.49 , 0.49]
Lindsay 2014	40	1.5	62	40.3	1.5	74	15.5%	-0.30 [-0.81 , 0.21]
Okesene-Gafa 2019	39.3	1.7	110	38.9	2.3	112	14.1%	0.40 [-0.13 , 0.93]
Pellonpera 2019	39.8	1.4	96	39.6	1.4	92	24.8%	0.20 [-0.20 , 0.60]
Total (95% CI)			**536**			**537**	**100.0%**	**0.01 [-0.19 , 0.21]**

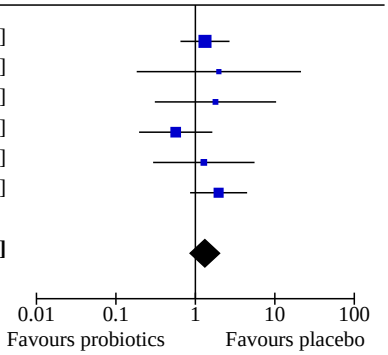
Heterogeneity: Chi² = 5.40, df = 4 (P = 0.25); I² = 26%
Test for overall effect: Z = 0.07 (P = 0.95)
Test for subgroup differences: Not applicable



Analysis 1.38. Comparison 1: Probiotics versus placebo, Outcome 38: Preterm birth

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	17	193	12	180	35.2%	1.32 [0.65 , 2.69]
Laitinen 2009	2	80	1	79	2.8%	1.98 [0.18 , 21.34]
Lindsay 2014	3	62	2	74	5.2%	1.79 [0.31 , 10.38]
Okesene-Gafa 2019	5	110	9	112	25.3%	0.57 [0.20 , 1.63]
Pellonpera 2019	4	96	3	92	8.7%	1.28 [0.29 , 5.55]
Wickens 2017	16	205	8	201	22.9%	1.96 [0.86 , 4.48]
Total (95% CI)		**746**		**738**	**100.0%**	**1.32 [0.86 , 2.01]**
Total events:	47		35			

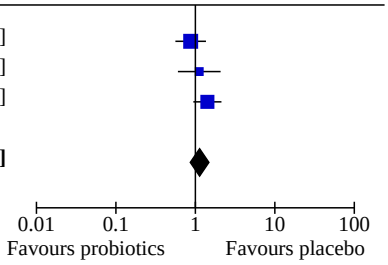
Heterogeneity: Chi² = 3.56, df = 5 (P = 0.61); I² = 0%
Test for overall effect: Z = 1.27 (P = 0.21)
Test for subgroup differences: Not applicable



Analysis 1.39. Comparison 1: Probiotics versus placebo, Outcome 39: Macrosomia

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	31	206	35	203	43.0%	0.87 [0.56 , 1.36]
Lindsay 2014	15	62	16	74	17.8%	1.12 [0.60 , 2.08]
Wickens 2017	46	205	32	202	39.3%	1.42 [0.94 , 2.13]
Total (95% CI)		**473**		**479**	**100.0%**	**1.13 [0.86 , 1.48]**
Total events:	92		83			

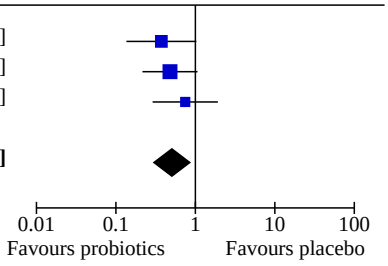
Heterogeneity: Chi² = 2.49, df = 2 (P = 0.29); I² = 20%
Test for overall effect: Z = 0.89 (P = 0.37)
Test for subgroup differences: Not applicable



Analysis 1.40. Comparison 1: Probiotics versus placebo, Outcome 40: Small-for-gestational age

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	5	205	13	199	33.6%	0.37 [0.14 , 1.03]
Okesene-Gafa 2019	8	110	17	112	42.9%	0.48 [0.22 , 1.06]
Pellonpera 2019	7	96	9	92	23.4%	0.75 [0.29 , 1.92]
Total (95% CI)		**411**		**403**	**100.0%**	**0.51 [0.30 , 0.85]**
Total events:	20		39			

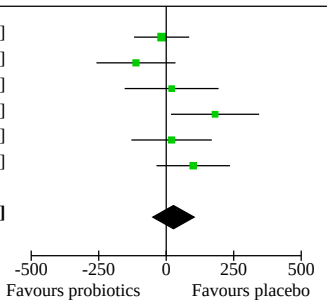
Heterogeneity: Chi² = 1.01, df = 2 (P = 0.60); I² = 0%
Test for overall effect: Z = 2.57 (P = 0.01)
Test for subgroup differences: Not applicable



Analysis 1.41. Comparison 1: Probiotics versus placebo, Outcome 41: Birthweight (g)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Random, 95% CI
Callaway 2019	3524	540	206	3541	514	203	23.3%	-17.00 [-119.16 , 85.16]
Laitinen 2009	3467	448	82	3579	500	80	16.2%	-112.00 [-258.31 , 34.31]
Lindsay 2014	3700	520	62	3680	510	74	13.0%	20.00 [-153.94 , 193.94]
Okesene-Gafa 2019	3685	565	110	3504	672	112	14.1%	181.00 [17.79 , 344.21]
Pellonpera 2019	3620	539	96	3600	503	92	15.8%	20.00 [-128.96 , 168.96]
Wickens 2017	3600	700	205	3500	700	202	17.6%	100.00 [-36.02 , 236.02]
Total (95% CI)			**761**			**763**	**100.0%**	**26.87 [-49.52 , 103.26]**

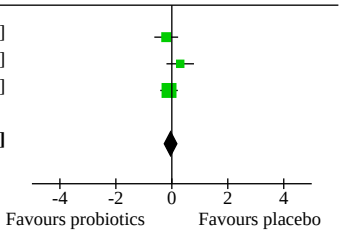
Heterogeneity: Tau² = 3814.67; Chi² = 8.69, df = 5 (P = 0.12); I² = 42%
Test for overall effect: Z = 0.69 (P = 0.49)
Test for subgroup differences: Not applicable



Analysis 1.42. Comparison 1: Probiotics versus placebo, Outcome 42: Head circumference (cm)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Laitinen 2009	34.8	1.39	82	35	1.37	80	27.9%	-0.20 [-0.63 , 0.23]
Okesene-Gafa 2019	35.5	1.6	109	35.2	2.1	112	20.8%	0.30 [-0.19 , 0.79]
Wickens 2017	35.3	1.8	205	35.4	1.4	201	51.3%	-0.10 [-0.41 , 0.21]
Total (95% CI)			**396**			**393**	**100.0%**	**-0.04 [-0.27 , 0.18]**

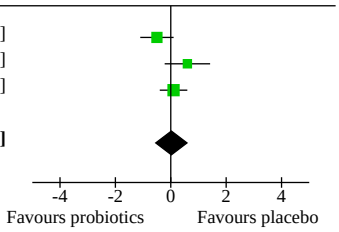
Heterogeneity: Chi² = 2.52, df = 2 (P = 0.28); I² = 21%
Test for overall effect: Z = 0.39 (P = 0.70)
Test for subgroup differences: Not applicable



Analysis 1.43. Comparison 1: Probiotics versus placebo, Outcome 43: Length (cm)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Random, 95% CI
Laitinen 2009	50.7	1.85	82	51.2	2.05	80	34.6%	-0.50 [-1.10 , 0.10]
Okesene-Gafa 2019	51.3	2.9	109	50.7	3.3	111	25.9%	0.60 [-0.22 , 1.42]
Wickens 2017	51.3	2.6	205	51.2	2.5	199	39.5%	0.10 [-0.40 , 0.60]
Total (95% CI)			**396**			**390**	**100.0%**	**0.02 [-0.54 , 0.59]**

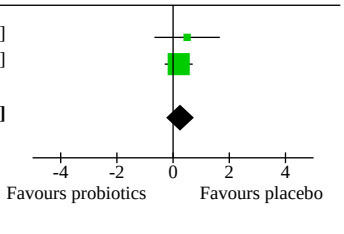
Heterogeneity: Tau² = 0.14; Chi² = 4.86, df = 2 (P = 0.09); I² = 59%
Test for overall effect: Z = 0.08 (P = 0.94)
Test for subgroup differences: Not applicable



Analysis 1.44. Comparison 1: Probiotics versus placebo, Outcome 44: Ponderal index (kg/m³)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Lindsay 2014	28.1	3.4	62	27.6	3.5	74	15.5%	0.50 [-0.66 , 1.66]
Wickens 2017	25.9	2.6	204	25.7	2.5	199	84.5%	0.20 [-0.30 , 0.70]
Total (95% CI)			**266**			**273**	**100.0%**	**0.25 [-0.21 , 0.70]**

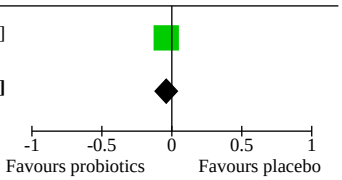
Heterogeneity: Chi² = 0.22, df = 1 (P = 0.64); I² = 0%
Test for overall effect: Z = 1.06 (P = 0.29)
Test for subgroup differences: Not applicable



Analysis 1.45. Comparison 1: Probiotics versus placebo, Outcome 45: Adiposity – fat mass (kg)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Okesene-Gafa 2019	0.4	0.18	57	0.44	0.24	53	100.0%	-0.04 [-0.12 , 0.04]
Total (95% CI)			**57**			**53**	**100.0%**	**-0.04 [-0.12 , 0.04]**

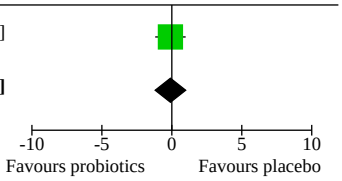
Heterogeneity: Not applicable
Test for overall effect: Z = 0.98 (P = 0.33)
Test for subgroup differences: Not applicable



Analysis 1.46. Comparison 1: Probiotics versus placebo, Outcome 46: Adiposity – % fat

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Callaway 2019	12.2	4.4	105	12.3	3.6	105	100.0%	-0.10 [-1.19 , 0.99]
Total (95% CI)			**105**			**105**	**100.0%**	**-0.10 [-1.19 , 0.99]**

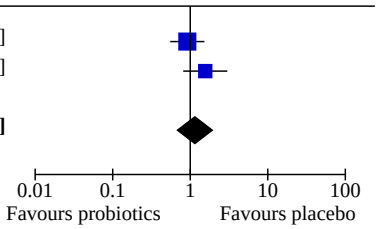
Heterogeneity: Not applicable
Test for overall effect: Z = 0.18 (P = 0.86)
Test for subgroup differences: Not applicable



Analysis 1.47. Comparison 1: Probiotics versus placebo, Outcome 47: Hypoglycaemia

Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
Callaway 2019	25	202	27	200	57.8%	0.92 [0.55 , 1.52]
Pellonpera 2019	20	95	12	89	42.2%	1.56 [0.81 , 3.00]
Total (95% CI)		**297**		**289**	**100.0%**	**1.15 [0.69 , 1.92]**
Total events:	45		39			

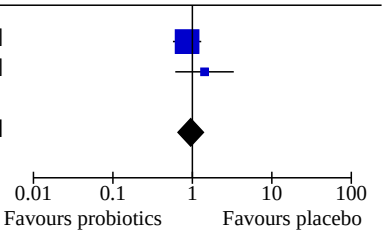
Heterogeneity: Tau² = 0.05; Chi² = 1.59, df = 1 (P = 0.21); I² = 37%
Test for overall effect: Z = 0.52 (P = 0.60)
Test for subgroup differences: Not applicable



Analysis 1.48. Comparison 1: Probiotics versus placebo, Outcome 48: Hyperbilirubinaemia

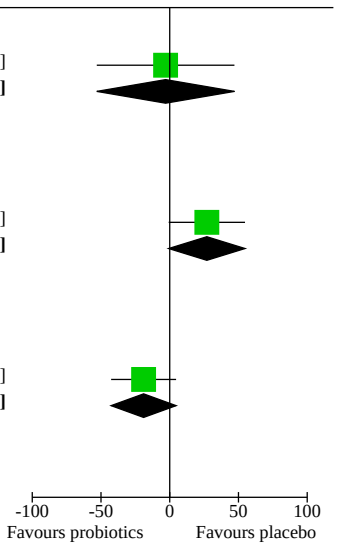
Study or Subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Callaway 2019	35	205	40	201	83.1%	0.86 [0.57 , 1.29]
Pellonpera 2019	12	96	8	91	16.9%	1.42 [0.61 , 3.32]
Total (95% CI)		**301**		**292**	**100.0%**	**0.95 [0.66 , 1.38]**
Total events:	47		48			

Heterogeneity: Chi² = 1.11, df = 1 (P = 0.29); I² = 10%
Test for overall effect: Z = 0.26 (P = 0.80)
Test for subgroup differences: Not applicable



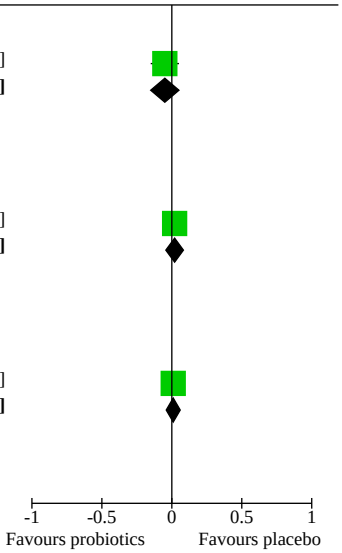
Analysis 1.49. Comparison 1: Probiotics versus placebo, Outcome 49: Infant weight gain (g/month)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
1.49.1 at 0–6 months								
Laitinen 2009	759	160	82	762	165	80	100.0%	-3.00 [-53.07 , 47.07]
Subtotal (95% CI)			**82**			**80**	**100.0%**	**-3.00 [-53.07 , 47.07]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.12 (P = 0.91)								
1.49.2 at 6–12 months								
Laitinen 2009	323	80	82	296	99	80	100.0%	27.00 [-0.76 , 54.76]
Subtotal (95% CI)			**82**			**80**	**100.0%**	**27.00 [-0.76 , 54.76]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.91 (P = 0.06)								
1.49.3 at 12–24 months								
Laitinen 2009	211	76	67	230	61	63	100.0%	-19.00 [-42.62 , 4.62]
Subtotal (95% CI)			**67**			**63**	**100.0%**	**-19.00 [-42.62 , 4.62]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.58 (P = 0.11)								



Analysis 1.50. Comparison 1: Probiotics versus placebo, Outcome 50: Infant height (cm/month)

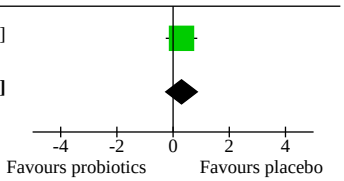
Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
1.50.1 at 0–6 months								
Laitinen 2009	2.84	0.35	78	2.89	0.29	78	100.0%	-0.05 [-0.15 , 0.05]
Subtotal (95% CI)			**78**			**78**	**100.0%**	**-0.05 [-0.15 , 0.05]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.97 (P = 0.33)								
1.50.2 at 6–12 months								
Laitinen 2009	1.4	0.19	78	1.38	0.21	78	100.0%	0.02 [-0.04 , 0.08]
Subtotal (95% CI)			**78**			**78**	**100.0%**	**0.02 [-0.04 , 0.08]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.62 (P = 0.53)								
1.50.3 at 12–24 months								
Laitinen 2009	0.95	0.14	67	0.94	0.15	63	100.0%	0.01 [-0.04 , 0.06]
Subtotal (95% CI)			**67**			**63**	**100.0%**	**0.01 [-0.04 , 0.06]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.39 (P = 0.69)								



Analysis 1.51. Comparison 1: Probiotics versus placebo, Outcome 51: Infant head circumference – 6 months (cm)

Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Laitinen 2009	44.3	1.7	58	44	1.4	61	100.0%	0.30 [-0.26 , 0.86]
Total (95% CI)			**58**			**61**	**100.0%**	**0.30 [-0.26 , 0.86]**

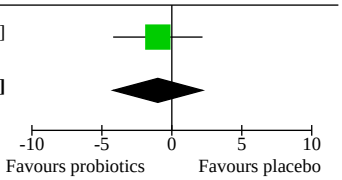
Heterogeneity: Not applicable
Test for overall effect: Z = 1.05 (P = 0.29)
Test for subgroup differences: Not applicable



Analysis 1.52. Comparison 1: Probiotics versus placebo, Outcome 52: Infant mean blood pressure – 6 months (mmHg)

Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Laitinen 2009	76	9.7	58	77	7.6	56	100.0%	-1.00 [-4.19 , 2.19]
Total (95% CI)			**58**			**56**	**100.0%**	**-1.00 [-4.19 , 2.19]**

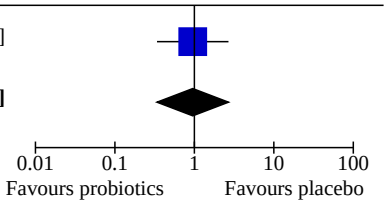
Heterogeneity: Not applicable
Test for overall effect: Z = 0.61 (P = 0.54)
Test for subgroup differences: Not applicable



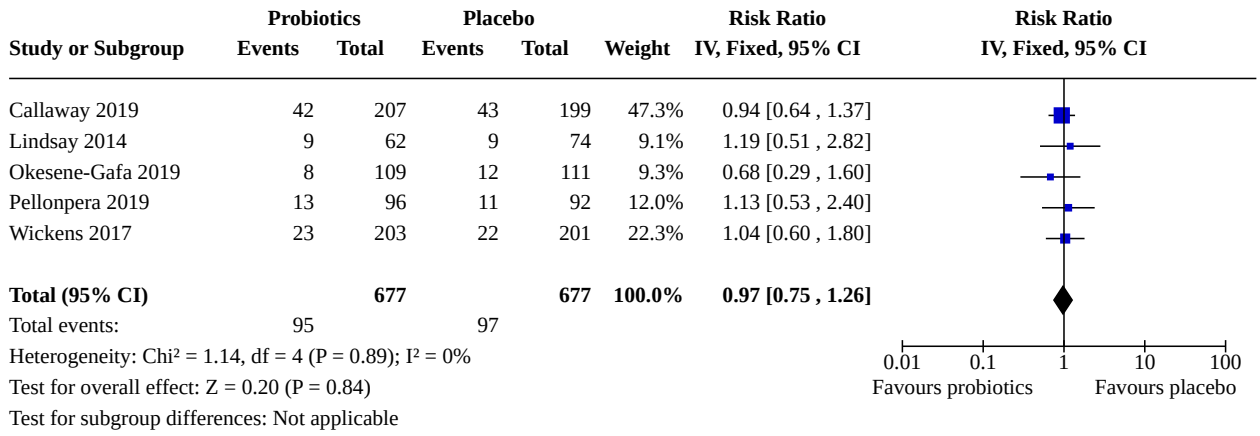
Analysis 1.53. Comparison 1: Probiotics versus placebo, Outcome 53: 32–33 split proinsulin > 85th percentile – 6 months

Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Laitinen 2009	6	62	7	69	100.0%	0.95 [0.34 , 2.69]
Total (95% CI)		**62**		**69**	**100.0%**	**0.95 [0.34 , 2.69]**
Total events:	6		7			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.09 (P = 0.93)
Test for subgroup differences: Not applicable



Analysis 1.54. Comparison 1: Probiotics versus placebo, Outcome 54: Neonatal intensive care unit admission



ADDITIONAL TABLES

Table 1. Diagnostic criteria for GDM

Parameter	IADPSG (IADPSG 2010)	Carpenter and Coustan (Carpenter 1982)	Modified Fourth International Workshop-Conference (Metzger 1998)	New Zealand Guidelines (Ministry of Health 2014)	Finnish Current Care Guidelines (The Finnish Medical Society Duodecim 2013)
OGTT (g)	75	100	75	75	75
Fasting (mmol/L)	5.1	5.3	4.8	5.5	5.3
1 hour (mmol/L)	10.0	10.0	10.0	—	10.0
2 hours (mmol/L)	8.5	8.6	8.7	9.0	8.6
3 hours (mmol/L)	—	7.8	—	—	—
Elevated values required	1	2	1	1	1

IADPSG: International Association of Diabetes and Pregnancy Study Groups; OGTT: oral glucose tolerance test.

APPENDICES

Appendix 1. Search methods for ICTRP and ClinicalTrials.gov

ICTRP

(each line was run separately)

probiotics AND pregnancy

probiotics AND pregnant

ClinicalTrials.gov

Advanced search

Interventional Studies | Pregnancy | probiotics

WHAT'S NEW

Date	Event	Description
20 March 2020	New citation required and conclusions have changed	In the previous version of the review, only 1 study was included that showed a decrease in the risk of gestational diabetes mellitus with probiotics. For this updated review, we identified 6 new studies that were eligible for inclusion that brought the total participant count from 256 to 1647. The meta-analysis performed on this much larger body of evidence revealed no clear difference in the risk of gestational diabetes with probiotics compared to placebo.
20 March 2020	New search has been performed	Search updated and 6 new studies found to be eligible for inclusion.

HISTORY

Protocol first published: Issue 7, 2012
Review first published: Issue 2, 2014

CONTRIBUTIONS OF AUTHORS

HB and MDN developed the protocol. LC edited and commented on the protocol. HB and MDN wrote the review, assessed the citations and studies found for inclusion, risk of bias and data analysis. LC assisted with data interpretation, and edited and commented on the review.

Contributions for 2019/20 update

SJD developed the updated protocol.

HB, MDN and LC edited and commented on the updated protocol.

SJD and MDN assessed studies for inclusion and risk of bias and collected data for most studies.

SJD and SAP assessed Callaway 2019 for inclusion and risk of bias and collected data to limit conflict of interest.

SJD wrote the updated review.

MDN, HB, LC and SAP assisted with data interpretation, and edited and commented on the review.

DECLARATIONS OF INTEREST

SJD: none.

HB: recieved a grant from the NHMRC, Australia (a competitive government research grant) to undertake a randomised control trial of probiotics for the prevention of gestational diabetes mellitus and I was an associate investigator this trial (Callaway 2019). In this review, HB was not involved in any decisions relating to this trial: assessment of the trial for inclusion, assessment of risk of bias and data extraction were carried out by individuals who were not directly involved in the trial. SJD and SAP carried out these tasks. Chr. Hansen A/S have donated the probiotics and matching placebo to the SPRING study (Callaway 2019) that was conducted by authors Barrett, Dekker Nitert and Callaway. While the authors are grateful for this donation from Chr.Hansen A/S, the conduct of the study, analysis and publication of the results is entirely independent of Chr. Hansen A/S.

SAP: none.

LC: was chief investigator in a trial examining the use of probiotics for preventing gestational diabetes mellitus (Callaway 2019), which was funded from a grant from the NHMRC, Australia (a competitive government research grant). In this review, LC was not involved in any decisions relating to this trial: assessment of the trial for inclusion, assessment of risk of bias and data extraction were carried out by individuals who were not directly involved in the trial. SJD and SAP carried out these tasks. Chr. Hansen A/S have donated the probiotics and matching placebo to the SPRING study (Callaway 2019) that was conducted by authors Barrett, Dekker Nitert and Callaway. While the

authors are grateful for this donation from Chr.Hansen A/S, the conduct of the study, analysis and publication of the results is entirely independent of Chr. Hansen A/S.

MDN: was scientific lead in a trial examining the use of probiotics for preventing gestational diabetes mellitus (Callaway 2019), which was funded from a grant from the NHMRC, Australia (a competitive government research grant). In this review, MDN was not involved in any decisions relating to this trial: assessment of the trial for inclusion, assessment of risk of bias and data extraction were carried out by individuals who were not directly involved in the trial. SJD and SAP carried out these tasks. Chr. Hansen A/S have donated the probiotics and matching placebo to the SPRING study (Callaway 2019) that was conducted by authors Barrett, Dekker Nitert and Callaway. While the authors are grateful for this donation from Chr.Hansen A/S, the conduct of the study, analysis and publication of the results is entirely independent of Chr. Hansen A/S.

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- University of Melbourne, Australia

  salary
- Mater Health, Australia

  salary

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  The Mater Foundation provided support for Helen L Barrett's work on this review.
- NHMRC Early Career Research Fellowship, Australia

  Helen L Barrett is the recipient of an NHMRC Early Career Research Fellowship (APP1120070), which provided support for her work on this review.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In this review update, we added in an additional search of ClinicalTrials.gov, the World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch/). We have also updated our outcomes to be in line with the Cochrane Pregnancy and Childbirth GDM Standardised Outcome Set, and we have incorporated 'Summary of findings' tables using GRADE to evaluate the evidence. Finally, we split our comparison into probiotics versus placebo and probiotics versus diet in place of probiotics versus all other interventions.

We also assessed all previously included eligible studies using Cochrane Pregnancy and Childbirth's trustworthiness screening tool.

INDEX TERMS

Medical Subject Headings (MeSH)

Bias; Cesarean Section [statistics & numerical data]; Diabetes, Gestational [*prevention & control]; Obesity; Overweight; Placebos [therapeutic use]; Pre-Eclampsia [*etiology]; Probiotics [adverse effects] [*therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Female; Humans; Pregnancy