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Probiotic treatment for women with gestational diabetes to improve maternal and infant health and well-being (Review)

Okesene-Gafa KAM, Moore AE, Jordan V, McCowan L, Crowther CA

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[Intervention Review]

Probiotic treatment for women with gestational diabetes to improve maternal and infant health and well-being

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ABSTRACT

Background

Gestational diabetes mellitus (GDM) is carbohydrate intolerance first recognised during pregnancy and associated with complications for mothers and babies. Probiotics are naturally occurring micro-organisms, which when ingested in adequate amounts, may confer health benefits. Evidence of the role of probiotics as treatment for GDM is limited.

Objectives

To evaluate the safety and effectiveness of probiotics in treating women with GDM on maternal and infant outcomes.

Search methods

We searched the Cochrane Pregnancy and Childbirth's Trials Register ClinicalTrials.gov, WHO International Clinical Trials Registry Platform (ICTRP) (24 July 2019), and reference lists of retrieved studies.

Selection criteria

Randomised controlled trials (RCTs) comparing the use of probiotics versus placebo/standard care for the treatment of GDM.

Data collection and analysis

Two review authors independently assessed study eligibility, extracted data, checked data accuracy, and assessed risk of bias of included trials. The certainty of evidence for selected maternal and infant/child outcomes was assessed using GRADE.

Main results

Nine RCTs (695 pregnant women with GDM) comparing probiotics versus placebo were identified. The overall risk of bias in the nine RCTs was low to unclear and the evidence was downgraded for imprecision due to the small numbers of women participating in the trials. The trials were carried out in hospitals and universities in Iran (seven trials), Thailand (one trial) and Ireland (one trial). All trials compared probiotics with placebo.

Maternal outcomes

We are uncertain if probiotics have any effect compared with placebo on hypertensive disorders of pregnancy, (risk ratio (RR) 1.50, 95% confidence interval (CI) 0.64 to 3.53; participants = 256; studies = 3; low-certainty evidence) and mode of birth as caesareans (average RR 0.64, 95% CI 0.30 to 1.35; participants = 267; studies = 3; low-certainty evidence) because the certainty of evidence is low and the 95% CIs span possible benefit and possible harm.

No trials reported primary outcomes of: mode of birth as vaginal/assisted and subsequent development of type 2 diabetes.

We are uncertain if probiotics have any effect compared with placebo on induction of labour (RR 1.33, 95% CI 0.74 to 2.37; participants = 127; studies = 1; very low-certainty evidence).

For other secondary maternal outcomes, we are uncertain if there are differences between probiotics and placebo for: postpartum haemorrhage; weight gain during pregnancy intervention and total gestational weight gain; fasting plasma glucose and need for extra pharmacotherapy (insulin). Probiotics may be associated with a slight reduction in triglycerides and total cholesterol.

In probiotics compared with placebo, there was evidence of reduction in markers for insulin resistance (HOMA-IR) and HOMA-B; and insulin secretion. There was also an increase in quantitative insulin sensitivity check index (QUICKI).

Probiotics were associated with minor benefits in relevant bio-markers with evidence of a reduction in inflammatory markers high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), and marker of oxidative stress malondialdehyde; and an increase in antioxidant total glutathione, but we are uncertain if there is any difference in total antioxidant capacity.

No trials reported secondary outcomes: perineal trauma, postnatal weight retention or return to pre-pregnancy weight and postnatal depression.

Infant/child/adult outcomes

We are uncertain if probiotics have any effect, compared with placebo, on the risk of large-for-gestational-age babies (RR 0.73, 95% CI 0.35 to 1.52; participants = 174; studies = 2; low-certainty evidence) or infant hypoglycaemia (RR 0.85, 95% CI 0.39 to 1.84; participants = 177; studies = 3; low-certainty evidence) because the certainty of evidence is low and the 95% CIs span possible benefit and possible harm.

No trials reported primary outcomes of: perinatal (fetal/neonatal) mortality; or neurosensory disability.

For other secondary outcomes, we are uncertain if there is any difference between probiotics and placebo in gestational age at birth, preterm birth, macrosomia, birthweight, head circumference, length, infant hypoglycaemia, and neonatal intensive care unit (NICU) admissions.

There was evidence of a reduction in infant hyperbilirubinaemia with probiotics compared with placebo.

No trials reported secondary outcomes: infant adiposity, and later childhood adiposity.

There were no adverse events reported by any of the trials.

Authors' conclusions

Low-certainty evidence means we are not certain if there is any difference between probiotic and placebo groups in maternal hypertensive disorders of pregnancy, caesareans; and large-for-gestational-age babies.

There were no adverse events reported by the trials.

Due to the variability of probiotics used and small sample sizes of trials, evidence from this review has limited ability to inform practice. Well-designed adequately-powered trials are needed to identify whether probiotics may improve maternal blood glucose levels and/or infant/child/adult outcomes; and whether they can be used to treat GDM.

PLAIN LANGUAGE SUMMARY

Probiotics as an added treatment for gestational diabetes to improve mother and baby outcomes

What is the issue?

Gestational diabetes mellitus (GDM) is carbohydrate intolerance resulting in high blood glucose levels, first recognised during pregnancy. Pregnant women with GDM are at risk of high blood pressure, labour induction, and caesareans. Their babies are at risk of being born large, birth difficulties, respiratory distress, low blood glucose at birth and jaundice that can cause brain injury. There is increased risks of having long-term diabetes in the mother, and the baby being overweight. Probiotics are micro-organisms naturally in food and are in fermented milk, yogurt, or capsules. There are many different probiotics; the two most used are *Lactobacillus* and *Bifidobacterium,* and if consumed in adequate amounts may confer health benefits.

Why is this important?

Probiotics need to be safe and maternal blood glucose levels carefully managed during pregnancy.

Women with GDM may receive dietary and physical activity education with monitoring blood glucose levels as initial management. When blood glucose levels are above a certain threshold, women with GDM are prescribed glucose-lowering medications including metformin and/or insulin. This review aimed to determine the safety and effectiveness of probiotics in treating women with GDM.

What evidence did we find?

We searched for evidence for randomised controlled trials (latest July 2019). We identified nine studies, involving 695 women with GDM. All trials compared probiotics with placebo. The certainty of the evidence was assessed as very low or low. The overall risk of bias was low to unclear.

Seven trials were conducted in Iran; one in Thailand, and one in Ireland. Trials took place in hospitals and universities.

We are uncertain if there is any difference between probiotic and placebo in rates of: high blood-pressure disorders (three studies, 256 participants, low-certainty evidence); caesarean section (three studies, 267 women, low-certainty evidence*);* and large-for-gestational-age babies (two studies, 174 participants, low-certainty evidence).

We are uncertain if there is any difference between probiotic and placebo for induction of labour (one study, 127 participants, very low-certainty evidence) and low blood glucose levels in the newborn (three studies, 177 participants, low-certainty evidence). We are also uncertain if there is any difference between probiotics and placebo for heavy bleeding immediately after birth, weight gain during pregnancy or total gestational weight gain.

We are uncertain if there is any difference in fasting blood glucose between probiotics and placebo (seven studies, 554 participants). Probiotics may be assoicated with a slight reduction in triglycerides and total cholesterol (four studies, 320 participants). There was reduction in insulin secretion with probiotics (seven studies, 505 participants). One trial (60 participants) showed no difference between groups in need for insulin.

Biomarkers, did show a reduction in insulin resistance (HOMA-IR), (seven studies, 505 participants) and insulin resistance and β cell function (HOMA-B) (two studies,130 participants) with probiotics. Quantitative insulin sensitivity check index (QUICKI) increased (four studies, 276 participants) with probiotics.

Inflammatory markers, hs-CRP (four studies, 248 participants) and interleukin 6 (two studies, 128 participants) were reduced with probiotics. Antioxidant total glutathione was increased (two studies, 120 participants) and the oxidative stress biomarker malondialdehyde was reduced with probiotics (three studies, 176 participants). We are uncertain if there is any difference in total antioxidant capacity (four studies 266 participants).

For the newborn baby, we are uncertain if there is any difference between groups for: birthweight, gestational age at birth, preterm births, large babies, head circumference and length scores, or need for admission to the neonatal intensive care unit. The number of babies with high levels of bilirubin was reduced with probiotics.

No adverse events were reported by the trials.

What does this mean?

Based on the clinical trials available, the evidence is limited to support the use of probiotics as treatment for women with GDM to improve pregnancy outcomes for mothers and their babies. Larger well-designed randomised controlled trials are needed to assess the effects of probiotics on management of glucose levels and when available, they can be included in the update of this review.

Summary of findings 1. Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and infant health and well-being - maternal outcomes

Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and fetal health and well-being - maternal outcomes

Patient or population: pregnant women diagnosed with gestational diabetes
Setting: Iran (8), Ireland (1), Thailand (1)
Intervention: probiotics (any type) administered by any route given during pregnancy to treat women with gestational diabetes
Comparison: placebo (similar appearance and taste to the probiotics) or standard care

Outcomes	№ of participants (studies) Follow up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with placebo	Risk difference with probiotic
Hypertensive disorders (including pre-eclampsia, pregnancy-induced hypertension, eclampsia)	256 (3 RCTs)	⊕⊕⊖⊖ LOW [1]	RR 1.50 (0.64 to 3.53)	Study population	
				63 per 1000	26 more per 1000 (26 fewer to 151 more)
Subsequent development of type 2 diabetes	(0 studies)		not estimable	No outcome data reported in the included studies.	
Mode of birth (caesarean)	267 (3 RCTs)	⊕⊕⊖⊖ LOW [2 3]	RR 0.64 (0.30 to 1.35)	Study population	
				351 per 1000	224 fewer per 1000 (105 fewer to 474 more)
Induction of labour	127 (1 RCT)	⊕⊖⊖⊖ VERY LOW [4]	RR 1.33 (0.74 to 2.37)	Study population	
				231 per 1000	76 more per 1000 (60 fewer to 316 more)
Perineal trauma	(0 studies)		not estimable	No outcome data reported in the included studies.	
Postnatal weight retention or return to pre-pregnancy weight	(0 studies)		not estimable	No outcome data reported in the included studies.	
Postnatal depression	(0 studies)		not estimable	No outcome data reported in the included studies.	

*__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; **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.

[1] Downgraded two levels due to serious concerns related to imprecision as only has 3 small studies with wide confidence intervals.
[2] Downgraded one level due to serious concerns related to imprecision as only has 3 small studies with wide confidence intervals.
[3] Downgraded one level due to serious concerns related to inconsistency as I^2 of 69%, studies showed different findings.
[4] Downgraded two levels due to serious concerns related to imprecision as only one small study with wide confidence intervals. We downgraded for indirectness as the population of one study will not reflect population of all women with GDM.

Summary of findings 2. Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and infant health and well-being- infant/child/adult outcome

Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and infant health and well-being - infant/child/adult outcomes

Patient or population: pregnant women diagnosed with gestational diabetes
Setting: Iran (1), Ireland (1)
Intervention: probiotic
Comparison: placebo

Outcomes	№ of participants (studies) Follow up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with placebo	Risk difference with probiotic
Perinatal (fetal and neonatal) mortality	(0 studies)		not estimable	No outcome data reported in the included studies.	
Large-for-gestational age > 90 centile	174 (2 RCTs)	⊕⊕⊖⊖ LOW [1]	RR 0.73 (0.35 to 1.52)	Study population	
				159 per 1000	43 fewer per 1000 (103 fewer to 83 more)
Composite serious neonatal outcomes (variously defined by trials, e.g. infant death, shoulder dystocia, bone fracture, or nerve palsy	(0 studies)		not estimable	No data reported for composite serious neonatal outcomes (variously defined by trials, e.g. infant death, shoulder dystocia, bone fracture, or nerve palsy in any of the included studies.	

Neurosensory disability	(0 studies)		not estimable	No outcome data reported in the included studies.	
Neonatal hypoglycaemia requiring treatment (variously defined)	177 (3 RCTs)	⊕⊕⊖⊖ LOW [1]	RR 0.85 (0.39 to 1.84)	Study population	
				135 per 1000	20 fewer per 1000 (82 fewer to 113 more)
Adiposity (neonatal/child/child as an adult)	(0 studies)		not estimable	No outcome data reported in the included studies.	
Diabetes(type1 or type2) or impaired glucose tolerance (child/adult)	(0 studies)		not estimable	No outcome data reported in the included studies.	

*__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; **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

[1] Downgraded two levels due to serious concerns related to imprecision as only has 2 small studies with wide confidence intervals.

BACKGROUND

Description of the condition

Gestational diabetes (GDM) is defined as "carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy" (Alberti 1998). The prevalence of GDM is thought to vary from 1.5% to 14% worldwide and varies between ethnic groups (ACOG 2001; Dabelea 2005; Ekeroma 2015; Ferrara 2007; Poston 2013), and countries or institutions depending on the diagnostic criteria for GDM being used (ADA 2010; Diabetes Care 2010; Ekeroma 2015; NICE 2015). The global epidemic of obesity (a risk factor for GDM) is continuing to rise in developed and developing countries (Swinburn 2011), with the concomitant increase in rates of pregnancy complications (WHO 2016), including GDM. Health risks for women with GDM include pre-eclampsia, induction of labour (Crowther 2005), caesarean section, and over half of women with GDM will develop type 2 diabetes within 10 years of the birth (Kim 2002). The risks for their infants include macrosomia (baby born much larger than average), respiratory distress syndrome, birth injuries such as nerve palsy, bone fracture and shoulder dystocia, jaundice, and hypoglycaemia, which if prolonged or severe can cause brain injury (Crowther 2005; Landon 2009). In addition, there is increasing recognition of the association between intrauterine fetal programming effects with adverse long-term health consequences for the infant, creating a vicious intergenerational cycle of obesity, diabetes, and metabolic syndrome (Boney 2005; Dabelea 2005).

Description of the intervention

Probiotics are micro-organisms that naturally occur in foods and when consumed in adequate amounts may confer health benefits for the host (FAO 2001). Probiotics are usually found in fermented milk products, yogurt or dietary supplements as well as in capsules. There are many different types of probiotics and the two most widely used genera are *Lactobacillus* and *Bifidobacterium* (Laitinen 2009).

The gut microbiota (micro-organisms that colonise the gut) have the potential to influence obesity and type 2 diabetes through modification of energy extraction, inflammation, hunger and satiety, as well as lipid and glucose metabolism (Flint 2012; Nieuwdorp 2014; Turnbaugh 2006). Type 2 diabetes has been associated with changes in the gut microbiome (Larsen 2010). Obese women have also been identified to have a different gut microbiome compared to lean women (Nieuwdorp 2014; Turnbaugh 2006). Gut microbiota differences also exist between pregnant overweight and normal weight women (Collado 2008), as well as in the third trimester of pregnancy compared to the first trimester, with the third trimester microbiome being similar to non pregnant individuals with metabolic syndrome (Koren 2012). Supplementation with probiotics has been shown to improve glycaemic control in men and women with type 2 diabetes (Andreasen 2010; Ejtahed 2012). Probiotics have been shown to prevent GDM in a sample of pregnant women in a general population (Luoto 2010), and probiotics with dietary counselling reduced mean plasma glucose concentrations and improved insulin sensitivity in another study of healthy pregnant women both antenatally and postpartum (Laitinen 2009). Probiotic milk products reduced pre-eclampsia in a large Norwegian cohort study (Braentsaeter 2011) and are considered safe to use in pregnancy (Allen 2010; Elias 2011). Probiotic capsules (*Lactobacillus rhamnosus*) in a double-blind randomised controlled trial showed significant and sustainable weight loss in obese non pregnant women (Sanchez 2014). A larger randomised controlled trial of probiotic versus placebo in pregnant women in Australia (Nitert 2013), to determine whether probiotics can prevent GDM in overweight and obese women has recently been published (Callaway 2019). A systematic review and meta-analysis looking at the effect of treatment of GDM on pregnancy outcomes showed that treatment significantly reduced the risks of fetal macrosomia, large-for-gestational-age births, shoulder dystocia and gestational hypertension, as well as a tendency to reduction of perinatal/neonatal mortality and birth trauma (Poolsup 2014). A Cochrane Review of probiotics for prevention of GDM included one study that reported lower rates of women diagnosed with GDM and lower birthweight with probiotics (Barrett 2014). GDM treatment to date has mostly comprised of dietary and glucose-lowering agents either insulin and or tablets (biguanides or second-generation sulphonylureas) (Coustan 2013). The role of probiotics in treating pregnant women with GDM has yet to be clearly established.

How the intervention might work

Probiotics in the 1960s were hypothesised to have the beneficial effects of producing substances that may promote the growth of other micro-organisms and was further defined in the 1980s as a microbial feed supplement that improves the intestinal balance of the host (FAO 2001). The discovery of the gut microbiome and its relationship to health and disease, together with DNA sequencing technology meant easier identification of the host genome and host micro-organisms or microbiome (Solt 2015). Microbiome changes influence gut content by allowing the predominance of some organisms over others, which in turn can cause a generalised increase in inflammatory markers in the host and increasing risks of diseases (Solt 2015). Modification of the gut microbiome (Flint 2012) by probiotics may be used as an intervention to prevent or treat metabolic diseases through various complex intracellular metabolic pathways within the gut (Nieuwdorp 2014; Turpin 2010). The mechanisms are complex from probiotics actively competing with pathological bacteria to dampening their inflammatory effect possibly by producing more butyrate; to improving the bile acid pool to reduce insulin resistance; or binding to mucosal receptors in the gut altering metabolic pathways responsible for the metabolic syndrome and satiety (Nieuwdorp 2014). Furthermore, probiotics have an anti-obesity action by influencing energy extraction in humans through increased lipolysis and reduction in lipoprotein lipase, which may reduce excess energy storage (Turpin 2010). The microbiomes of obese people have been found to have the ability to convert non digestible carbohydrates to digestible short-chain fatty acids, with increased uptake in the gut increasing energy harvest, storage and consequently increasing adiposity (Flint 2012). High adiposity in human and animal studies has been associated with increased systemic inflammation, which impacts adversely on pregnancy outcomes especially increasing risks of pre-eclampsia (Braentsaeter 2011), and increased insulin resistance. Probiotics have been shown to reduce the rates of severe pre-eclampsia (Braentsaeter 2011), reduce insulin resistance (Asemi 2013) and improve insulin sensitivity (Laitinen 2009). Other beneficial effects of probiotics include reduction of psychological distress in healthy volunteers (Messaoudi 2011), and consumption of probiotic yoghurt improved mood (Benton 2007) possibly by reducing systemic inflammatory markers (Dinan 2011). Futhermore, individuals with depression

have been shown to have a different microbiome to healthy individuals (Jiang 2015), as well as high levels of inflammatory cytokines (Dinan 2011), with probiotics predicted to dampen the negative effects of inflammation causing depression. Trials of probiotics in preterm neonates have demonstrated a reduction in necrotising enterocolitis and mortality (AlFaleh 2014).

Why it is important to do this review

The prevalence of GDM is increasing and the implementation of the International Association of Diabetes and Pregnancy Study Group diagnostic (IADPSG) criteria could also be contributing to the increase in women diagnosed with GDM. (Cundy 2014; Ekeroma 2015). All women with GDM may receive lifestyle advice (Metzger 2007), and for some women, this may be an effective treatment to maintain glycaemic control without the addition of pharmacotherapy (Brown 2017). The use of probiotics may prove a useful adjunct to lifestyle interventions and reduce the need for pharmacotherapy possibly by influencing metabolic pathways that lead to development of GDM (Nieuwdorp 2014). This review will establish the effectiveness of such an intervention in particular for women with GDM.

OBJECTIVES

To evaluate the safety and effectiveness of probiotics in treating pregnant women with gestational diabetes mellitus (GDM) on maternal and infant outcomes.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs). Cluster-randomised trials were eligible for inclusion but none were identified. Quasi-randomised and cross-over trials were not eligible for inclusion. There were no restrictions to language or year of publication.

Types of participants

Pregnant women diagnosed with gestational diabetes (diagnosis as defined by the individual trial). Trials of women with type 1 or type 2 diabetes diagnosed prior to pregnancy were excluded.

Types of interventions

Probiotics (any type) administered by any route given during pregnancy to treat women with gestational diabetes and where the control group received placebo or standard care (as defined by the trialist).

Types of outcome measures

Primary outcomes

Maternal

- Hypertensive disorders of pregnancy (including pre-eclampsia, pregnancy-induced hypertension, eclampsia)
- Subsequent development of type 2 diabetes (as defined by trialist)
- Mode of birth

Infant

- Perinatal (fetal and neonatal) mortality
- Large-for-gestational age (birthweight greater than the 90th centile; or as defined by individual trial)
- Composite of serious neonatal outcomes (variously defined by trials, e.g. infant death, shoulder dystocia, bone fracture or nerve palsy)
- Neurosensory disability (defined by trialists)

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 (e.g. adiponectin, free-fatty acids, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), insulin)
- Sense of well-being and quality of life (any validated Well-being and Quality of life scores)
- Views of the intervention
- Breastfeeding (e.g. at discharge, six weeks postpartum)
- Use of additional pharmacotherapy
- Glycaemic control during/end of treatment (as defined by trialists)
- Maternal hypoglycaemia
- Maternal mortality

Long-term maternal outcomes

- Postnatal depression (any validated postnatal depression scores e.g. Edinburgh Postnatal Depression Scale (EPDS))
- Postnatal weight retention or return to pre-pregnancy 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 (BP), hypertension, cardiovascular disease, 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

- 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 requiring treatment (variously defined)
- Hyperbilirubinaemia
- Neonatal hypocalcaemia
- Polycythaemia
- Relevant biomarker changes associated with the intervention (e.g. cord c peptide, cord insulin)

Later childhood

- Weight and z score
- Height and z score
- Head circumference and z score
- Adiposity (including BMI, skinfold thickness)
- BP
- Type 1 diabetes mellitus
- Type 2 diabetes mellitus
- Impaired glucose tolerance
- Dyslipidaemia or metabolic syndrome
- Educational achievement

Adulthood outcomes

- Weight
- Height
- Adiposity (including skin folds, fat mass)
- Cardiovascular health (as defined by trialists, including BP, hypertension, cardiovascular disease, metabolic syndrome)
- Type 1 diabetes mellitus
- Type 2 diabetes mellitus
- Impaired glucose tolerance
- Dyslipidaemia or metabolic syndrome
- Employment, education and social status/achievement

Health services

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

Search methods for identification of studies

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

Electronic searches

We searched Cochrane Pregnancy and Childbirth's Trials Register by contacting their Information Specialist (24 July 2019).

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, please follow this link.

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

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE (Ovid);
3. weekly searches FSTA;
4. weekly searches Biosis;
5. weekly searches of Embase (Ovid);
6. monthly searches of CINAHL (EBSCO);
7. handsearches of 30 journals and the proceedings of major conferences;
8. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Search results were screened by two people and the full text of all relevant trial reports identified through the searching activities described above is reviewed. 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, Excluded, Awaiting Classification or Ongoing).

In addition, we searched ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP) (24 July 2019) for unpublished, planned and ongoing trial reports using search methods detailed in Appendix 1.

Searching other resources

We searched the reference lists of all retrieved studies.

We did not apply any language or date restrictions.

Data collection and analysis

The methods was based on the standard template used by Cochrane Pregnancy and Childbirth.

Selection of studies

Two review authors Karaponi OKesene-Gafa (KOG) and Abigail Moore (AM) independently assessed for inclusion all potential studies identified as a result of the search strategy. Any disagreement was resolved through discussion with senior author Professor Caroline A Crowther (CAC).

Data extraction and management

We extracted relevant data using the Cochrane Pregnancy and Childbirth Group's data extraction form. We collected information on type of intervention, frequency and route of administration; trialists' declarations of interest and trial dates. For eligible studies, two review authors extracted the data using the agreed form. Discrepancies were resolved through discussion. Data were entered into Review Manager software (RevMan 2014) and checked for accuracy. We contacted trial authors for the original reports to provide further details if required.

Assessment of risk of bias in included studies

Two review authors (KOG and AM) independently assessed risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We resolved any disagreement by discussion or by involving our senior author (CAC).

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

We described for each included study the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce 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); we planned to exclude studies judged to be of high risk of bias.
- unclear risk of bias.

(2) Allocation concealment (checking for possible selection bias)

We described for each included study the method used to conceal allocation to interventions prior to assignment and will assess whether 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);
- unclear risk of bias.

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

We described for each included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies were 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 for participants and personnel.

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

We described for each included study the methods used, if any, to blind outcome assessors from knowledge of which intervention a participant received. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed methods used to blind outcome assessment 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 for each included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. We stated whether attrition and exclusions were reported and 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 supplied by the trial authors, we re-included missing data in the analyses.

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 for each included study 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 is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- high risk of bias (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest were reported incompletely and so cannot be used; study fails 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 for each included study any important concerns we had about other possible sources of bias.

We assessed whether each study was free of other problems that could 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 studies were at high risk of bias, according to the criteria given in the *Handbook* (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.

Assessment of the quality of the evidence using the GRADE approach

For the main comparison or probiotic versus placebo, the quality of the evidence will be assessed using the GRADE approach, outlined in the GRADE handbook and Chapters 11 and 12 of the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2011), for the outcomes listed below.

Maternal

- Hypertensive disorders of pregnancy (including pre-eclampsia, pregnancy-induced hypertension, eclampsia)
- Subsequent development of type 2 diabetes (as defined by trialist)
- Mode of birth
- Induction of labour
- Perineal trauma
- Postnatal weight retention or return to pre-pregnancy weight
- Postnatal depression

Infant/child/adult

- Perinatal (fetal and neonatal) mortality
- Large-for-gestational age (birthweight greater than the 90th centile; or as defined by individual trial)
- Composite of serious neonatal outcomes (variously defined by trials, e.g. infant death, shoulder dystocia, bone fracture or nerve palsy)
- Neurosensory disability (defined by trialists)
- Neonatal hypoglycaemia
- Adiposity (neonatal/child/adult)
- Diabetes (type 1 or type 2) or impaired glucose tolerance (child/adult)

We used the GRADEpro Guideline Development tool to import data from Review Manager 5.3 (RevMan 2014) in order to create 'Summary of findings' tables. A summary of the intervention effect and a measure of quality 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.

Measures of treatment effect

Dichotomous data

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

Continuous data

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

Unit of analysis issues

Cluster-randomised trials

We did not identify any cluster-randomised trials in this review. If cluster-randomised trials are identified in future updates of this review, we will include them in the analyses along with individually-randomised trials. We will make adjustments using the methods described in the *Handbook* [Section 16.3.4 or 16.3.6] (Higgins 2011) using an estimate of the intra-cluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial or from a study of a similar population. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. We will consider it reasonable to combine the results from both cluster-randomised trials and individually-randomised trials if there is little heterogeneity between the study designs and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.

Multiple pregnancies

There were no multiple pregnancies identified in this review. In future updates of this review, if studies involving multiple pregnancies are identified, we will present maternal data as per woman randomised and neonatal data per infant.

Multiple-arm studies

There were no studies with multiple arms identified in this review. If in future updates of this review, if studies with multiple intervention arms are identified, we will avoid 'double counting' of participants by combining groups to create a single pair-wise comparison if possible. Where this is not possible, we will split the 'shared' group into two or more groups with smaller sample size and include two or more (reasonably independent) comparisons.

Dealing with missing data

For included studies, we noted levels of attrition. We planned to explore 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 all participants were analysed 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 Tau^2, I^2 and Chi^2 statistics. We regarded heterogeneity as substantial if the I^2 was greater than 30% and either a 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

Had we included more than 10 studies in the meta-analysis, we planned to investigate reporting biases (such as publication bias) using funnel plots. We planned to assess funnel plot asymmetry visually. If asymmetry was suggested by a visual assessment, we planned to perform exploratory analyses to investigate it.

Data synthesis

We carried out statistical analysis using the Review Manager software (RevMan 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 were examining 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 substantial statistical heterogeneity was detected, we used random-effects meta-analysis to produce an overall summary, if an average treatment effect across trials was considered clinically meaningful. The random-effects summary was treated as the average of the range of possible treatment effects and we planned to discuss the clinical implications of treatment effects differing between trials. If the average treatment effect was not clinically meaningful, we did not combine trials.

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

Subgroup analysis and investigation of heterogeneity

Had we identified substantial heterogeneity, we planned to investigate it using subgroup analyses and sensitivity analyses. We will consider whether an overall summary is meaningful, and if it is, use random-effects analysis to produce it.

We planned to carry out the following subgroup analyses.

- Different types of probiotic (probiotic A versus probiotic B)
- Mode of administration of probiotic (capsule versus yoghurt versus nutritional supplement)
- Dosage (high versus low dose)
- Diagnostic criteria used for GDM (IADPSG, American College of Obstetrics and Gynaecology, World Health Organization, Carpenter and Coustan, Australian Diabetes in Pregnancy Society, other criteria not specified above, diagnostic criteria not specified)

Subgroup analysis will be restricted to the review's primary outcomes.

We will assess subgroup differences by interaction tests available within RevMan (RevMan 2014). We will report the results of subgroup analyses quoting the Chi^2 statistic and P value, and the interaction test I^2 value.

Sensitivity analysis

In order to examine robustness of individual decisions being made to this systematic review, we planned to carry out sensitivity analysis restricting our analyses to:

- studies at a low risk of bias (for allocation concealment);
- full-text papers;
- number of participants > 300;
- RCTs (excluding cluster-randomised trials in order to investigate the effect of the randomisation unit);
- studies without high levels of missing data.

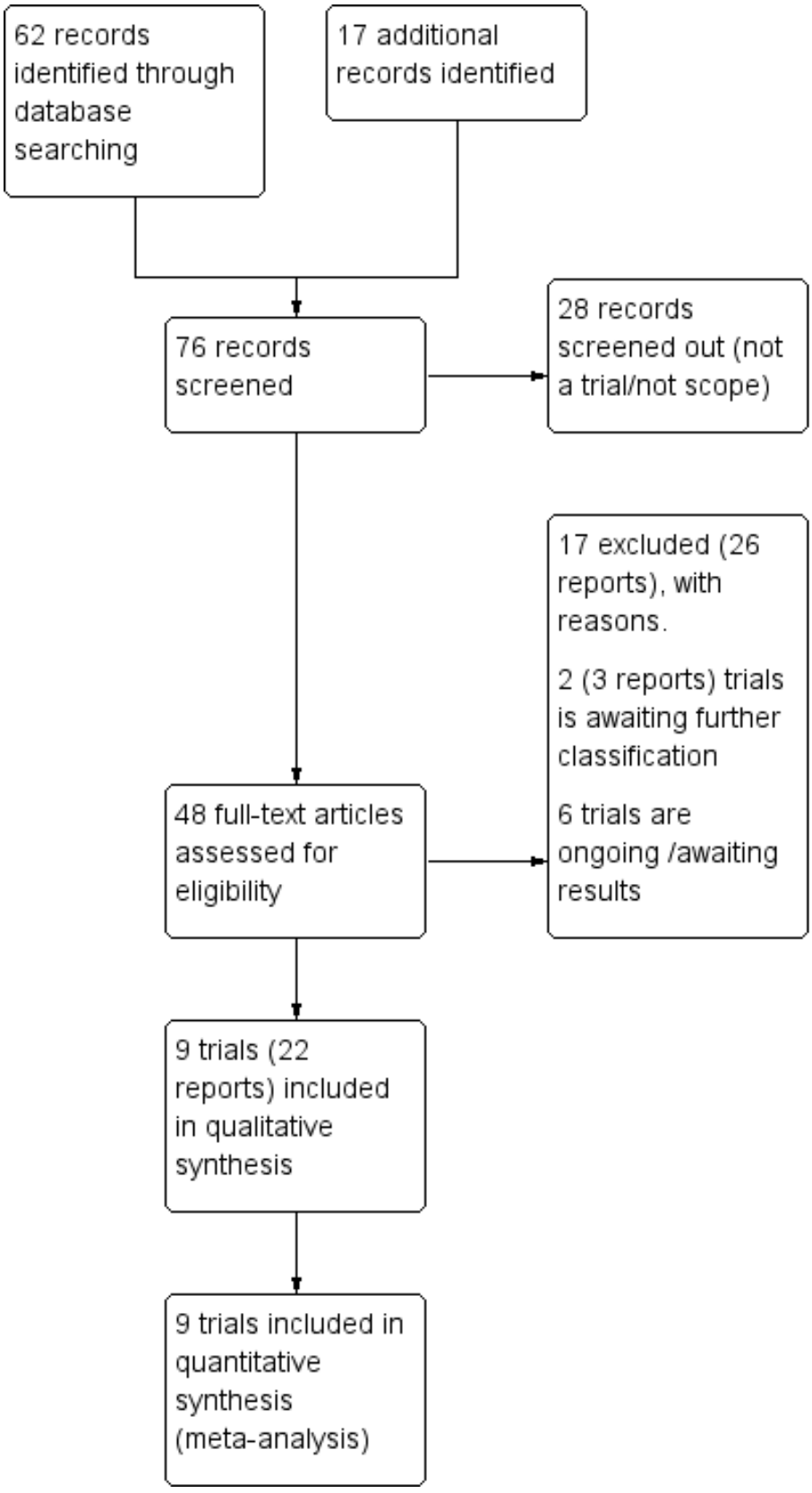
RESULTS

Description of studies

Results of the search

See: Figure 1

Figure 1. Study flow diagram.



We retrieved 31 trial reports from the Cochrane Pregnancy and Childbirth database searches plus an additional 17 from other sources. We included nine trials (22 reports) and excluded 17. Two trials (three reports) are awaiting further classification (Characteristics of studies awaiting classification), and six trials are ongoing (Characteristics of ongoing studies).

Included studies

Nine trials were selected and analysed (Ahmadi 2016; Badehnoosh 2018; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Karamali 2018; Kijmanawat 2019; Lindsay 2015; Nabhani 2018). All trials randomised women with gestational diabetes mellitus (GDM) to probiotics or placebo.

Information regarding the included trials is reported in the Characteristics of included studies tables.

Design

All studies were randomised controlled clinical trials comparing probiotics with placebo. Probiotics used in most studies were different in strengths and combinations (refer to interventions and comparisons).

Sample sizes

From the nine included trials, sample sizes ranged from 60 to 149 participants. The total number of participants were 695 randomised and 674 analysed. Total number of participants per study randomised (final analysis) were: Hajifaraji 2017; randomised 64 (analysed 56); Kijmanawat 2019 randomised 60 (analysed 57); Badehnoosh 2018, Karamali 2016, Karamali 2018 randomised 60 (analysed 60) in each trial. Ahmadi 2016 randomised 70 (analysed 70); Jafarnejad 2016 randomised 82 (analysed 72) and the trial with largest number of participants was Lindsay 2015 which randomised and analysed 149; and Nabhani 2018 randomised and analysed 90.

Setting

Seven studies were carried out in hospital or university settings in Iran (Ahmadi 2016; Badehnoosh 2018; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Karamali 2018; Nabhani 2018. One of the studies was carried out in Bangkok (Thailand) (Kijmanawat 2019), and one in Dublin (Ireland) (Lindsay 2015).

Dates of studies

Ahmadi 2016 took place between February and May 2016; Badehnoosh 2018 between April to September 2016, Hajifaraji 2017 during spring and summer 2014 (April to August); Jafarnejad 2016 between May 2014 to October 2015; Karamali 2016 between November 2015 to January 2016; Karamali 2018 between April and December 2016; Kijmanawat 2019 between July 2016 and February 2017; Lindsay 2015 between March 2012 and May 2014; Nabhani 2018 between January 2015 and September 2016.

Participants

All participants were women diagnosed with GDM according to the criteria chosen by each research team at between 24 to 28 weeks' gestation. Ahmadi 2016, Badehnoosh 2018, Hajifaraji 2017, Karamali 2016, Karamali 2018, Kijmanawat 2019, Nabhani 2018 used the American Diabetes Association (ADA) criteria after taking a 75 g oral glucose tolerance test (OGTT), and having a fasting blood glucose of ≥ 92 mg/dL, one-hour OGTT ≥ 180 mg/dL and two-hour OGTT ≥ 153 mg/dL. Kijmanawat 2019, as well as using the ADA diagnostic criteria also used a fasting plasma glucose ≥ 92 mg/dL at the first prenatal visit as a diagnosis for GDM. Jafarnejad 2016 used the Australasian Diabetes in Pregnancy Society (ADIPS) criteria with 75 g OGTT with results of fasting venous plasma glucose level, ≥ 5.5 mmol/L^{-1} or two-hour venous plasma glucose level, ≥ 8.0 mmol/L^{-1}. Lindsay 2015 used the Carpenter Coustan criteria results of a three-hour 100 g OGTT with fasting ≥ 95 mg/dL, one-hour ≥ 180 mg/dL, two-hours ≥ 155 mg/dL, three-hour 140 mg/dL for newly diagnosed impaired glucose tolerance (IGT) (1 raised value) or GDM (≥ 2 raised values).

Participants were between 18 to 45 years of age.

Three trials specifically reported participants as nulliparous (Badehnoosh 2018; Hajifaraji 2017; Karamali 2016). The other six trials did not clearly report baseline information related to parity (Ahmadi 2016; Jafarnejad 2016; Karamali 2018; Kijmanawat 2019; Lindsay 2015; Nabhani 2018).

Interventions and comparisons

Probiotics used in studies were of different strengths and combinations and given to participants in capsule form daily for either four, six or eight weeks. Participants in Ahmadi 2016 were given *Lactobacillus casei* and, *Bifidobacterium bifidum* (2×10^9 colony-forming units (CFU)/g each) plus 0·8 g inulin for six weeks. Badehnoosh 2018 gave participants *Lactobacillus acidophilus*, *L casei* and *B bifidum* (2×10^9 CFU/g each) for six weeks. Hajifaraji 2017 used (4Biocap capsules) containing 180 mg (4×10^9 CFU) standard power including freeze-dried cultures of *Lactobacillus acidophilus* LA-5, Bifibacterium BB12, *Streptococcus thermophilus* STY-31, and *Lactobacillus delbrueckii bulgaricus* LBY-27 + dextrose anhydrate filler and magnesium stearate lubricant for eight weeks. Jafarnejad 2016 used VSL#3, a freeze-dried probiotic preparation containing eight strains of lactic acid bacteria (*S thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *L acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *L delbrueckii subsp. Bulgaricus* (112.5×10^9 CFU/capsule), plus microcrystalline cellulose, stearic acid, magnesium stearate, and vegetable capsule (hydroxypropyl methylcellulose), silicon dioxide for eight weeks. Karamali 2016 and Karamali 2018 used three viable freeze-dried strains: *L acidophilus* (2×10^9 CFU/g), *L. casei* (2×10^9 CFU/g), *L casei* (2×10^9 CFU/g) and *B bifidum* (2×10^9 CFU/g) for six weeks. Kijmanawat 2019 gave participants *L acidophilus* and *B bifidum* (1×10^9) CFU for four weeks. Lindsay 2015 used 100 mg *Lactobacillus salivarus* UCC118 (10^9 CFU/capsule) for four to six weeks. Nabhani 2018 used *L acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus gasseri* (1.5–7.0 x 10^9–10 CFU/g) – with fructo-oligosaccharide (38.5 mg) with lactose (300 mg), magnesium stearate, talc, colloidal silicon dioxide (each of them 5.5 mg), flavourings and sweeteners that have neutral effects for six weeks.

All studies had placebo capsules as a comparison. Placebo in studies are explained in more detail in the Blinding (performance bias and detection bias) section of the review.

To support the interventions, four trials sent daily reminder text messages to participants (Ahmadi 2016; Badehnoosh 2018; Karamali 2016; Karamali 2018). Four trials carried out weekly phone interviews (Hajifaraji 2017; Jafarnejad 2016; Kijmanawat 2019;

Nabhani 2018). One study did not use phone interviews in their processes (Lindsay 2015).

Outcomes

Three trials (Badehnoosh 2018; Karamali 2018; Lindsay 2015) reported hypertensive disorders of pregnancy including pre-eclampsia and pregnancy-induced hypertension. No trials reported eclampsia. Three trials (Badehnoosh 2018; Karamali 2018; Lindsay 2015) reported caesarean section rates. Two trials (Badehnoosh 2018; Lindsay 2015) reported large-for-gestational age > 90 centile. One trial (Lindsay 2015) reported induction of labour and postpartum haemorrhage. Six trials (Ahmadi 2016; Badehnoosh 2018; Jafarnejad 2016; Karamali 2016; Karamali 2018; Kijmanawat 2019) reported weight gain during pregnancy (during the intervention) . Three trials (Badehnoosh 2018; Kijmanawat 2019; Lindsay 2015) reported total gestational weight gain.

For relevant biomarker for oxidative stress, three trials (Badehnoosh 2018; Hajifaraji 2017; Karamali 2018) reported malondialdehyde (MDA); two trials (Badehnoosh 2018; Karamali 2018) reported total glutathione (GSH), and one trial (Hajifaraji 2017) reported uric acid.

For inflammatory bio markers: four trials (Badehnoosh 2018; Hajifaraji 2017; Jafarnejad 2016; Karamali 2018) reported high-sensitivity C-reactive protein (hs-CRP); one trial (Jafarnejad 2016) reported interleukin 10 (IL-10) and interferon c (IFN-c); two trials (Hajifaraji 2017; Jafarnejad 2016) reported interferon 6 (IL-6) and tumour necrosis factor alpha (TNF-α).

For antioxidants: one trial (Karamali 2018) reported nitrous oxide; four trials (Badehnoosh 2018; Hajifaraji 2017; Karamali 2018; Nabhani 2018) reported total antioxidant capacity (TAC), and one trial (Hajifaraji 2017) reported serum GSH reductase (GSHR), erythrocyte superoxide dismutase (SOD) and erythrocyte glutathione peroxidase (GPx).

In biomarkers for insulin resistance: seven trials (Ahmadi 2016; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Kijmanawat 2019; Lindsay 2015; Nabhani 2018) reported Homeostatic Model Assessment of Insulin Resistance (HOMA-IR); and two trials (Ahmadi 2016; Karamali 2016) reported HOMA-B (β-cell function).

The biomarker for insulin sensitivity QUICKI (quantitative insulin-sensitivity check index) was reported by four trials (Ahmadi 2016; Hajifaraji 2017; Karamali 2016; Nabhani 2018).

Insulin secretion was reported by seven trials (Ahmadi 2016; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Kijmanawat 2019; Lindsay 2015; Nabhani 2018).

For lipids: four trials (Ahmadi 2016; Karamali 2016; Lindsay 2015; Nabhani 2018) reported triglycerides (TAG); two trials (Ahmadi 2016; Karamali 2016) reported very low-density lipoprotein (VLDL); and four trials (Ahmadi 2016; Karamali 2016; Lindsay 2015; Nabhani 2018) reported low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and total cholesterol.

Use of additional pharmacotherapy was reported by one trial (Badehnoosh 2018).

For glycaemic control: seven trials (Ahmadi 2016; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Kijmanawat 2019; Lindsay 2015; Nabhani 2018) reported fasting plasma glucose.

For neonatal outcomes: three trials (Badehnoosh 2018; Karamali 2018; Lindsay 2015) reported gestational age at birth; two trials (Badehnoosh 2018; Karamali 2018) reported preterm birth; three trials (Badehnoosh 2018; Karamali 2018; Lindsay 2015) reported macrosomia; one trial (Lindsay 2015) reported small-for-gestational age (SGA); four trials (Badehnoosh 2018; Karamali 2018; Kijmanawat 2019; Lindsay 2015) reported birthweight; three trials (Badehnoosh 2018; Karamali 2018; Lindsay 2015) reported head circumference, length and infant hypoglycaemia (requiring treatment, variously defined); two trials (Badehnoosh 2018; Karamali 2018) reported hyperbilirubinaemia; one trial (Lindsay 2015) reported Cord C peptide; and two trials (Badehnoosh 2018; Lindsay 2015) reported on neonatal intensive care unit (NICU) or nursery admissions.

All trials reported no significant issues or important clinical adverse effects with probiotics.

Sources of funding

Grants from: the Vice Chancellor for Research AUMS, Iran funded Ahmadi 2016; Vice Chancellor for Research, IUMS, Tehran, Iran funded Badehnoosh 2018; Tehran, Shahid Beheshti, University Medical Sciences funded Hajifaraji 2017; Vice Chancellor for Research, IUMS, Tehran, Iran funded Karamali 2016 and Karamali 2018; Thailand Research Fund (TRF) funded Kijmanawat 2019; National Maternity Hospital Medical Fund with support from the Ivo Drury Award and the European Union's Seventh Framework Program (FP7/2007-2013), project Early Nutrition under grant agreement number 289346 funded Lindsay 2015; and Tabriz University of Medical Sciences, Iran, and Nutrition Research Center funded Nabhani 2018.

No details of funding for one trial (Jafarnejad 2016),

Declarations of conflict of interest

A total of nine trials declared no conflict of interest except two trials that declared conflict of interest of at least one of its members (Kijmanawat 2019) (SR received grant support from Merck Sharp and Dohme, research equipment support from ResMed, and speaker honoraria from Sanofi, Novo Nordisk and Medtronic), (Lindsay 2015) (F.S. was a shareholder in Alimentary Health Ltd and has received grants from GlaxoSmithKline and the Procter and Gamble Company in the past).

Excluded studies

There were 17 articles that were excluded including one conference abstract.

In seven of the trials (Asemi 2013; Asemi 2013a; Barthow 2016; Luoto 2010; Nitert 2013; Okesene-Gafa 2018; Wickens 2017), the participants in the randomised controlled trial (RCTs) were not women with GDM. Four articles (Al-Dughaishi 2016; Gomez 2015; Lindsay 2013; Lindsay 2014) were not RCTs. Two of the papers (Barrett 2012; Barrett 2014) were systematic reviews; the latter a Cochrane Review. One of the papers (Muktabhant 2015) was a Cochrane Review on diet and exercise. Two of the trials (Fei 2014; Zhang 2018) used prebiotics and not probiotics.

Studies awaiting classification

Two trials (Gonai 2014; Jamilian 2019) are awaiting classification.

Risk of bias in included studies

Risk of bias is summarised in Figure 2 and Figure 3.

Figure 2. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

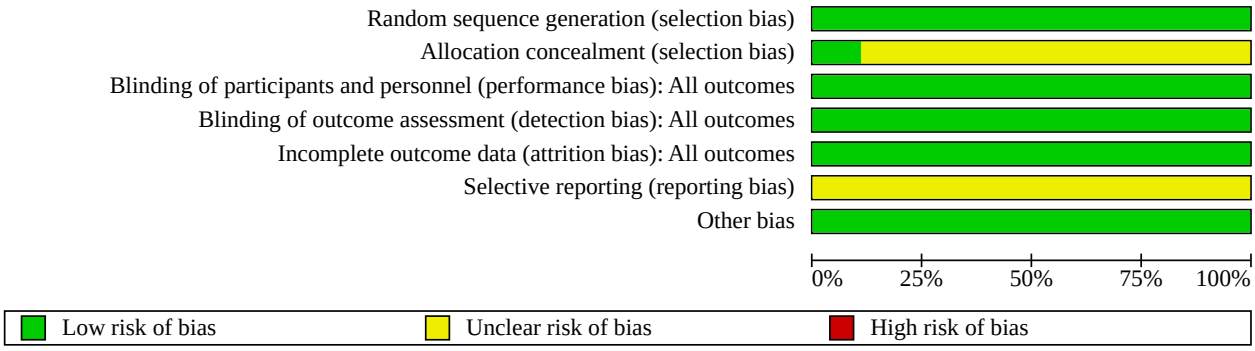
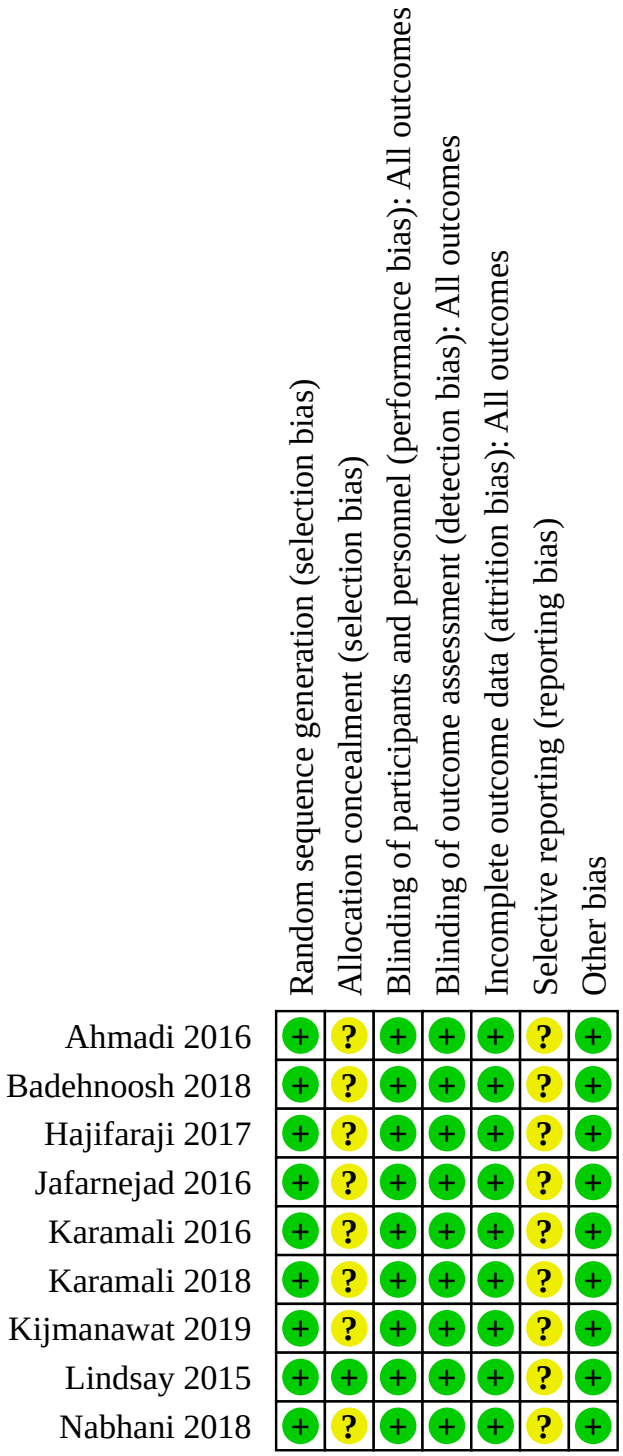


Figure 3. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Random sequence generation

We assessed all nine studies as low risk of bias for random sequence generation because they appeared to have adequate randomisation processes.

Eight trials reported that their random sequence was computer-generated (Ahmadi 2016; Badehnoosh 2018; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Karamali 2018; Lindsay 2015; Nabhani 2018).

One of the trials reported that a statistician generated the randomisation sequence using blocks (Kijmanawat 2019).

Three trials used computer block randomisation (Hajifaraji 2017, Kijmanawat 2019, Nabhani 2018).

Two trials specified that a separate researcher assistant (counsellor/therapist/trained personnel) carried out the randomisation and allocated the capsule packages according to the random sequence generated by the computer program (Hajifaraji 2017; Jafarnejad 2016).

Allocation concealment

Of the nine trials, one was rated as low risk of bias for allocation concealment (Lindsay 2015). The remaining eight studies were rated as unclear as we were unable to determine if researchers were aware of the allocation sequence when recruiting participants (Ahmadi 2016; Badehnoosh 2018; Jafarnejad 2016; Karamali 2016; Nabhani 2018; Hajifaraji 2017; Karamali 2018; Kijmanawat 2019).

Blinding of participants and personnel (performance bias)

All nine trials were specifically reported as double-blind, placebo-controlled randomised trials and were all graded as low risk of performance bias.

Six trials specified that probiotics and placebo were indistinguishable from each other (Ahmadi 2016; Hajifaraji 2017; Karamali 2016; Kijmanawat 2019; Lindsay 2015; Nabhani 2018). One trial stated their placebo capsules were identical to probiotics and contained 40 mg microcrystalline cellulose (Jafarnejad 2016).

Two trials did not offer adequate details of their placebo capsules: one trial stated that the placebo contained starch (Badehnoosh 2018); the other trial stated that their placebo contained gelatin (Kijmanawat 2019. One trial reported use of placebo with no specifics (Karamali 2018).

Four trials reported that a coder or supplier of capsules anonymously labelled the packages as A or B, whereas the contents of the packages were unknown to the researcher allocating the treatment in four trials (Hajifaraji 2017; Jafarnejad 2016; Lindsay 2015; Nabhani 2018). In one of these studies the packages (A or B) were placed in sequentially-numbered, sealed opaque envelopes (Lindsay 2015).

Blinding of outcome assessment (detection bias)

We assessed all nine trials as low risk of detection bias since they all reported adequate blinding of outcome assessors.

Incomplete outcome data

All nine studies were graded as low risk of bias, as there were minimal dropouts and no differential attrition. Two trials also stated that they used intention-to-treat analysis (Ahmadi 2016; Hajifaraji 2017.

Selective reporting

We assessed all nine trials as unclear risk of reporting bias because none of them had published study protocols, nor were any of them registered prospectively in any clinical trials registry, therefore we had insufficient information to judge which outcomes were pre-specified outcomes and if they were reported in full.

Other potential sources of bias

One trial had a significant difference in baseline cholesterol level between the probiotics and placebo groups. After adjusting for biochemical values, maternal age and body mass index (BMI) at baseline, there was no significant differences in these results (Ahmadi 2016).

One trial had significant differences in baseline levels of fasting plasma glucose (FPG) and HDL cholesterol between the two groups, but after further adjusting these variables as well as for baseline maternal age and BMI, the results were similar in both groups except for HOMA-B (P = 0.08) (Karamali 2016).

One trial had a slightly lower rate of Caucasian ethnicity and obesity and a higher rate of primiparity in the probiotic compared to placebo group, although these differences were not significant (Lindsay 2015).

One trial showed that there was a difference in energy, protein and total fat intakes (P < 0.05); thus, final analyses were adjusted for the measures of energy intake, BMI and baseline values (Nabhani 2018).

One trial stated that the women in their trial were taking 400 µg early pregnancy and 60 mg/day of ferrous sulphate from the second trimester (Badehnoosh 2018).

We assessed all nine studies as low risk of other sources of bias.

Effects of interventions

See: **Summary of findings 1** Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and infant health and well-being - maternal outcomes; **Summary of findings 2** Probiotic compared to placebo for treating women with gestational diabetes for improving maternal and infant health and well-being- infant/child/adult outcome

Probiotics versus placebo

Primary outcomes

Maternal

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

We are uncertain if there is any difference in hypertensive disorders in the probiotics compared to the placebo group, due to the wide 95% confidence intervals (CIs) which span possible benefit and potential harm (risk ratio (RR) 1.50, 95% CI 0.64 to 3.53; participants = 256; studies = 3; I^2 = 0%; low-certainty evidence; Analysis 1.1).

Subsequent development of type 2 diabetes

This outcome was not reported by the included trials.

Mode of birth (caesarean)

We are uncertain if there is any difference in caesarean sections as a mode of birth in the probiotics compared to the placebo group, because the quality of the evidence is low and the 95% CI is consistent with possible benefit and possible harm (average RR 0.64, 95% CI 0.30 to 1.35; participants = 267; studies = 3; I^2 = 69%; low-certainty evidence; Analysis 1.2).

Infant

Perinatal (fetal and neonatal) mortality

This outcome was not reported by the included trials.

Large-for-gestational age (LGA) > 90 centile

We are uncertain if there is any difference in LGA in the probiotics compared to the placebo group because the quality of evidence is low and the 95% CI spans possible benefit and potential harm (RR 0.73, 95% CI 0.35 to 1.52; participants = 174; studies = 2; I^2 = 0%; low-certainty evidence; Analysis 1.3).

Composite serious neonatal outcomes (variously defined by trials, e.g. infant death, shoulder dystocia, bone fracture, or nerve palsy)

This outcome was not reported by the included trials.

Neurosensory disability

This outcome was not reported by the included trials.

Secondary outcomes

Maternal

Induction of labour

We are uncertain if there is any difference in induction of labour in probiotic versus placebo because the certainty of evidence is very low and the 95% CI is consistent with possible benefit and possible harm (RR 1.33, 95% CI 0.74 to 2.37; participants = 127; studies = 1; Analysis 1.4).

Perineal trauma

This outcome was not reported by the included trials.

Placental abruption

This outcome was not reported by the included trials.

Postpartum haemorrhage

We are uncertain if there is any difference in incidence of postpartum haemorrhage (RR 0.77, 95% CI 0.36 to 1.62; participants = 126; studies = 1; Analysis 1.5).

Postpartum infection

This outcome was not reported by the included trials.

Weight gain during pregnancy

We are uncertain if there is any difference in weight gain from the beginning of intervention to the end of the intervention in the probiotics compared with placebo groups (mean difference (MD) 1.38, 95% CI -0.49 to 3.24; participants = 379; studies = 6; I^2 = 0%; Analysis 1.6).

Total gestational weight gain (kg)

We are uncertain if there is any difference in total gestational weight gain in the probiotics compared with the placebo groups (MD 0.24, 95% CI -0.30 to 0.78; participants = 239; studies = 3; I^2 = 0%; Analysis 1.7).

Adherence to intervention

This outcome was not reported by the included trials.

Behaviour changes associated with the intervention

This outcome was not reported by the included trials.

Relevant biomarker changes associated with the intervention:

Homeostatic model assessment for Insulin resistance (HOMA-IR)

There was evidence of a reduction in marker for HOMA-IR in the probiotics compared to the placebo group (MD -0.30, 95% CI -0.35 to -0.25; participants = 505; studies = 7; I^2 = 70%; Analysis 1.8).

Homeostatic model assessment for beta cell function (HOMA-B)

There was evidence of a reduction in HOMA-B in the probiotic compared to the placebo group (MD -25.38, 95% CI -38.32 to -12.44; participants = 130; studies = 2; I^2 = 0%; Analysis 1.8).

Quantitative insulin sensitivity check index (QUICKI)

There was some evidence of an increase in QUICKI levels in the probiotic compared to the placebo group (MD 0.01, 95% CI 0.00 to 0.02; participants = 276; studies = 4; I^2 = 0%; Analysis 1.8).

Triglycerides (TAG) (mg/dL)

There was evidence of a reduction in triglycerides in the probiotic compared with the placebo group (MD -19.19, 95% CI -35.69 to -2.70; participants = 320; studies = 4; I^2 = 46%) Analysis 1.8).

Very low-density lipoprotein (VLDL) cholesterol (mg/dL)

There was evidence of a reduction in VLDL cholesterol with probiotics compared with placebo group (MD -7.80, 95% CI -12.93 to -2.66; participants = 130; studies = 2; I^2 = 26%; Analysis 1.8).

Low-density lipoprotein (LDL) cholesterol (mg/dL)

We are uncertain if there is any difference in LDL cholesterol with probiotics compared with placebo (MD -5.36, 95% CI -12.83 to 2.12; participants = 320; studies = 4; I^2 = 0%; Analysis 1.8).

High-density lipoprotein (HDL) cholesterol (mg/dL)

There was evidence of a reduction in HDL cholesterol with probiotics compared to placebo (MD -3.48, 95% CI -6.02 to -0.93; participants = 320; studies = 4; I^2 = 76%; Analysis 1.8).

Total cholesterol (mg/dL)

There was evidence of a reduction in total cholesterol with probiotics compared to placebo (MD -10.63, 95% CI -19.73 to -1.54; participants = 320; studies = 4; I^2 = 56%; Analysis 1.8).

High-sensitivity C-reactive protein (hs-CRP) (µg/mL)

There was evidence of a reduction in maternal inflammatory marker hs-CRP in probiotics compared to the placebo group (MD -1.29, 95% CI -1.72 to -0.86; participants = 248; studies = 4; I^2 = 44%; Analysis 1.8).

Nitrous oxide (NO) (µmol/L)

There was no evidence of a clear difference in levels of NO (vasodilator) with probiotics compared to placebo groups (MD 1.70, 95% CI -0.94 to 4.34; participants = 120; studies = 2; I^2 = 0%; Analysis 1.8).

Malondialdehyde (MDA) (µmol/L)

There was evidence of a decrease in MDA (marker of oxidative stress) levels in the probiotics compared to the placebo group (MD -0.85, 95% CI -1.20 to -0.50; participants = 176; studies = 3; I^2 = 0%; Analysis 1.8).

Total glutathione (GSH) (µmol/L)

There was evidence of increased GSH levels (antioxidant) with probiotics compared with placebo (MD 44.95, 95% CI 13.36 to 76.55; participants = 120; studies = 2; I^2 = 0%; Analysis 1.8).

Total glutathione reductase (GSHR) (ng/mL)

There was evidence of increased GSHR levels (an antioxidant) with probiotics compared with placebo (MD 5.78, 95% CI 0.30 to 11.26; participants = 56; studies = 1; I^2 = 0%; Analysis 1.8).

Total antioxidant capacity (TAC) (mmol/L)

There may be little to no difference in TAC with probiotics compared to placebo (MD 0.02, 95% CI -0.05 to 0.10; participants = 266; studies = 4; I^2 = 92%; Analysis 1.8). This meta-analysis has a high level of heterogeneity which may be due to the different methods used to measure TAC. We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Interleukin 10 (IL-10) (pg/mL)

Only one trial Jafarnejad 2016 reported IL-10 levels. We are uncertain if there is a difference in IL-10 levels between probiotics compared to placebo group (MD -0.27, 95% CI -2.93 to 2.39; participants = 72; studies = 1; Analysis 1.8).

Inteferon c (IFN-c)

Only one trial Jafarnejad 2016 reported on IFN-c and it is not certain if there is any difference in levels between probiotics compared to placebo (MD -1.90, 95% CI -9.38 to 5.58; participants = 72; studies = 1; Analysis 1.8).

Inter leukin 6 (IL-6) (pg/mL)

There was evidence of a reduction in IL-6 in the probiotic compared to the placebo group (MD -0.89, 95% CI -1.17 to -0.60; 128 participants; 2 trials Tau^2 = 0.00; I^2 = 0%; Analysis 1.8).

Tumour necrosis factor alpha (TNF-α) (pg/mL)

There was evidence of reduction in TNF-α in the probiotic compared to the placebo group: mean difference (MD -0.53, 95% CI -0.78 to -0.27; participants = 128; studies = 2; I^2 = 80%; Analysis 1.8).

Serum uric acid (mg/dL)

Only one trial looked at serum uric acid (Hajifaraji 2017). We are uncertain if there is any difference between probiotics and placebo (MD -0.21, 95% CI -0.52 to 0.10; participants = 56; studies = 1; Analysis 1.8).

Erythrocyte superoxide dismutase (SOD) (U/gHBb)

Only one trial looked at levels of erythrocyte SOD (Hajifaraji 2017). We are uncertain if there is any difference in erythrocyte SOD with probiotics compared with placebo (MD 189.20, 95% CI -57.31 to 435.71; participants = 56; studies = 1; Analysis 1.8).

Erythrocyte glutathione peroxidase (GPx) (U/gHb)

One trial calculated Erythrocyte GPx Hajifaraji 2017 and there was evidence of an increase in erythrocyte GPX with probiotics compared with placebo (MD 6.93, 95% CI 1.34 to 12.52; participants = 56; studies = 1; Analysis 1.8).

Sense of well-being and quality of life

These outcomes were not reported by the included trials.

Views of the intervention

This outcome was not reported by the included trials.

Breastfeeding at discharge or six weeks postpartum

This outcome was not reported by the included trials.

Use of additional pharmacotherapy

Use of additional pharmacotherapy was reported by one trial. We are uncertain if there is any difference between probiotics and placebo in requiring additional pharmacotherapy (insulin) (RR 0.67, 95% CI 0.12 to 3.71; participants = 60; studies = 1; Analysis 1.9).

Glycaemic control during/end of treatment (as defined by trialist)

This outcome was not reported by the included trials.

Fasting blood glucose(mg/dL)

We are uncertain if there is any difference in fasting plasma glucose in those in the probiotics arm compared to placebo (MD -0.42, 95% CI -1.66 to 0.82; participants = 554; studies = 7; I^2 = 46%; Analysis 1.10).

Postprandial blood glucose

This outcome was not reported by the included trials.

Maternal hypoglycaemia

This outcome was not reported by the included trials.

Maternal mortality

This outcome was not reported by the included trials.

Long-term maternal outcomes

None of the included studies reported any of our pre-specified long-term maternal outcomes.

Infant outcomes

Stillbirth

This outcome was not reported by the included trials.

Neonatal mortality

This outcome was not reported by the included trials.

Gestational age at birth (days)

We are uncertain if there is any difference in gestational age at birth between probiotics and placebo (MD 1.37 days, 95% CI -1.33 to 4.07; participants = 267; studies = 3; I^2 = 0%; Analysis 1.11).

Preterm birth

We are uncertain if there is any difference in rates of preterm births in the probiotics compared to placebo groups (RR) 1.00, 95% CI 0.18 to 5.59; participants = 120; studies = 2; I^2 = 0%; Analysis 1.12).

Apgar score less than seven in five minutes

This outcome was not reported by the included trials.

Macrosomia (> 4000 g)

We are uncertain if there is any difference in macrosomia (> 4000 g) in the probiotics compared to placebo groups (RR 0.84, 95% CI 0.50 to 1.43; participants = 267; studies = 3; I^2 = 48%; Analysis 1.13).

Small-for-gestational age (SGA)

Only one trial (Lindsay 2015) reported on SGA. We are uncertain if there is any difference in SGA in the probiotics group compared to placebo (RR 1.04, 95% CI 0.39 to 2.76; participants = 114; studies = 1; Analysis 1.14).

Birthweight (g) and z scores

We are uncertain if there is any difference in birthweight in the probiotics groups compared to the placebo groups (MD -79.14 g, 95% CI -183.00 to 24.73; participants = 324; studies = 4; Analysis 1.15).

Head circumference (cm) and z scores

We are uncertain if there is any difference in head circumference of infants in the probiotic group compared to the placebo group (MD -0.02 cm, 95% CI -0.52 to 0.48; participants = 249; studies = 3; I^2 = 0%; Analysis 1.16).

Length(cm) and z scores

We are uncertain if there is any difference in length of infants in the probiotic groups compared to the placebo groups (MD -0.35 cm, 95% CI -1.03 to 0.33; participants = 248; studies = 3; I^2 = 0%; Analysis 1.17).

Ponderal index

This outcome was not reported by the included trials.

Adiposity

This outcome was not reported by the included trials.

Shoulder dystocia

This outcome was not reported by the included trials.

Bone fracture

This outcome was not reported by the included trials.

Nerve palsy

This outcome was not reported by the included trials.

Respiratory distress syndrome

This outcome was not reported by the included trials.

Neonatal hypoglycaemia requiring treatment (variously defined)

We are uncertain if there is any difference in neonatal hypoglycaemia in the probiotics groups compared with the placebo groups because the quality of evidence is low and the 95% CI is consistent with possible benefit and possible harm (RR 0.85, 95% CI 0.39 to 1.84; participants = 177; studies = 3; I^2 = 0%; Analysis 1.18; low-certainty evidence; Summary of findings 2).

Hyperbilirubinemia

There was evidence of a reduction in infant hyperbilirubinaemia with probiotics compared to placebo (RR 0.18, 95% CI 0.05 to 0.57; participants = 120; studies = 2; I^2 = 0%; Analysis 1.19).

Neonatal hypocalcaemia

This outcome was not reported by the included trials.

Polycythaemia

This outcome was not reported by the included trials.

Relevant infant biomarkers associated with intervention (cord C peptide, cord insulin)

C-peptide

A marker of insulin secretion cord C peptide was reported by only one trial Lindsay 2015. We are uncertain if there is any difference in C-peptide secretions, in probiotics compared to placebo (MD -0.05, 95% CI -0.44 to 0.34; participants = 100; studies = 1; Analysis 1.20).

Later childhood

None of the included studies reported any of our pre-specified outcomes relating to later childhood.

Adulthood outcomes

None of the included studies reported any of our pre-specified adulthood outcomes.

Health services

Number of antenatal visits or admissions

This outcome was not reported by the included trials.

Number of hospital or heath professional visits (including midwives, obstetricians, physicians, dietician, diabetic nurse)

This outcome was not reported by the included trials.

Admission to NICU/nursery

We are uncertain if there is any difference between probiotics and placebo in NICU or nursery admissions (average RR 1.71, 95% CI 0.45 to 6.53; participants = 202; studies = 2; I^2 = 66%) (Analysis 1.21).

One of the included trials (Badehnoosh 2018) only reported newborn hospitalisations and we made the assumption that any neonatal hospitalisations would be to the neonatal nursery and this study was added to the analysis for admission to NICU/nursery.

Length of antenatal stay

This outcome was not reported by the included trials.

Length of postnatal stay (maternal)

This outcome was not reported by the included trials.

Length of postnatal stay (baby)

This outcome was not reported by the included trials.

Cost of maternal care

This outcome was not reported by the included trials.

Cost of offspring care (including NICU admissions)

This outcome was not reported by the included trials.

Costs associated with the interventions

This outcome was not reported by the included trials.

Costs associated with the interventions

This outcome was not reported by the included trials.

DISCUSSION

Summary of main results

Nine randomised controlled trials (RCTs) involving 695 women with gestational diabetes mellitus (GDM) and their babies met the inclusion criteria for this review. All trials compared probiotics (some used the same and others used different strengths and compositions and administered at different lengths of time) with placebo.

For mothers, we are uncertain if probiotics have any effect on the risk of hypertensive disorders, mode of birth or induction of labour compared with placebo because the evidence is low to very low certainty, and the 95% confidence intervals are consistent with possible benefit and possible harm (Summary of findings 1). We identified no evidence relating to the risk of developing type 2 diabetes, perineal trauma, postnatal weight retention or postnatal depression.

For infants, we are uncertain if probiotics lead to more or fewer large-for-gestational age infants, or if probiotics have any effect on the risk of neonatal hypoglycaemia, compared with placebo, because the evidence is low-certainty and the 95% confidence intervals are consistent with possible benefit and possible harm (Summary of findings 2). We identified no evidence relating to perinatal mortality, serious neonatal outcomes, neurosensory disability, or adiposity or diabetes later in life.

In other secondary maternal outcomes, in the probiotics compared to the placebo group, there was a reduction in inflammatory markers hs-CRP and interleukin 6. There was an increase in antioxidant total glutathione and reduction in maker of oxidative stress malondialdehyde. There may be a reduction in triglyceride and total cholesterol, but we are uncertain of a difference in fasting plasma glucose. For neonatal/infant outcomes we are uncertain whether probiotics compared to placebo have any effect on birthweight, gestational age at birth, preterm births, macrosomia, head circumference and length; or increase in NICU admissions.

Limitations of the studies at the outcome level were their small sample sizes. At the reporting level, a number of RCTs focused on probiotics to prevent development of GDM but not as a treatment for GDM and were therefore excluded.

Overall completeness and applicability of evidence

The trials in this review were conducted in women with GDM. The studies recruited pregnant women with GDM mostly from Iran (Ahmadi 2016; Badehnoosh 2018; Hajifaraji 2017; Jafarnejad 2016; Karamali 2016; Karamali 2018; Nabhani 2018), with only two studies outside Iran where one was conducted in Bangkok (Thailand) (Kijmanawat 2019), and the other in Dublin (Ireland) (Lindsay 2015). The largest trial involved 149 women (Lindsay 2015); most of the other trials had smaller numbers of women (60 to 95). All trials reported the outcomes specified in the trials.

Included studies did not report a number of our main GRADE outcomes, nor a large number of our secondary outcomes.

Quality of the evidence

Overall, the nine trials were judged as low to unclear risk of bias. The risk of selection bias was generally unclear because there was insufficient information to judge whether allocation concealment had been carried out adequately. Additionally, the risk of reporting bias was assessed as unclear due to a lack of published study protocols against which to compare the outcomes selected for reporting in the trial results.

Using GRADE methodology, the evidence was assessed as low to very low certainty. Downgrading decisions for mode of birth was mainly due to imprecision and inconsistency (low-certainty evidence), and for induction of labour (a secondary outcome) (very low-certainty evidence), downgraded one level due to indirectness and two levels for imprecision.

Potential biases in the review process

To reduce the potential for publication bias, the Information Specialist of the Cochrane Pregnancy and Childbirth group was asked to conduct a systematic detailed search process for this review. It is possible that additional trials assessing the use of probiotics as a treatment of GDM may be available that may have been carried out but not yet published, or have recently been published but are not included in this review. Should they be identified, they will be included in future updates of this review.

Agreements and disagreements with other studies or reviews

This is the first Cochrane Review of this topic and hence, agreements and disagreements with other reviews is not possible. Most of the Cochrane or non-Cochrane Reviews or systematic reviews of randomised controlled trial already carried out were to determine whether probiotics compared to placebo prevented development of GDM. One meta-analysis looked at effect of probiotics on metabolic health in pregnant women who were or were not diagnosed with GDM (Zheng 2018).

AUTHORS' CONCLUSIONS

Implications for practice

Low-certainty evidence showed that we were uncertain of any difference between probiotic and placebo groups in maternal hypertensive disorders of pregnancy and mode of birth by caesarean section; and neonatal/infant outcome of large for gestational age.

There were no reported adverse events reported by the trials.

Due to the variability of probiotics used and small sample sizes of the trials, the evidence from this review has limited ability to inform practice. Future studies need to consider the use of a specific probiotic type and strength, to be able to identify the impact on glucose metabolism and core clinical outcomes and to facilitate standardised reporting.

Implications for research

Further adequately powered, well-designed randomised controlled trials are needed to clarify the effects of probiotics on glucose metabolism in pregnant women with gestational diabetes mellitus (GDM). There are currently six ongoing trials, with over 500 participants to be recruited. Once completed, these trials will add to the evidence base. These trials have been designed to examine some of our clinical outcomes but it would appear that they are not designed to measure long-term infant outcomes. There is still a need for trials to be designed to address this need.

There is also a need for trials to be in agreement as to what probiotics should be used for research to avoid uncertainty of benefit with variability in probiotic type and strength. Clear evidence of benefit of these specific probiotics in improving fasting, pre-prandial and/or postprandial glucose should also theoretically translate into improvement in important maternal and neonatal/infant clinical outcomes. In future trials, it is important to consider collecting important immediate and long-term maternal and offspring outcomes as outlined in this review, but were not available. Important bio-markers of insulin resistance (HOMA-IR and HOMA-B), insulin sensitivity (QUICKI), as well as inflammatory markers, antioxidants and markers of oxidative stress can be explored further to determine their role in modifying the effect of insulin resistance during pregnancy and whether they also play a role in improving glucose metabolism and consequently improve clinical outcomes. Another important factor to be considered in future trials is agreed criteria for diagnosing GDM.

We look forward to the results of current ongoing studies. The results of these studies will be included in future updates of this review.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Ahmadi 2016

Study characteristics	
Methods	Parallel randomised double-blind placebo-controlled trial
Participants	Pregnant women with GDM (n = 70) **Setting** Kosar Clinic in Arak, Iran **Dates of study** February 2016 to May 2016 **Inclusion criteria** Pregnant women with GDM, age 18-40years, diagnosed with GDM by the American Diabetes Association guidelines by the 1-step 2-hour, 75 g OGTT, at 24-28 weeks gestation, having a fasting blood glucose of ≥ 92 mg/dL, 1-hour OGTT ≥ 180 mg/dLand 2-hour OGTT ≥ 153 mg/dL. **Exclusion criteria** Women taking synbiotic or probiotic supplements, including probiotic yogurt, kefir and other fermented foods. Women taking insulin, with placenta abruption, pre-eclampsia, eclampsia, hypothyroidism and hyperthyroidism, smokers, and those with kidney or liver diseases.
Interventions	Probiotic/symbiotic - capsules containing *Lactobacillus acidophilus*, *Lactobacillus casei* and, *Bifidobacterium bifidum* (2×10^9 CFU/g each) plus 0·8 g inulin (n = 30) Placebo capsules containing starch without bacteria and inulin (n = 35) The appearance of the placebo was indistinguishable with regard to colour, shape, size and packaging, smell and taste from the probiotic capsule.
Outcomes	Primary outcomes: markers of insulin metabolism: FPG, serum insulin, quantitative insulin sensitivity check index (QUICKI), Homoeostatic model assessment for insulin resistance (HOMA-IR), Homoeostatic model assessment for B-cell function (HOMA-B) Secondary outcomes: measurements of lipid concentrations: serum TAG, VLDL cholesterol, Total cholesterol, HDL cholesterol, LDL cholesterol.
Notes	**Funding:** a grant from the Vice Chancellor for Research AUMS, Iran. **Conflicts of interest:** authors declare no conflict of interest. Participants were also sent reminder text messages daily to take their probiotics. Total time for the intervention was 6 weeks.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated random numbers were used to assign the participants.

Ahmadi 2016 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	The randomised allocation sequence, enrolling participants and allocating them to interventions were conducted by a trained staff at the gynaecology clinic. Quote: "Randomisation and allocation were concealed from researchers and participants".
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Randomisation and allocation were concealed from the researchers and participants until all the analyses were completed.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	As above
Incomplete outcome data (attrition bias) All outcomes	Low risk	70 women randomised. 3 women in the placebo group withdrew for personal reasons, 1 woman in the intervention group also withdrew for personal reasons. 70 women analysed
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	This is a journal article. The groups were balanced at baseline. (mean age height, baseline weight and BMI as well as their means after 6 weeks intervention were not significant between the synbiotic group and the placebo group. Comparison of the 3-day dietary intake throughout the study revealed no significant differences in micronutrient and macronutrient intakes, energy carbohydrates, proteins, fats, SFA, PUFA, MUFA, total dietary fibre, Fe, Mg, Zn, and Mn between the groups. (There was a significant difference in baseline cholesterol levels between the 2 groups. The baseline concentration of the biochemical values, maternal age and BMI at baseline were controlled for the analysis. After adjustments, no significant differences in the results were identified.)

Badehnoosh 2018

Study characteristics	
Methods	Parallel randomised control trial
Participants	Pregnant women with GDM (n = 60) **Setting** Akbarabadi Clinic in Tehran, Iran **Dates of Study** April to September 2016 **Inclusion criteria** Pregnant women, primigravidae, age 18-40 years, with GDM by the 1-step 2-hour 75 g OGTT with GDM diagnosed according to the American Diabetes Association guidelines (FPG ≥ 92 mg/dL, 1-hour OGTT ≥ 180 mg/dL and 2-hour OGTT ≥ 153 mg/dL). **Exclusion criteria**

Badehnoosh 2018 *(Continued)*

	Placenta abruption, pre-eclampsia, eclampsia, hypo- and hyperthyroidism, urinary tract infection, smokers, those with kidney or liver diseases and required commencing insulin therapy during the intervention. Taking any probiotic products including probiotic yogurt and kefir during the trial.
Interventions	**Probiotics:** probiotic supplements 6 weeks containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* (2 x 10^9 CFU/g each) strains (n = 30) **Placebo:** capsules containing starch (n = 30).
Outcomes	hs-CRP, FPG, NO, MDA, TAC GSH, MDA/TAC ratio, caesarean sections, preterm delivery, need for insulin intervention after intervention, pre-eclampsia, polyhydramnios, maternal hospitalisation, macrosomia, gestational age, newborn: weight, length, head circumference, LGA, 1- and 5-minute Apgar score, hyperbilirubinaemia, hospitalisations, hypoglycaemia
Notes	**Funding:** grant from the Vice Chancellor for Research, IUMS, Tehran, Iran. **Conflicts of interest:** authors declare no conflicts of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation assignment was carried out using computer-generated random numbers
Allocation concealment (selection bias)	Unclear risk	The randomised allocation sequence, enrolling patients and allocating them to interventions were done by a trained midwife at the gynaecology clinic. Randomisation and allocation were concealed from the researchers and participants until the final analyses were completed.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Researchers and participants were blinded by the use of placebo.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Researchers were blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	60 women randomised. Total 60 women analysed. All participants completed the study, none lost to follow-up
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	All groups were balanced at the baseline

Hajifaraji 2017

Study characteristics	
Methods	Double-blind, placebo-controlled randomised trial
Participants	Pregnant women with GDM (n = 64)

Hajifaraji 2017 *(Continued)*

	Setting Gynecology and Endocrinology clinics at Tabriz Al-Zahra referral University hospital, Tabriz, Iran **Dates of study** Spring and summer 2014 **Inclusion criteria** Nulliparity, newly diagnosed gestational diabetes at 24 and 28 weeks + 6 days, GDM diagnosed by a fasting BG of 92 mg/dL to125 mg/dL at time of diagnosis. Age 18 to 45 years, with BMI ≥ 18.5 kg/m2. No history of IGT in early pregnancy, T2DM, chronic diseases, smoking, alcohol, ingestion of probiotics (including yoghurt, kefir, other fermented food) within 2 weeks of intervention, or antibiotic use 1 month prior to intervention. No acute GI problems for a month prior to intervention. **Exclusion criteria** Needing insulin therapy or other medications (fasting BG > 105 and BG postprandial > 120 mg/dL) during intervention. Ingestion of other forms of probiotics (yoghurt, kefir, fermented foods), antibiotics, glucocorticoids, immunosuppressive drugs, having acute GI problems, and not taking adequate number of tablets.
Interventions	**Probiotic:** capsules (4Biocap) containing 180 mg (> 4 x 10^9 CFU) standard power including freeze dried cultures of *Lactobacillus acidophilus LA-5*, *Bifibacterium BB-12, Streptococcus thermophiles* STY-31, and *Lactobacillus delbrueckii bulgaricus* LBY-27 + dextrose anhydrate filler and magnesium stearate lubricant (n = 32). **Placebo:** capsules similar design, shape and colour to the probiotics (n = 32).
Outcomes	Systolic and diastolic BP changes from baseline, to 2 weeks, 4 weeks, 6 weeks and 8 weeks after recruitment. Serum inflammatory markers: serum hs-CRP, lnTNF-α, Serum IL-6. Oxidative stress markers: serum TAC, serum MDA, serum GSHR, erythrocyte GPX, serum uric acid, erythrocyte SOD. Weight changes, FPG, serum insulin levels, HOMA-IR, QUICKI index
Notes	**Funding:** Tehran, Shahid Beheshti University Medical Sciences. Tehran Darou pharmaceuticals, Tehran, Iran – provided the 4-Biocap (probiotic supplement). **Conflicts of interest:** authors declare no conflict of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated random sequence. To confirm double-blinding, a coder secretly labelled the capsule packages A or B.
Allocation concealment (selection bias)	Unclear risk	All 64 females were allocated using block randomisation techniques to either probiotic or placebo; it is unclear whether the allocation was concealed.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants and clinicians were blinded to the intervention.

Hajifaraji 2017 *(Continued)*

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Outcome assessors were blinded to the intervention.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Of the 64 women who were randomised, 56 were analysed in the final analysis. Reasons for discontinuation: probiotic arm, (3 loss to follow-up); declined to continue (1); needing drug therapy (2): n = 29 (91%). In the placebo arm: (5 loss to follow-up); declined to continue (2), preterm pregnancy (1), needing drug therapy (2).
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	Baseline characteristics were balanced across groups.

Jafarnejad 2016

Study characteristics	
Methods	Parallel randomised control trial (Block randomisation)
Participants	Pregnant women with GDM (n = 82) **Setting:** Tuba Endocrine clinic (Sari, Iran) **Dates of study** May 2014 to October 2015 **Inclusion criteria** Pregnant women diagnosed with GDM diagnosed by 2-hour, 75 g OGTT: fasting venous plasma glucose level, $5.5 mmol \cdot L^{-1}$ or higher or 75-g OGTT 2-hour venous plasma glucose level, 8.0 $mmol \cdot L^{-1}$ or higher **Exclusion criteria** Unwillingness to follow a prescribed diet, pre-GDM (either type 1 or type 2 DM), the need for insulin treatment, and pregnancy co-morbidities other than obesity, hypertension, and/or dyslipidaemia
Interventions	**Probiotics**: VSL#3 is a freeze-dried pharmaceutical probiotic preparation containing 112.5×10^9 CFU/capsule of 8 strains of lactic acid bacteria (*Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii subsp. Bulgaricus*), microcrystalline cellulose, stearic acid, magnesium stearate, and vegetable capsule (hydroxypropyl methylcellulose), silicon dioxide (n = 41). **Placebo:** capsules containing 40mg microcrystalline cellulose; placebo boxes had identical appearances (n = 41)
Outcomes	FPG, HbA1c, HOMA-IR, insulin levels, IL-6, IL-10, TNF-α, hs-CRP, IFN-ɣ
Notes	**Funding:** no detail **Conflicts of interest:** authors declare that they have no competing interests.

Jafarnejad 2016 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation performed by trained personnel at the endocrine clinic.
Allocation concealment (selection bias)	Unclear risk	Allocation of the intervention or control group was concealed from the researchers.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Neither the participants nor the investigators were aware of the treatment assignments.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Allocation blinded from the researchers.
Incomplete outcome data (attrition bias) All outcomes	Low risk	82 women randomised. 4 women excluded from probiotic group (2 women needed insulin and 2 birthed prematurely < 35 weeks); 6 women in the placebo group were excluded (5 needed insulin treatment and 1 birthed prematurely).
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	Groups were balanced at the baseline

Karamali 2016

Study characteristics	
Methods	Parallel randomised controlled trial
Participants	Pregnant women with GDM (n = 60) **Dates of study** November 2015 and January 2016 **Setting** Kosar Clinicin Arak, Iran **Inclusion criteria** Pregnant women with GDM diagnosed by 1 step 75 OGTT based on ADA criteria of FPG ≥ 92 mg/dL (5.1 mmol); 1-hour OGTT ≥ 180 mg/dL (10 mmol); or 2-hour OGTT ≥ 153 mg/dL (8.5 mmol) **Exclusion criteria** Women with: preterm premature rupture of membranes, placental abruption, pre-eclampsia, eclampsia, hypo or hyperthyroidism, a history of T2DM, a family history of GDM, smokers, and those with kidney or liver disease, or taking probiotics, antibiotics or glucocorticoids, or requiring insulin therapy.
Interventions	**Probiotics:** group took a daily capsule that contained 3 viable freeze-dried strains: *Lactobacillus acidophilus* (2×10^9 CFU/g), L. casei (2×10^9 CFU/g) and *Bifidobacterium bifidum* (2×10^9 CFU/g) for 6 weeks (n = 30).

Karamali 2016 *(Continued)*

	Placebo: group took daily capsules filled with cellulose and indistinguishable in colour, shape, size and packaging, as well as in smell and taste, from the probiotic capsules (n = 30).
Outcomes	FPG, serum insulin levels, HOMAR-IR, and HOMAR B cell function (only for unadjusted results, when results adjusted for baseline differences between groups there was no significant different in HOMAR B), insulin sensitivity check index, serum triglycerides and VLDL cholesterol concentrations.
Notes	**Funding:** grant from the Vice Chancellor for Research, IUMS, Tehran, Iran. All capsules were produced by the Tak Gen Zist Pharmaceutical Company in Tehran, Iran, and approved by the Food and Drug Administration. **Conflicts of interest:** authors declare no conflicts of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random assignment of the participants was conducted using computer-generated random numbers
Allocation concealment (selection bias)	Unclear risk	Quote: "Allocations were concealed from the researchers and participants until the final analyses were completed".
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "The trial was blinded with placebo capsules were indistinguishable in colour, shape, size and packaging, as well as in smell and taste, from the probiotic capsules".
Blinding of outcome assessment (detection bias) All outcomes	Low risk	As above
Incomplete outcome data (attrition bias) All outcomes	Low risk	60 women randomised. 3 women in the placebo group were lost to follow-up (withdrew for personal reasons). None of the participants in the intervention arm were lost to follow-up.
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	Significant differences in baseline levels of FPG and HDL cholesterol between the 2 groups, but after further adjusting these variables as well as for baseline maternal age and BMI. the results were similar in both groups except for HOMA-B (P = 0.08).

Karamali 2018

Study characteristics

Methods	Randomised, double-blind, placebo-controlled clinical trial
Participants	Pregnant women with GDM (n = 60) **Setting** Akbarabadi clinic affiliated to Iran University of Medical Sciences (IUMS), Tehran, Iran. **Dates of Study**

Karamali 2018 *(Continued)*

	April 2016 and December 2016. **Inclusion criteria** Pregnant women diagnosed with GDM using the ADA guidelines, aged 18-40 years (n = 30). **Exclusion criteria** Pre-eclampsia, eclampsia, hypo- and hyperthyroidism, smokers and those with kidney or liver diseases and required commencing insulin therapy during intervention; taking any probiotic and/or synbiotic products including probiotic yogurt and kefir during the trial (n = 30).
Interventions	Synbiotic capsule containing *Lactobacillus acidophilus* strain T16 (IBRC-M10785), *L. casei* strain T2 (IBRC-M10783) and *Bifidobacterium bifidum* strain T1 (IBRC-M10771) (2×10^9 CFU/g each) plus 800 mg inulin (HPX) or placebo for 6 weeks.
Outcomes	Primary outcomes: inflammatory markers. Secondary outcomes: biomarkers of oxidative stress and pregnancy outcomes.
Notes	**Funding:** funded by a grant from the Vice Chancellor for Research, IUMS, Tehran, Iran **Conflicts of interest:** authors declared no conflict of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was by computer-generated random numbers.
Allocation concealment (selection bias)	Unclear risk	Unclear if researchers were aware of the upcoming allocation when recruiting participants.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blind placebo-controlled trial in the text
Blinding of outcome assessment (detection bias) All outcomes	Low risk	As above
Incomplete outcome data (attrition bias) All outcomes	Low risk	There were no losses to follow-up and all participant data were analysed.
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	None

Kijmanawat 2019

Study characteristics	
Methods	Randomised, double-blind, placebo-controlled trial

Kijmanawat 2019 *(Continued)*

Participants	Pregnant women with GDM (n = 60) **Setting** Antenatal Care Clinic, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand **Dates of Study** July 2016 and February 2017. **Inclusion criteria** Pregnant women with GDM, between 24-28 weeks' gestation, age 18 to 45 years. GDM by the International Association of Diabetes and Pregnancy Study Groups criteria as follows: FPG ≥ 92 mg/dL at the first prenatal visit, or an abnormal glucose tolerance test at 24–28 weeks-of-gestation using a 75-g oral glucose load (defined as 1 or more of the following abnormal glucose values: FPG ≥ 92 mg/dL, 1-hour ≥ 180 mg/dL, 2-hour ≥ 153 mg/dL). **Exclusion criteria** Fetal or chromosomal abnormalities, chronic diseases (such as immunodeficiency, hypertension, pre-gestational diabetes, kidney disease or liver disease). Consumption of probiotic food products, such as yogurt, fermented foods and bean paste during the 2 weeks before enrolment; exposure to antibiotics during the 4 weeks before enrolment.
Interventions	Probiotic capsule (Infloran) contained 1,000 million CFU of *Lactobacillus acidophilus* and 1,000 million CFU of *Bifidobacterium bifidum* (n = 30) Placebo capsule contained gelatin (n = 30)
Outcomes	Primary outcome: mean differences in FPG, insulin and insulin resistance index. Secondary outcomes: mean difference in maternal weight gain across the study period.
Notes	**Funders** The Thailand Research Fund (TRF). **Conflict of interest** SR receives grant support from Merck Sharp and Dohme, research equipment support from ResMed, and speaker honoraria from Sanofi, Novo Nordisk and Medtronic. Other authors declared no conflict of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random allocation sequence was carried out in blocks of 4 by the statistician.
Allocation concealment (selection bias)	Unclear risk	Researchers arranged the enrolment and intervention assignment of participants. Unclear if they knew.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The capsules and their packages were unidentified to participants, researchers and primary investigators.

Kijmanawat 2019 *(Continued)*

Blinding of outcome assessment (detection bias) All outcomes	Low risk	As above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	60 women were randomised. Discontinued from the probiotic group (2): diagnosis of systemic lupus erythematosus requiring steroid treatment (1), antibiotic use during the study period (1). Discontinued from the placebo group: (1) due to a subsequent diagnosis of systemic lupus erythematosus requiring steroid treatment. 57/60 (95%) participants were analysed.
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	Baseline characteristics were balanced between both groups.

Lindsay 2015

Study characteristics	
Methods	Parallel randomised controlled trial
Participants	Pregnant women with GDM (n = 149). **Setting** National Maternity Hospital, Dublin, Ireland **Dates of Study** March 2012 to May 2014. Final birth July 2014 **Inclusion criteria** Pregnant women attending the National Maternity Hospital who were newly diagnosed with either IGT (1 raised value) or GDM (2 raised values) following a 3-hour 100-g OGTT in the current pregnancy, age > 18 years, < 34 weeks' gestation, singleton pregnancy and adequate English **Exclusion criteria** Pregestational diabetes, were aged < 18 years, were 34 weeks' gestation, had a multiple pregnancy or fetal anomaly, were commenced on insulin or metformin therapy immediately after diagnosis, or had a poor understanding of the English language
Interventions	Probiotic: capsule contained 100 mg of *Lactobacillus salivarius* UCC118 at a target dose of 10^9 CFU (n = 74). Placebo: identical in appearance to probiotics (n = 75).
Outcomes	Maternal outcomes: FPG, C-peptide, triglycerides; requirement for pharmacological therapy, Total, LDL, and HDL cholesterol, insulin, and triglycerides, HOMAR-IR, gestational weight gain, hypertension, delivery complications Neonatal/infant outcomes: birthweight, glucose, c-peptide, lipids, 5-minute Apgar score, and admission to NICU

Lindsay 2015 *(Continued)*

Notes	**Funding**: National Maternity Hospital Medical Fund with support from the Ivo Drury Award and the European Union's Seventh Framework Program (FP7/2007-2013), project Early Nutrition under grant agreement number 289346. **Conflicts of interest**: F.S. is a shareholder in Alimentary Health Ltd and has received grants from GlaxoSmithKline and the Procter and Gamble Company in the past. The remaining authors report no conflict of interest.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated simple randomisation process in a ratio of 1:1.
Allocation concealment (selection bias)	Low risk	Allocation to either 1 of the capsules was conducted by an independent researcher. The allocation sequence was sequentially numbered in sealed, opaque envelopes and was concealed from the research dietitian enrolling and assessing the participants.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Probiotics and placebo identical capsules were supplied and researchers and participants were unaware of the allocation.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All clinical and laboratory staff who were involved with care of study participants or analysis of samples remained blinded to the allocation sequence
Incomplete outcome data (attrition bias) All outcomes	Low risk	149 women randomised. In the probiotic arm, 9 women were lost to follow-up, 4 discontinued intervention when they changed their minds. In the placebo arm, 9 women were lost to follow-up and 4 changes their minds. 79 were analysed
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	There was a slightly lower rate of Caucasian ethnicity and obesity and a higher rate of primiparity in the probiotic compared to placebo group but these differences were not significant. Otherwise the groups were balanced at the baseline

Nabhani 2018

Study characteristics

Methods	Double-blind, placebo-controlled, randomised clinical trial
Participants	Pregnant women with GDM (n = 95). **Setting** Diabetes East Health Centre, Ahwaz, Iran **Dates of study** January 2015 and September 2016

Nabhani 2018 *(Continued)*

	Inclusion criteria Pregnant women aged 18-40 years, diagnosed with GDM according to Diabetes Association guideline criteria, by a 1-step 2-hour, 75 g OGT at 24-28 weeks' gestation. (Fasting ≥ 92 mg/dL, 1 hour ≥ 180 mg/dL, 2 hour ≥ 153 mg/dL.) **Exclusion criteria** Use of anti-diabetic drugs, (metformin, insulin) Current smokers, placenta abruption, pre-eclampsia, eclampsia, hypo/hyperthyroidism, kidney, liver, inflammatory or immunodeficiency diseases, thyroid disorders, lactose intolerant. Also using any kinds of oestrogen, progesterone, cholesterol-lowering drugs or diuretics; consuming any type of probiotics in the past 1 month prior to GDM diagnosis. Also if currently taking probiotic food or supplements during study period, taking antibiotics or glucocorticoids.
Interventions	Synbiotic capsule (LactoFem) contained 500 mg of *Lactobacillus* probiotic strains consisting of: *L. acidophilus* (5 x 10^{10} CFU/g), *L. plantarum* (1.5 x 10^{10} CFU/g), *L. fermentum* (7 x 10^{9} CFU/g), *L. gasseri* (2 x 10^{10} CFU/g) and 38.5 mg of FOS as prebiotic substance. Other components included lactose (300 mg), magnesium stearate, talc, colloidal silicon dioxide (each of them 5.5 mg), flavourings and sweeteners that have neutral effects (n = 48). Placebo capsules contained similar as above: lactose (300 mg), magnesium stearate, talc, colloidal silicon dioxide (each of them 5.5 mg), flavourings and sweeteners that have neutral effects) without the probiotics. The appearance, texture, weight and smell of capsules and packages were identical for both synbiotics and placebo and they were only different in their codes A or B that were placed on their packs (n = 47).
Outcomes	Biochemical factors of FPG, serum insulin, HOMA-IR, quantitative insulin sensitivity check index (QUICKI), lipid profile (LDL-C, HDL-C, TG) and TC, TAC and blood pressure indices.
Notes	**Source of funding** Tabriz University of Medical Sciences, Tabriz, Iran and Nutrition Research Center. (LactoFem) were produced by ZistTakhmir Pharmaceutical Company in Tehran, Iran, and registered at the Food and Drug Administration. **Conflicts of interest:** No conflicts of interest reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random sequence by random allocation software. Computer block randomisation
Allocation concealment (selection bias)	Unclear risk	All researchers were unaware of the allocation.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The appearance, texture, weight and smell of capsules and packages were identical for both synbiotics and placebo and they were only different in their codes A or B that were placed on their packs
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All researchers were blinded to the allocation.

Nabhani 2018 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	Synbiotic: discontinued n = 3 (1 preterm labour before 3 weeks, 1 change of mind, 1 did not consume supplements according to plan). Placebo group – discontinued intervention n = 2 (1 changed mind, 1 preterm labour before 35 weeks)
Selective reporting (reporting bias)	Unclear risk	No retrospective trial registration. Insufficient information to judge if pre-specified outcomes are reported in full.
Other bias	Low risk	Analysis of dietary intakes showed that there were no significant differences between the 2 groups for the macro- and micronutrient intakes, except for the energy, protein and total fat intakes (P < 0.05); thus, final analyses were adjusted for the measures of energy intake, BMI and baseline values.

BG: blood glucose;**BMI:** body mass index; **BP:** blood pressure; **CFU:** colony-forming units; **CRP:** C-reactive protein; **erythrocyte GPX:** red blood cell glutathione peroxidase; **erythrocyte SOD:** red blood cell superoxide dismutase; **Fe:** iron; **FOS:** Fructo-oligosaccharides; **FPG:** fasting plasma glucose; **GDM:** gestational diabetes mellitus; **GI:** gastrointestinal; **GSH:** glutathione; **HDL:** high-density lipoprotein; **HOMA:** Homeostasis Model Assessment; **IGT:** impaired glucose tolerance**; IL-6:** interleukin 6;**LGA:** large-for-gestational age; **MDA:** malondialdehyde; **MET:** metabolic equivalents; **MFA:** monounsaturated fatty acids; **Mg:** magnesium; **NICU:** neonatal intensive care unit; **NO:** nitric oxide; **LDL:** low-density lipoprotein; **OGTT:** oral glucose tolerance test; **PUFA:** polyunsaturated fatty acids; **SFA:** saturated fatty acids; **TAC:** total antioxidant capacity; very low-density lipoprotein; **TAG:** triglycerides; **TNF:** tumour necrosis factor; **ZN:** zinc.

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

Study	Reason for exclusion
Al-Dughaishi 2016	Not an RCT
Asemi 2013	RCT not in GDM women
Asemi 2013a	RCT in T2DM
Barrett 2012	Systematic Review of probiotics for preventing GDM
Barrett 2014	Cochrane review on probiotics for preventing GDM
Barthow 2016	RCT not in GDM women
Fei 2014	RCT using a prebiotic soybean oligosaccharide.
Gomez 2015	Not an RCT
Hamano 2013	Non-blinded study comparing probiotic yoghurt and standard diet.
Lindsay 2013	Observational study
Lindsay 2014	RCT of probiotics in women with obesity not GDM
Luoto 2010	RCT of probiotics but not in GDM women
Muktabhant 2015	Cochrane Review on Diet or exercise not GDM women
Nitert 2013	SPRING RCT in overweight and obese and not in women with GDM
Okesene-Gafa 2018	RCT not in women with GDM

Study	Reason for exclusion
Wickens 2017	Probiotics not in women with GDM to see if they reduce prevalence of GDM
Zhang 2018	RCT (3 arms) using prebiotics (Xylooligosaccharide) and not probiotics

GDM: gestational diabetes mellitus; **RCT:** randomised controlled trial; **T2DM:** type 2 diabetes.

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

Gonai 2014

Methods	Randomised trial
Participants	Pregnant women with GDM **Setting** Japan (Kawasaki) **Dates of Study** Not defined **Inclusion criteria** Pregnant Japanese women age > 40 years, plasma glucose > 140 mg/dL, past GDM history, BMI > 30 and past delivery of macrosomia. **Exclusion criteria** Not stated (appears pre-existing diabetes excluded)
Interventions	**Probiotics** (lactobacilli GG yoghurt) **plus dietary intervention** **Standard care:** regular dietary intervention
Outcomes	Fasting plasma glucose and glucagon like peptide-1 (GLP-1) just prior to delivery and OGTT 2 months after delivery.
Notes	Hypothesis: dietary intervention like lactobacillus yoghurt may improve GDM patients' metabolic status or newborn outcomes through.

Jamilian 2019

Methods	Randomised trial
Participants	Pregnant women with GDM **Setting** Iran **Dates of Study** **Inclusion criteria** Pregnant women, age 18-40 years, at 24-28 weeks of gestation, diagnosed with GDM by a 2-hour 75g OGTT, based on the American Diabetes Association guidelines.

Jamilian 2019 *(Continued)*

	Exclusion criteria Intake of probiotics or synbiotics, vitamin D supplements anytime during the last three months prior to the intervention; requiring insulin treatment, smoker, hyperthyroidism, pre-eclampsia, eclampsia, hypo- and hyperthyroidism.
Interventions	**Vitamin D + probiotics:** 50,000 IU vitamin D3 **plus** probiotics containing ($8x10^9$ CFU/g) *Lactobacillus acidophilus*, *Bi-dobacterium bidum*, *L. reuteri*, and *Lactobacillus fermentum* **Probiotics:** Receive one probiotic per day containing (8×10^9 CFU/g) *Lactobacillus acidophilus*, *Bidobacterium bidum*, *L. reuteri*, and Lactobacillus fermentum. **Placebo:** paraffin (as placebo for vitamin D) and starch (placebo for probiotics). These were identical in appearance (size, shape, colour), taste and smell.
Outcomes	Primary outcomes: markers of insulin metabolism Secondary outcomes: lipid profiles; biomarkers of inflammation and oxidative stress. (Serum hydroxyvitamin D, serum insulin concentrations, HOMA-IR, Quantitative insulin sensitivity index (QUICKI), fasting plasma glucose, serum triglycerides, VLDV, LDL, HDL-cholesterol, hs-CRP, Nitric oxide (NO), total antioxidant capacity (TAC), total glutathione, malondialdehyde (MDA) concentrations). Clinical outcomes: polyhydramnios, preterm birth (birth < 37 weeks). Newborn outcome: hyperbilirubinaemia.
Notes	Aims: to assess effects of vitamin D together with probiotics (8×10^9 CFU/day) compared to placebo on markers of metabolism and pregnancy outcomes of women with GDM. Total treatment time of six weeks.

CFU: colony-forming units; **BMI:** body mass index; **GDM:** gestational diabetes mellitus; **HOMA:** Homeostasis Model Assessment;**OGTT:** oral glucose tolerance test..

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

Asemi 2019

Study name	The effects of selenium and probiotics co-supplementation on pregnancy outcomes, inflammatory factors, oxidative stress biomarkers and gene expression related to inflammation in women with gestational diabetes
Methods	Randomised double-blind placebo-controlled trial.
Participants	Pregnant women with GDM, aged 18-40 years, referred to Kosar Clinic affiliated to Arak University of Medical Sciences. Exclusion: overt diabetes mellitus. Taking any supplements before the intervention. Unwillingness to cooperate.
Interventions	Combined probiotics *Lactobacillus acidophilus*, *Bifidobacterium Bifidum* and *Bifidobacterium langum* (all 2×10^9) daily and selenium supplements (Webber Naturals Canada) 200 µg orally daily for 6 weeks. Other group receives placebo (Barij Essence, Kashan, Iran) daily orally for 6 weeks.
Outcomes	Primary outcomes: markers of insulin metabolism Secondary outcomes: lipid profiles, gene expression related to insulin and lipids.
Starting date	Recruitment dates: no information.

Asemi 2019 *(Continued)*

Contact information	Contact Zatollah Asemi, asemi_z@kaums.ac.ir
Notes	Title: The effects of selenium and probiotics co-supplementation on pregnancy outcomes, inflammatory factors, oxidative stress biomarkers and gene expression related to inflammation in women with gestational diabetes. Total sample size: 60. IRCT registration number: IRCT20170513033941N55

CTRI/2018/08/015432

Study name	Effect of probiotic supplementation on blood glucose of gestational diabetic mellitus (GDM) mothers
Methods	Double-blind randomised trial.
Participants	Pregnant women, primigravidae with a singleton pregnancy, with a normal fetal scan, at 12-14 weeks' gestation, with an OGTT confirming GDM as per respective O&G consultants. Exclusion: pregnant GDM mothers with gestational age > 28 weeks, pre-GDM, medications that influence insulin resistance (glucocorticoids, immunosuppressants), taking any form of probiotics < 1 month before recruitment.
Interventions	Receive 1 probiotic capsule a day that contains recommended strains of bacteria from the onset of GDM Routine care without probiotics receive counselling session on diet and physical activity interventions for pregnant women with GDM. Trained dietitians would give the counselling sessions on modified diet with portions and size; printed educational materials that included common diet guidelines and physical activity information on GDM. In the postnatal period, they will receive session on importance of compliance of lifestyle modifications such as diet and physical activity after delivery.
Outcomes	Primary outcomes: blood glucose levels (fasting and postprandial), urine analysis for pus cells. Secondary outcomes: maternal: mode of delivery, preterm delivery, perineal tear, pregnancy-induced hypertension, hydramnios, vaginal infections. Neonatal/infant: birthweight, congenital malformations, birth injuries, shoulder dystocia, hypoglycaemia, NICU admissions.
Starting date	
Contact information	
Notes	Title: The effect of maternal probiotic supplementation on glycaemic control and pregnancy outcomes among GDM. Total sample size: 202. CTRI/2018/08/015432. Kavitha Ramanathan: Study not yet recruiting. Locality Chennai, India.

IRCT20121224011862N2

Study name	The effect of probiotic yogurt and conventional yogurt in blood glucose control and outcomes of pregnancy in women with gestational diabetes
Methods	Double-blind randomised clinical trial

IRCT20121224011862N2 *(Continued)*

Participants	Pregnant women with gestational diabetes 24-28 weeks' gestation with a singleton. Exclusion criteria: history of diabetes in previous pregnancy or fetal abnormality.
Interventions	Intervention group will consume probiotic yoghurt containing 10^6colony of *Lactobacillus acidophilus* and *Bifidobacterium lactis* for 8 weeks. Control group will consume 300 g/day of conventional yoghurt.
Outcomes	HBA1c and fasting blood glucose before and after 8 weeks.
Starting date	
Contact information	Contact: Farnaz Sahaf email: sahaf@tbzmed.ac.ir
Notes	The effect of probiotic yogurt and conventional yogurt in blood glucose control and outcomes of pregnancy in women with gestational diabetes. Recruitment centres: Alzahra Hospital, South Artesh Street, East Azarbaijan, Iran and Taleghani Hospital, Rah Ahan, Tabriz, East Azerbaijan Province, Iran. Funding: Tabriz University of Medical Sciences IRCT registration number: IRCT20121224011862N2 Registration date: 2018-05-19, 1397/02/29 Registration timing: registered_while_recruiting Last update: 2018-05-19, 1397/02/29 Current status: recruiting

IRCT2015110310089N4

Study name	Comparison of probiotic capsules and placebo on fasting blood sugar and metabolic parameters in pregnant woman with gestational diabetes or impaired glucose tolerance test
Methods	Randomised controlled double-blind trial
Participants	Pregnant women with GDM 18-49 years (GDM diagnosed by a 3-hourOGTT based on carpenter criteria by internist). Inclusions criteria: single pregnancy; new diagnosis of IGT or GDM in the current pregnancy; < 34 weeks' gestation; without insulin or metformin therapy. Exclusions criteria: "pre-gestational diabetes; fetal anomaly; without follow; do not use probiotics" Locality: prenatal clinic in Al-Zahra hospital of Guilan University of Medical Sciences.
Interventions	Probiotics with brand Lactofem
Outcomes	Primary outcomes: probiotics user, placebo user. Secondary outcomes: fasting blood glucose, triglyceride, total cholesterol, low density lipoprotein, high density lipoprotein, blood pressure.
Starting date	Expected starting date 22.12.2015, expected completion date 21.12.2016. Currently no information.
Contact information	Contact: Arezoo Fani, email: arezoo.fani@yahoo.com
Notes	IRCT registration number: IRCT2015110310089N4 Registration date: 2016-02-01, 1394/11/12 Registration timing: registered while recruiting

IRCT20171010036697N1

Study name	The effect of probiotic supplementation on gene expression related to insulin, lipid and inflammation in patients with gestational diabetes
Methods	Double-blind randomised placebo-controlled clinical trial
Participants	Pregnant women 18 to 40 years with gestational diabetes Exclusion: unwillingness to cooperate
Interventions	Intervention group: probiotic supplements containing 4 strains ofLactobacillus acidophilus (2 × 109 CFU/g), Lactobacillus casei (2×109 CFU/g) and *Bifidobacterium bifidum* (2 × 10^9 CFU/g), *Lactobacillus fermentum* (2 × 10^9 CFU/g), daily, for 6 weeks orally. Control group: placebo, daily, for 6 weeks orally.
Outcomes	Primary outcomes at baseline and 6 weeks after intervention, PCR measurements for: expressed levels of GLUT-1 gene, PPAR-? gene. Secondary outcomes at baseline and 6 weeks post intervention: expressed levels LDLR gene, IL-1 gene, TNF-a gene, LPa gene and IL-8 gene.
Starting date	
Contact information	
Notes	Funding: Vice Chancellor for research, Kashan University of Medical Sciences

Nachum 2019

Study name	The effect of oral probiotics on glycaemic control of women with gestational diabetes mellitus
Methods	Randomised, double-blind, placebo-controlled trial, phase 4.
Participants	Inclusion: pregnant women diagnosed with GDM from 13 to 32.6 weeks, > 18 years old, singleton pregnancy.
Interventions	Dietary supplement (Femina II) 2 capsules per day until delivery Placebo: 2 capsules per day until delivery.
Outcomes	Primary outcomes: the rate of women who will require pharmacotherapy for glycaemic control. The mean value of the daily glucose charts after 2 weeks of treatment with the study products. Secondary outcomes: the rate of women with controlled diabetes; mean daily glucose charts; mean daily pre-prandial glucose levels; mean daily postprandial glucose levels; level of glycated molecules; rate of: women with mean pre-prandial values ≥ 95 mg/dL, mean post-prandial values ≥ 130 mg/dL, and mean daily glucose > 100 mg/dL; caesareans; labour inductions; birthweight > 4000 g/> 90th percentile; admission to NICU; 1-minute and 5-minute Apgar score; neonatal hypoglycaemia; neonatal hypomagnesaemia; cord blood PH levels; neonatal malformations and developmental disorders; birthweight; head circumference; maternal adverse effects; duration of time until pharmacotherapy for glycaemic control is indicated.
Starting date	Not yet recruiting
Contact information	Zohar Nachum; nachum_zo@clalit.org.il

Nachum 2019 *(Continued)*

Notes	Objectives: to examine the effect of a mixture of probiotic strains given daily on maternal glycaemic parameters, and pregnancy outcomes among women with GDM.

CFU: colony-forming units; **GDM:** gestational diabetic mellitus; **IGT:** impaired glucose tolerance**; NICU:** neonatal intensive care unit;**OGTT:** oral glucose tolerance test; **PCR:** polymerase chain reaction.

DATA AND ANALYSES

Comparison 1. Probiotic versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Hypertensive disorders (including pre-eclampsia, pregnancy-induced hypertension, eclampsia)	3	256	Risk Ratio (M-H, Fixed, 95% CI)	1.50 [0.64, 3.53]
1.2 Mode of birth (caesarean)	3	267	Risk Ratio (M-H, Random, 95% CI)	0.64 [0.30, 1.35]
1.3 Large-for-gestational age > 90 centile	2	174	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.35, 1.52]
1.4 Induction of labour	1	127	Risk Ratio (M-H, Fixed, 95% CI)	1.33 [0.74, 2.37]
1.5 Postpartum haemorrhage	1	126	Risk Ratio (M-H, Fixed, 95% CI)	0.77 [0.36, 1.62]
1.6 Weight gain during pregnancy (kg)	6	379	Mean Difference (IV, Fixed, 95% CI)	1.38 [-0.49, 3.24]
1.7 Total gestational weight gain (kg)	3	239	Mean Difference (IV, Fixed, 95% CI)	0.24 [-0.30, 0.78]
1.8 Relevant biomarker changes associated with the intervention	9		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.8.1 HOMA-IR	7	505	Mean Difference (IV, Fixed, 95% CI)	-0.30 [-0.35, -0.25]
1.8.2 HOMA-B	2	130	Mean Difference (IV, Fixed, 95% CI)	-25.38 [-38.32, -12.44]
1.8.3 Insulin (microIU/L)	7	505	Mean Difference (IV, Fixed, 95% CI)	-1.04 [-1.27, -0.80]
1.8.4 QUICKI	4	276	Mean Difference (IV, Fixed, 95% CI)	0.01 [0.00, 0.02]
1.8.5 TAG (Triglycerides) (mg/dL)	4	320	Mean Difference (IV, Fixed, 95% CI)	-19.19 [-35.69, -2.70]
1.8.6 VLDL cholesterol (mg/dL)	2	130	Mean Difference (IV, Fixed, 95% CI)	-7.80 [-12.93, -2.66]

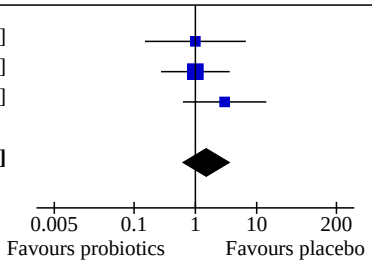
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.8.7 LDL-cholesterol (mg/dL)	4	320	Mean Difference (IV, Fixed, 95% CI)	-5.36 [-12.83, 2.12]
1.8.8 HDL-cholesterol (mg/dL)	4	320	Mean Difference (IV, Fixed, 95% CI)	-3.48 [-6.02, -0.93]
1.8.9 Total cholesterol (mg/dL)	4	320	Mean Difference (IV, Fixed, 95% CI)	-10.63 [-19.73, -1.54]
1.8.10 hs-CRP (μg/mL)	4	248	Mean Difference (IV, Fixed, 95% CI)	-1.29 [-1.72, -0.86]
1.8.11 NO (nitrous oxide)μmol/L	2	120	Mean Difference (IV, Fixed, 95% CI)	1.69 [-0.95, 4.33]
1.8.12 MDA (malondialdehyde) (μmol/L)	3	176	Mean Difference (IV, Fixed, 95% CI)	-0.85 [-1.20, -0.50]
1.8.13 GSH (total glutathione μmol/L)	2	120	Mean Difference (IV, Fixed, 95% CI)	44.95 [13.36, 76.55]
1.8.14 Glutathione reductase (GSHR)(ng/mL)	1	56	Mean Difference (IV, Fixed, 95% CI)	5.78 [0.30, 11.26]
1.8.15 TAC (total antioxidant capacity)mmol/L	4	266	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.05, 0.10]
1.8.16 IL-10(pg/mL)	1	72	Mean Difference (IV, Fixed, 95% CI)	-0.27 [-2.93, 2.39]
1.8.17 IFN-c	1	72	Mean Difference (IV, Fixed, 95% CI)	-1.90 [-9.38, 5.58]
1.8.18 IL-6(pg/mL)	2	128	Mean Difference (IV, Fixed, 95% CI)	-0.89 [-1.17, -0.60]
1.8.19 TNF-α(pg/mL)	2	128	Mean Difference (IV, Fixed, 95% CI)	-0.53 [-0.78, -0.27]
1.8.20 Serum uric acid (mg/dL)	1	56	Mean Difference (IV, Fixed, 95% CI)	-0.21 [-0.52, 0.10]
1.8.21 Erythrocyte superoxide dismutase (SOD) (U/gHBb)	1	56	Mean Difference (IV, Fixed, 95% CI)	189.20 [-57.31, 435.71]
1.8.22 Erythrocyte glutathione peroxidase (GPx) (U/gHb)	1	56	Mean Difference (IV, Fixed, 95% CI)	6.93 [1.34, 12.52]
1.9 Use of additional pharmacotherapy	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
1.9.1 Insulin therapy	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.10 Glycaemic control during/ end of treatment (as defined by trialists)	7		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.10.1 Fasting blood glucose(mg/dL)	7	554	Mean Difference (IV, Fixed, 95% CI)	-0.42 [-1.66, 0.82]
1.10.2 Postprandial blood glucose(mg/dL)	0	0	Mean Difference (IV, Fixed, 95% CI)	Not estimable
1.11 Gestational age at birth (days)	3	267	Mean Difference (IV, Fixed, 95% CI)	1.37 [-1.33, 4.07]
1.12 Preterm birth	2	120	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.18, 5.59]
1.13 Macrosomia (> 4000 g)	3	267	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.50, 1.43]
1.14 Small-for-gestational age (SGA)	1	114	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.39, 2.76]
1.15 Birthweight (g)	4	324	Mean Difference (IV, Fixed, 95% CI)	-79.14 [-183.00, 24.73]
1.16 Head circumference (cm)	3	249	Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.52, 0.48]
1.17 Length (cm)	3	248	Mean Difference (IV, Fixed, 95% CI)	-0.35 [-1.03, 0.33]
1.18 Infant hypoglycemia requiring treatment (variously defined)	3	177	Risk Ratio (M-H, Fixed, 95% CI)	0.85 [0.39, 1.84]
1.19 Hyperbilirubinemia	2	120	Risk Ratio (M-H, Fixed, 95% CI)	0.18 [0.05, 0.57]
1.20 Relevant infant biomarker's associated with intervention (cord C peptide, cord insulin)	1	100	Mean Difference (IV, Fixed, 95% CI)	-0.05 [-0.44, 0.34]
1.20.1 Cord C peptide (ng/mL)	1	100	Mean Difference (IV, Fixed, 95% CI)	-0.05 [-0.44, 0.34]
1.21 Admission to NICU/nursery	2	202	Risk Ratio (M-H, Random, 95% CI)	1.71 [0.45, 6.53]

Analysis 1.1. Comparison 1: Probiotic versus placebo, Outcome 1: Hypertensive disorders (including pre-eclampsia, pregnancy-induced hypertension, eclampsia)

Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	2	30	2	30	25.0%	1.00 [0.15 , 6.64]
Karamali 2018	4	30	4	30	50.0%	1.00 [0.28 , 3.63]
Lindsay 2015	6	68	2	68	25.0%	3.00 [0.63 , 14.34]
Total (95% CI)		**128**		**128**	**100.0%**	**1.50 [0.64 , 3.53]**
Total events:	12		8			

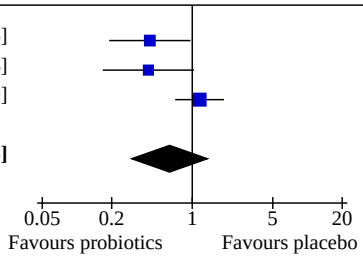
Heterogeneity: Chi² = 1.31, df = 2 (P = 0.52); I² = 0%
Test for overall effect: Z = 0.93 (P = 0.35)
Test for subgroup differences: Not applicable



Analysis 1.2. Comparison 1: Probiotic versus placebo, Outcome 2: Mode of birth (caesarean)

Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Badehnoosh 2018	6	30	14	30	31.1%	0.43 [0.19 , 0.96]
Karamali 2018	5	30	12	30	28.4%	0.42 [0.17 , 1.04]
Lindsay 2015	24	73	21	74	40.4%	1.16 [0.71 , 1.89]
Total (95% CI)		**133**		**134**	**100.0%**	**0.64 [0.30 , 1.35]**
Total events:	35		47			

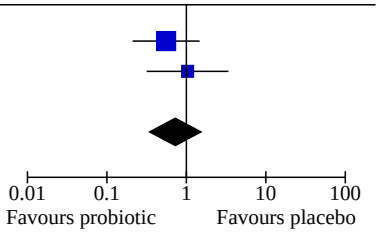
Heterogeneity: Tau² = 0.30; Chi² = 6.47, df = 2 (P = 0.04); I² = 69%
Test for overall effect: Z = 1.18 (P = 0.24)
Test for subgroup differences: Not applicable



Analysis 1.3. Comparison 1: Probiotic versus placebo, Outcome 3: Large-for-gestational age > 90 centile

Study or Subgroup	Probiotic Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	5	30	9	30	64.7%	0.56 [0.21 , 1.46]
Lindsay 2015	5	56	5	58	35.3%	1.04 [0.32 , 3.38]
Total (95% CI)		**86**		**88**	**100.0%**	**0.73 [0.35 , 1.52]**
Total events:	10		14			

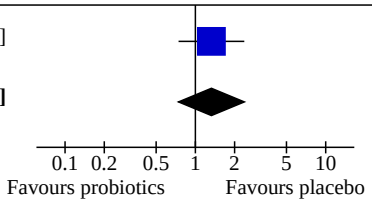
Heterogeneity: Chi² = 0.64, df = 1 (P = 0.42); I² = 0%
Test for overall effect: Z = 0.85 (P = 0.40)
Test for subgroup differences: Not applicable



Analysis 1.4. Comparison 1: Probiotic versus placebo, Outcome 4: Induction of labour

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Lindsay 2015	19	62	15	65	100.0%	1.33 [0.74 , 2.37]
Total (95% CI)		**62**		**65**	**100.0%**	**1.33 [0.74 , 2.37]**
Total events:	19		15			

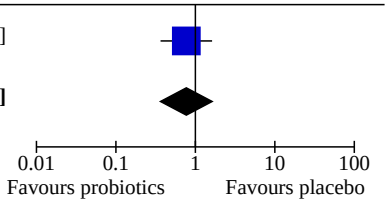
Heterogeneity: Not applicable
Test for overall effect: Z = 0.96 (P = 0.34)
Test for subgroup differences: Not applicable



Analysis 1.5. Comparison 1: Probiotic versus placebo, Outcome 5: Postpartum haemorrhage

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Lindsay 2015	10	63	13	63	100.0%	0.77 [0.36 , 1.62]
Total (95% CI)		**63**		**63**	**100.0%**	**0.77 [0.36 , 1.62]**
Total events:	10		13			

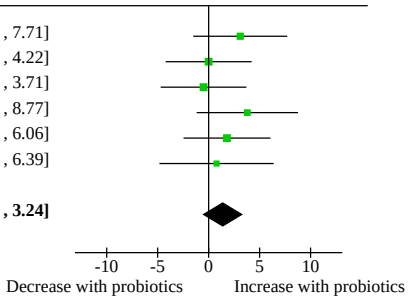
Heterogeneity: Not applicable
Test for overall effect: Z = 0.69 (P = 0.49)
Test for subgroup differences: Not applicable



Analysis 1.6. Comparison 1: Probiotic versus placebo, Outcome 6: Weight gain during pregnancy (kg)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Ahmadi 2016	80.3	12.3	35	77.2	6.5	35	16.4%	3.10 [-1.51 , 7.71]
Badehnoosh 2018	77.4	9.1	30	77.4	7.5	30	19.6%	0.00 [-4.22 , 4.22]
Jafarnejad 2016	75.7	8.3	37	76.2	9.8	35	19.7%	-0.50 [-4.71 , 3.71]
Karamali 2016	79.3	10.5	30	75.5	9.1	30	14.1%	3.80 [-1.17 , 8.77]
Karamali 2018	77	10.4	30	75.2	5.8	30	19.2%	1.80 [-2.46 , 6.06]
Kijmanawat 2019	65.18	11.42	28	64.4	10.11	29	11.1%	0.78 [-4.83 , 6.39]
Total (95% CI)			**190**			**189**	**100.0%**	**1.38 [-0.49 , 3.24]**

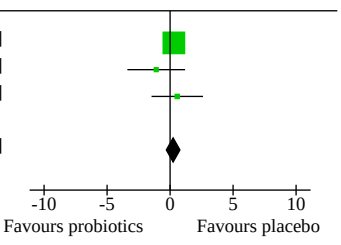
Heterogeneity: Chi² = 2.70, df = 5 (P = 0.75); I² = 0%
Test for overall effect: Z = 1.45 (P = 0.15)
Test for subgroup differences: Not applicable



Analysis 1.7. Comparison 1: Probiotic versus placebo, Outcome 7: Total gestational weight gain (kg)

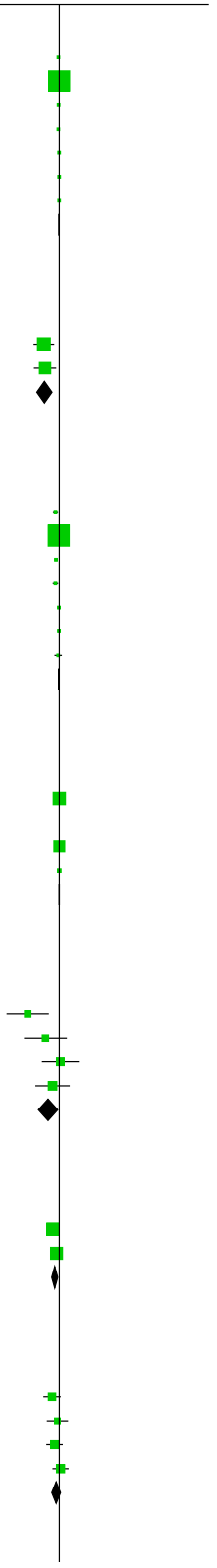
Study or Subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Badehnoosh 2018	11.9	1.4	30	11.6	0.8	30	87.5%	0.30 [-0.28 , 0.88]
Kijmanawat 2019	10.72	4	28	11.82	4.8	29	5.5%	-1.10 [-3.39 , 1.19]
Lindsay 2015	8.53	5.67	59	7.96	5.83	63	7.0%	0.57 [-1.47 , 2.61]
Total (95% CI)			**117**			**122**	**100.0%**	**0.24 [-0.30 , 0.78]**

Heterogeneity: Chi² = 1.46, df = 2 (P = 0.48); I² = 0%
Test for overall effect: Z = 0.88 (P = 0.38)
Test for subgroup differences: Not applicable



Analysis 1.8. Comparison 1: Probiotic versus placebo, Outcome 8: Relevant biomarker changes associated with the intervention

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
1.8.1 HOMA-IR								
Ahmadi 2016	2.7	1	35	4.2	2.8	35	0.3%	-1.50 [-2.49 , -0.51]
Hajifaraji 2017	1.11	0.09	29	1.4	0.11	27	96.6%	-0.29 [-0.34 , -0.24]
Jafarnejad 2016	3.7	1.5	37	4.9	1.2	35	0.7%	-1.20 [-1.83 , -0.57]
Karamali 2016	2.5	1	30	4.1	2.7	30	0.3%	-1.60 [-2.63 , -0.57]
Kijmanawat 2019	2.07	0.94	28	2.34	1.3	29	0.8%	-0.27 [-0.86 , 0.32]
Lindsay 2015	2.65	1.06	48	2.85	1.78	52	0.8%	-0.20 [-0.77 , 0.37]
Nabhani 2018	2.8	1.9	45	3.03	1.6	45	0.5%	-0.23 [-0.96 , 0.50]
Subtotal (95% CI)			**252**			**253**	**100.0%**	**-0.30 [-0.35 , -0.25]**
Heterogeneity: Chi² = 20.06, df = 6 (P = 0.003); I² = 70%								
Test for overall effect: Z = 11.38 (P < 0.00001)								
1.8.2 HOMA-B								
Ahmadi 2016	40.7	13.8	35	67	51.4	35	53.9%	-26.30 [-43.93 , -8.67]
Karamali 2016	42.4	16.6	30	66.7	50.6	30	46.1%	-24.30 [-43.36 , -5.24]
Subtotal (95% CI)			**65**			**65**	**100.0%**	**-25.38 [-38.32 , -12.44]**
Heterogeneity: Chi² = 0.02, df = 1 (P = 0.88); I² = 0%								
Test for overall effect: Z = 3.84 (P = 0.0001)								
1.8.3 Insulin (microIU/L)								
Ahmadi 2016	11.6	3.8	35	18.1	12.6	35	0.3%	-6.50 [-10.86 , -2.14]
Hajifaraji 2017	5.15	0.41	29	6.12	0.5	27	96.6%	-0.97 [-1.21 , -0.73]
Jafarnejad 2016	16.6	5.9	37	22.3	4.9	35	0.9%	-5.70 [-8.20 , -3.20]
Karamali 2016	11.2	4.4	30	17.8	12.3	30	0.3%	-6.60 [-11.27 , -1.93]
Kijmanawat 2019	9.88	4.15	28	10.53	5.33	29	0.9%	-0.65 [-3.13 , 1.83]
Lindsay 2015	13.04	5.08	48	13.58	7.73	52	0.9%	-0.54 [-3.09 , 2.01]
Nabhani 2018	11.6	12.9813	45	13.5	17.3083	45	0.1%	-1.90 [-8.22 , 4.42]
Subtotal (95% CI)			**252**			**253**	**100.0%**	**-1.04 [-1.27 , -0.80]**
Heterogeneity: Chi² = 25.45, df = 6 (P = 0.0003); I² = 76%								
Test for overall effect: Z = 8.60 (P < 0.00001)								
1.8.4 QUICKI								
Ahmadi 2016	0.33	0.02	35	0.32	0.02	35	50.9%	0.01 [0.00 , 0.02]
Hajifaraji 2017	0.16	0	29	0.16	0	27		Not estimable
Karamali 2016	0.33	0.02	30	0.32	0.02	30	43.7%	0.01 [-0.00 , 0.02]
Nabhani 2018	0.3	0.09	45	0.3	0.04	45	5.4%	0.00 [-0.03 , 0.03]
Subtotal (95% CI)			**139**			**137**	**100.0%**	**0.01 [0.00 , 0.02]**
Heterogeneity: Chi² = 0.44, df = 2 (P = 0.80); I² = 0%								
Test for overall effect: Z = 2.77 (P = 0.006)								
1.8.5 TAG (Triglycerides) (mg/dL)								
Ahmadi 2016	156.5	81.5	35	210.5	72.5	35	20.8%	-54.00 [-90.14 , -17.86]
Karamali 2016	191.1	71.2	30	214.8	73.7	30	20.2%	-23.70 [-60.37 , 12.97]
Lindsay 2015	252.44	84.15	48	250.66	76.17	52	27.3%	1.78 [-29.77 , 33.33]
Nabhani 2018	153.8	70	45	165.3	72	45	31.6%	-11.50 [-40.84 , 17.84]
Subtotal (95% CI)			**158**			**162**	**100.0%**	**-19.19 [-35.69 , -2.70]**
Heterogeneity: Chi² = 5.58, df = 3 (P = 0.13); I² = 46%								
Test for overall effect: Z = 2.28 (P = 0.02)								
1.8.6 VLDL cholesterol (mg/dL)								
Ahmadi 2016	31.3	16.3	35	42.1	14.4	35	50.7%	-10.80 [-18.01 , -3.59]
Karamali 2016	38.2	14.2	30	42.9	14.7	30	49.3%	-4.70 [-12.01 , 2.61]
Subtotal (95% CI)			**65**			**65**	**100.0%**	**-7.80 [-12.93 , -2.66]**
Heterogeneity: Chi² = 1.36, df = 1 (P = 0.24); I² = 26%								
Test for overall effect: Z = 2.98 (P = 0.003)								
1.8.7 LDL-cholesterol (mg/dL)								
Ahmadi 2016	95.8	31.3	35	108.2	32	35	25.4%	-12.40 [-27.23 , 2.43]
Karamali 2016	110.2	37.7	30	113.4	33.9	30	17.0%	-3.20 [-21.34 , 14.94]
Lindsay 2015	137.28	34.03	48	145.4	37.9	52	28.1%	-8.12 [-22.22 , 5.98]
Nabhani 2018	112.1	32.3	45	110	34.3	45	29.5%	2.10 [-11.67 , 15.87]
Subtotal (95% CI)			**158**			**162**	**100.0%**	**-5.36 [-12.83 , 2.12]**
Heterogeneity: Chi² = 2.20, df = 3 (P = 0.53); I² = 0%								
Test for overall effect: Z = 1.40 (P = 0.16)								



Analysis 1.8. (Continued)

Heterogeneity: Chi² = 2.20, df = 3 (P = 0.53); I² = 0%
Test for overall effect: Z = 1.40 (P = 0.16)

1.8.8 HDL-cholesterol (mg/dL)

Ahmadi 2016	46.8	8.1	35	54.8	10.8	35	32.3%	-8.00 [-12.47 , -3.53]
Karamali 2016	49.9	10.2	30	57.4	12.7	30	19.0%	-7.50 [-13.33 , -1.67]
Lindsay 2015	64.97	14.69	48	65.35	13.53	52	21.0%	-0.38 [-5.93 , 5.17]
Nabhani 2018	47	11	45	44.8	12.3	45	27.8%	2.20 [-2.62 , 7.02]
Subtotal (95% CI)			**158**			**162**	**100.0%**	**-3.48 [-6.02 , -0.93]**

Heterogeneity: Chi² = 12.28, df = 3 (P = 0.006); I² = 76%
Test for overall effect: Z = 2.68 (P = 0.007)

1.8.9 Total cholesterol (mg/dL)

Ahmadi 2016	173.9	42.1	35	205.2	43.8	35	20.4%	-31.30 [-51.43 , -11.17]
Karamali 2016	198.3	47.5	30	213.7	46.5	30	14.6%	-15.40 [-39.19 , 8.39]
Lindsay 2015	252.51	37.12	48	260.63	43.31	52	33.3%	-8.12 [-23.89 , 7.65]
Nabhani 2018	190.15	39.2	45	187.9	39	45	31.7%	2.25 [-13.91 , 18.41]
Subtotal (95% CI)			**158**			**162**	**100.0%**	**-10.63 [-19.73 , -1.54]**

Heterogeneity: Chi² = 6.74, df = 3 (P = 0.08); I² = 56%
Test for overall effect: Z = 2.29 (P = 0.02)

1.8.10 hs-CRP (µg/mL)

Badehnoosh 2018	4.5	2.4	30	7	3.9	30	6.9%	-2.50 [-4.14 , -0.86]
Hajifaraji 2017	7.46	3.01	29	9.76	4.12	27	5.1%	-2.30 [-4.20 , -0.40]
Jafarnejad 2016	4.93	0.92	37	6	1.1	35	83.7%	-1.07 [-1.54 , -0.60]
Karamali 2018	5.9	4.3	30	8.4	3.9	30	4.3%	-2.50 [-4.58 , -0.42]
Subtotal (95% CI)			**126**			**122**	**100.0%**	**-1.29 [-1.72 , -0.86]**

Heterogeneity: Chi² = 5.32, df = 3 (P = 0.15); I² = 44%
Test for overall effect: Z = 5.89 (P < 0.00001)

1.8.11 NO (nitrous oxide)µmol/L

Badehnoosh 2018	43	2.1	30	45.2	26.9	30	7.5%	-2.20 [-11.86 , 7.46]
Karamali 2018	41.8	2.9	30	39.8	7.1	30	92.5%	2.00 [-0.74 , 4.74]
Subtotal (95% CI)			**60**			**60**	**100.0%**	**1.69 [-0.95 , 4.33]**

Heterogeneity: Chi² = 0.67, df = 1 (P = 0.41); I² = 0%
Test for overall effect: Z = 1.25 (P = 0.21)

1.8.12 MDA (malondialdehyde) (µmol/L)

Badehnoosh 2018	3.4	0.8	30	4	1.7	30	27.2%	-0.60 [-1.27 , 0.07]
Hajifaraji 2017	3.89	1.319	29	4.96	1.678	27	19.5%	-1.07 [-1.86 , -0.28]
Karamali 2018	2.3	0.6	30	3.2	1.2	30	53.3%	-0.90 [-1.38 , -0.42]
Subtotal (95% CI)			**89**			**87**	**100.0%**	**-0.85 [-1.20 , -0.50]**

Heterogeneity: Chi² = 0.87, df = 2 (P = 0.65); I² = 0%
Test for overall effect: Z = 4.76 (P < 0.00001)

1.8.13 GSH (total glutathione µmol/L)

Badehnoosh 2018	409.8	39	30	382.8	126.6	30	44.4%	27.00 [-20.40 , 74.40]
Karamali 2018	514.9	68.9	30	455.6	96.3	30	55.6%	59.30 [16.93 , 101.67]
Subtotal (95% CI)			**60**			**60**	**100.0%**	**44.95 [13.36 , 76.55]**

Heterogeneity: Chi² = 0.99, df = 1 (P = 0.32); I² = 0%
Test for overall effect: Z = 2.79 (P = 0.005)

1.8.14 Glutathione reductase (GSHR)(ng/mL)

Hajifaraji 2017	28.38	11.4704	29	22.6	9.405	27	100.0%	5.78 [0.30 , 11.26]
Subtotal (95% CI)			**29**			**27**	**100.0%**	**5.78 [0.30 , 11.26]**

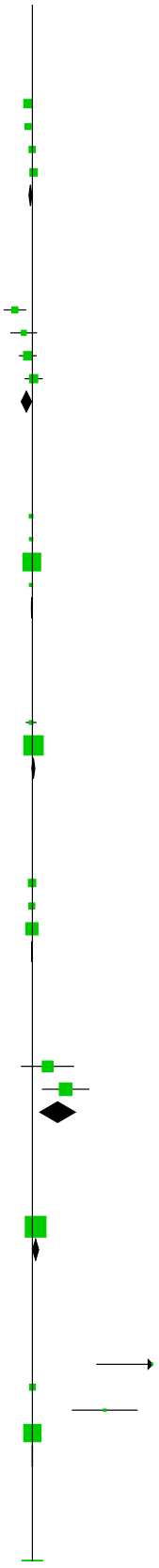
Heterogeneity: Not applicable
Test for overall effect: Z = 2.07 (P = 0.04)

1.8.15 TAC (total antioxidant capacity)mmol/L

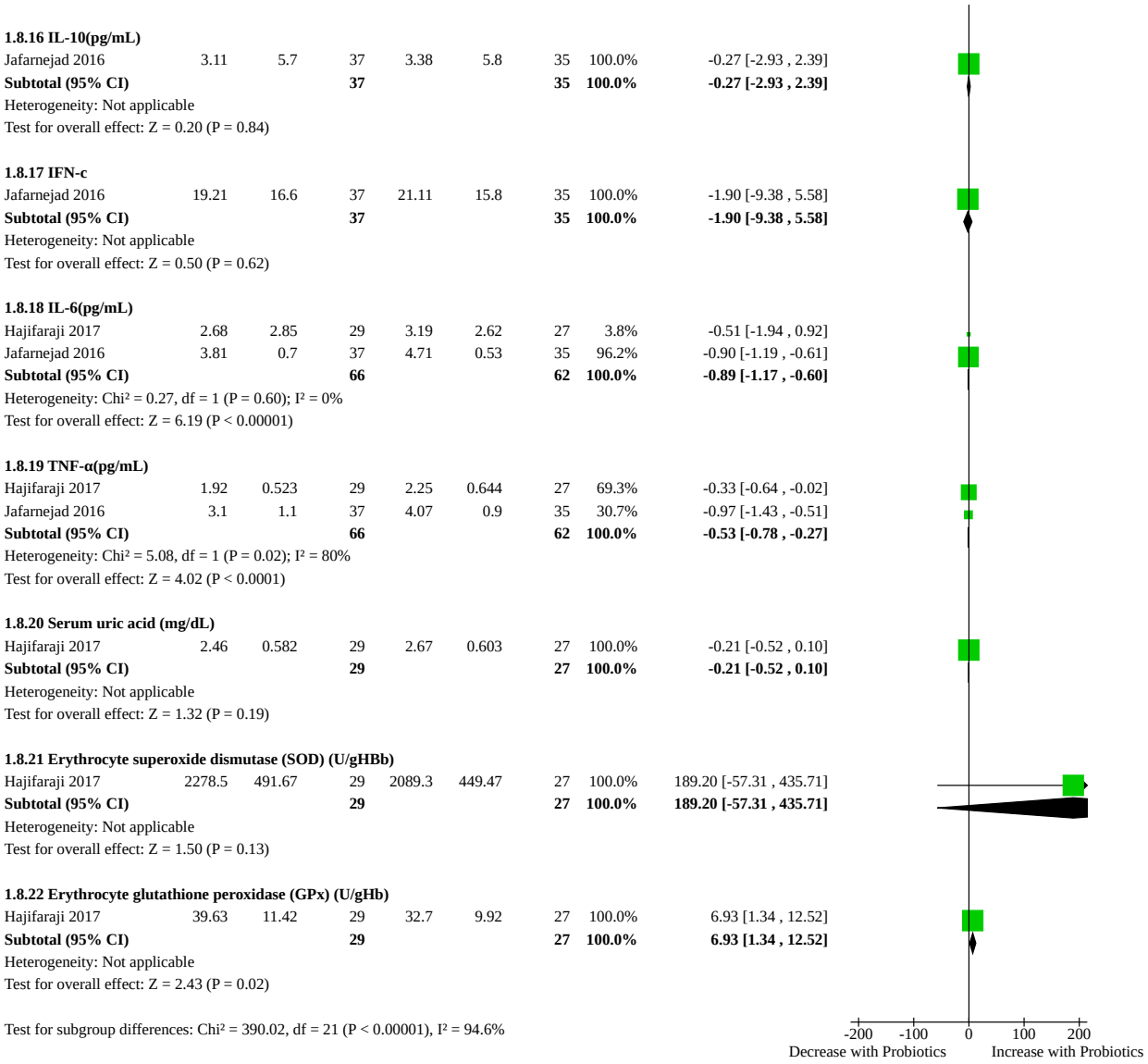
Badehnoosh 2018	1050.5	119.7	30	835.5	255.7	30	0.0%	215.00 [113.97 , 316.03]
Hajifaraji 2017	1.66	0.292	29	1.55	0.359	27	18.7%	0.11 [-0.06 , 0.28]
Karamali 2018	1040.2	129.6	30	911.2	100.2	30	0.0%	129.00 [70.38 , 187.62]
Nabhani 2018	1.3	0.2	45	1.3	0.2	45	81.3%	0.00 [-0.08 , 0.08]
Subtotal (95% CI)			**134**			**132**	**100.0%**	**0.02 [-0.05 , 0.10]**

Heterogeneity: Chi² = 37.27, df = 3 (P < 0.00001); I² = 92%
Test for overall effect: Z = 0.55 (P = 0.58)

1.8.16 IL-10(pg/mL)

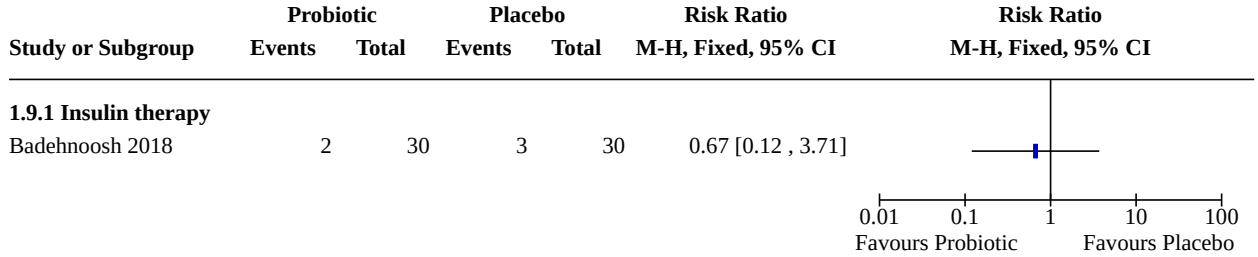


Analysis 1.8. (Continued)



1.8.16 IL-10(pg/mL)								
Jafarnejad 2016	3.11	5.7	37	3.38	5.8	35	100.0%	-0.27 [-2.93 , 2.39]
Subtotal (95% CI)			**37**			**35**	**100.0%**	**-0.27 [-2.93 , 2.39]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.20 (P = 0.84)								
1.8.17 IFN-c								
Jafarnejad 2016	19.21	16.6	37	21.11	15.8	35	100.0%	-1.90 [-9.38 , 5.58]
Subtotal (95% CI)			**37**			**35**	**100.0%**	**-1.90 [-9.38 , 5.58]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.50 (P = 0.62)								
1.8.18 IL-6(pg/mL)								
Hajifaraji 2017	2.68	2.85	29	3.19	2.62	27	3.8%	-0.51 [-1.94 , 0.92]
Jafarnejad 2016	3.81	0.7	37	4.71	0.53	35	96.2%	-0.90 [-1.19 , -0.61]
Subtotal (95% CI)			**66**			**62**	**100.0%**	**-0.89 [-1.17 , -0.60]**
Heterogeneity: Chi² = 0.27, df = 1 (P = 0.60); I² = 0%								
Test for overall effect: Z = 6.19 (P < 0.00001)								
1.8.19 TNF-α(pg/mL)								
Hajifaraji 2017	1.92	0.523	29	2.25	0.644	27	69.3%	-0.33 [-0.64 , -0.02]
Jafarnejad 2016	3.1	1.1	37	4.07	0.9	35	30.7%	-0.97 [-1.43 , -0.51]
Subtotal (95% CI)			**66**			**62**	**100.0%**	**-0.53 [-0.78 , -0.27]**
Heterogeneity: Chi² = 5.08, df = 1 (P = 0.02); I² = 80%								
Test for overall effect: Z = 4.02 (P < 0.0001)								
1.8.20 Serum uric acid (mg/dL)								
Hajifaraji 2017	2.46	0.582	29	2.67	0.603	27	100.0%	-0.21 [-0.52 , 0.10]
Subtotal (95% CI)			**29**			**27**	**100.0%**	**-0.21 [-0.52 , 0.10]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.32 (P = 0.19)								
1.8.21 Erythrocyte superoxide dismutase (SOD) (U/gHBb)								
Hajifaraji 2017	2278.5	491.67	29	2089.3	449.47	27	100.0%	189.20 [-57.31 , 435.71]
Subtotal (95% CI)			**29**			**27**	**100.0%**	**189.20 [-57.31 , 435.71]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.50 (P = 0.13)								
1.8.22 Erythrocyte glutathione peroxidase (GPx) (U/gHb)								
Hajifaraji 2017	39.63	11.42	29	32.7	9.92	27	100.0%	6.93 [1.34 , 12.52]
Subtotal (95% CI)			**29**			**27**	**100.0%**	**6.93 [1.34 , 12.52]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.43 (P = 0.02)								
Test for subgroup differences: Chi² = 390.02, df = 21 (P < 0.00001), I² = 94.6%								

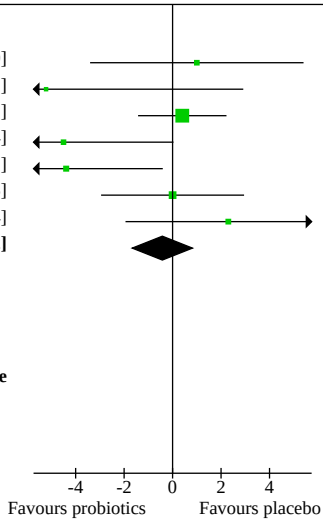
Analysis 1.9. Comparison 1: Probiotic versus placebo, Outcome 9: Use of additional pharmacotherapy



Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Risk Ratio M-H, Fixed, 95% CI
1.9.1 Insulin therapy					
Badehnoosh 2018	2	30	3	30	0.67 [0.12 , 3.71]

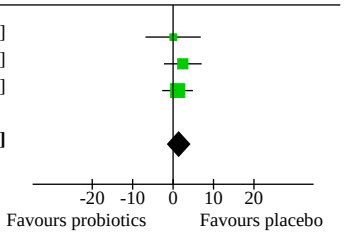
Analysis 1.10. Comparison 1: Probiotic versus placebo, Outcome 10: Glycaemic control during/ end of treatment (as defined by trialists)

Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
1.10.1 Fasting blood glucose(mg/dL)								
Ahmadi 2016	94.5	8.4	35	93.5	10.3	35	7.9%	1.00 [-3.40 , 5.40]
Hajifaraji 2017	88.37	11.0396	29	93.59	18.7581	27	2.3%	-5.22 [-13.36 , 2.92]
Jafarnejad 2016	89.3	3.4	37	88.9	4.4	35	46.3%	0.40 [-1.42 , 2.22]
Karamali 2016	87.7	7.1	30	92.2	10.5	30	7.5%	-4.50 [-9.04 , 0.04]
Kijmanawat 2019	83.92	6.48	28	88.31	8.74	29	9.7%	-4.39 [-8.37 , -0.41]
Lindsay 2015	83.7	8.82	74	83.7	9.54	75	17.7%	0.00 [-2.95 , 2.95]
Nabhani 2018	89.2	11.7	45	86.9	8.6	45	8.6%	2.30 [-1.94 , 6.54]
Subtotal (95% CI)			**278**			**276**	**100.0%**	**-0.42 [-1.66 , 0.82]**
Heterogeneity: Chi² = 11.09, df = 6 (P = 0.09); I² = 46%								
Test for overall effect: Z = 0.67 (P = 0.50)								
1.10.2 Postprandial blood glucose(mg/dL)								
Subtotal (95% CI)			**0**			**0**		**Not estimable**
Heterogeneity: Not applicable								
Test for overall effect: Not applicable								
Test for subgroup differences: Not applicable								



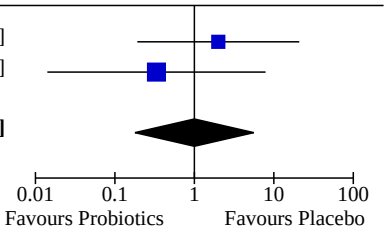
Analysis 1.11. Comparison 1: Probiotic versus placebo, Outcome 11: Gestational age at birth (days)

Study or Subgroup	Probiotic Mean	SD	Total	Placebo Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Badehnoosh 2018	273.7	17.5	30	273.7	7.7	30	15.6%	0.00 [-6.84 , 6.84]
Karamali 2018	275.4	10.5	30	273	7.7	30	33.7%	2.40 [-2.26 , 7.06]
Lindsay 2015	278.34	12.25	73	277.23	11.2	74	50.7%	1.11 [-2.69 , 4.91]
Total (95% CI)			**133**			**134**	**100.0%**	**1.37 [-1.33 , 4.07]**
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² = 0%								
Test for overall effect: Z = 0.99 (P = 0.32)								
Test for subgroup differences: Not applicable								



Analysis 1.12. Comparison 1: Probiotic versus placebo, Outcome 12: Preterm birth

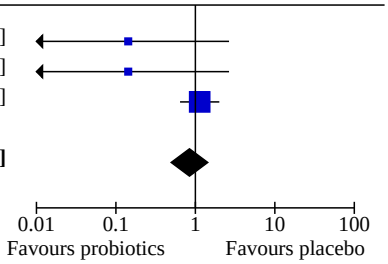
Study or Subgroup	Probiotics Events	Total	Placebo Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	2	30	1	30	40.0%	2.00 [0.19 , 20.90]
Karamali 2018	0	30	1	30	60.0%	0.33 [0.01 , 7.87]
Total (95% CI)		**60**		**60**	**100.0%**	**1.00 [0.18 , 5.59]**
Total events:	2		2			
Heterogeneity: Chi² = 0.80, df = 1 (P = 0.37); I² = 0%						
Test for overall effect: Z = 0.00 (P = 1.00)						
Test for subgroup differences: Not applicable						



Analysis 1.13. Comparison 1: Probiotic versus placebo, Outcome 13: Macrosomia (> 4000 g)

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	0	30	3	30	14.7%	0.14 [0.01 , 2.65]
Karamali 2018	0	30	3	30	14.7%	0.14 [0.01 , 2.65]
Lindsay 2015	19	73	17	74	70.7%	1.13 [0.64 , 2.00]
Total (95% CI)		**133**		**134**	**100.0%**	**0.84 [0.50 , 1.43]**
Total events:	19		23			

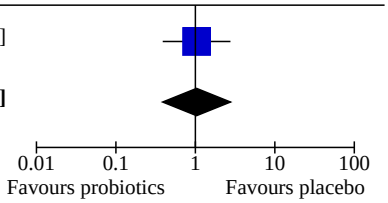
Heterogeneity: Chi² = 3.88, df = 2 (P = 0.14); I² = 48%
Test for overall effect: Z = 0.63 (P = 0.53)
Test for subgroup differences: Not applicable



Analysis 1.14. Comparison 1: Probiotic versus placebo, Outcome 14: Small-for-gestational age (SGA)

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Lindsay 2015	7	56	7	58	100.0%	1.04 [0.39 , 2.76]
Total (95% CI)		**56**		**58**	**100.0%**	**1.04 [0.39 , 2.76]**
Total events:	7		7			

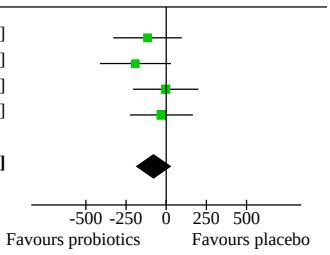
Heterogeneity: Not applicable
Test for overall effect: Z = 0.07 (P = 0.94)
Test for subgroup differences: Not applicable



Analysis 1.15. Comparison 1: Probiotic versus placebo, Outcome 15: Birthweight (g)

Study or Subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Badehnoosh 2018	3321.7	443.54	30	3438	398.4	30	23.7%	-116.30 [-329.64 , 97.04]
Karamali 2018	3181.6	459.8	30	3373.3	412.1	30	22.1%	-191.70 [-412.65 , 29.25]
Kijmanawat 2019	3120	411.09	28	3123	369.81	29	26.1%	-3.00 [-206.23 , 200.23]
Lindsay 2015	3570	640	73	3600	570	74	28.1%	-30.00 [-226.01 , 166.01]
Total (95% CI)			**161**			**163**	**100.0%**	**-79.14 [-183.00 , 24.73]**

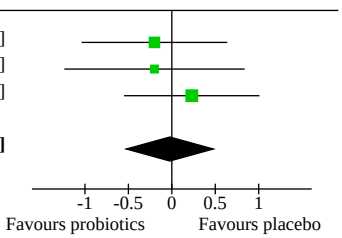
Heterogeneity: Chi² = 1.89, df = 3 (P = 0.59); I² = 0%
Test for overall effect: Z = 1.49 (P = 0.14)
Test for subgroup differences: Not applicable



Analysis 1.16. Comparison 1: Probiotic versus placebo, Outcome 16: Head circumference (cm)

Study or Subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Badehnoosh 2018	35.8	1.8	30	36	1.5	30	35.6%	-0.20 [-1.04 , 0.64]
Karamali 2018	35.2	2.1	30	35.4	2	30	23.2%	-0.20 [-1.24 , 0.84]
Lindsay 2015	35.32	2.88	64	35.09	1.36	65	41.2%	0.23 [-0.55 , 1.01]
Total (95% CI)			**124**			**125**	**100.0%**	**-0.02 [-0.52 , 0.48]**

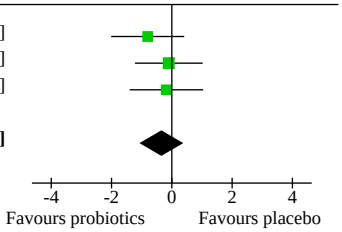
Heterogeneity: Chi² = 0.69, df = 2 (P = 0.71); I² = 0%
Test for overall effect: Z = 0.09 (P = 0.93)
Test for subgroup differences: Not applicable



Analysis 1.17. Comparison 1: Probiotic versus placebo, Outcome 17: Length (cm)

Study or Subgroup	Probiotic Mean	Probiotic SD	Probiotic Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean Difference IV, Fixed, 95% CI
Badehnoosh 2018	50.4	2.8	30	51.2	1.9	30	31.8%	-0.80 [-2.01 , 0.41]
Karamali 2018	50	2.5	30	50.1	1.9	30	36.9%	-0.10 [-1.22 , 1.02]
Lindsay 2015	51.09	3.96	64	51.27	3.01	64	31.4%	-0.18 [-1.40 , 1.04]
Total (95% CI)			**124**			**124**	**100.0%**	**-0.35 [-1.03 , 0.33]**

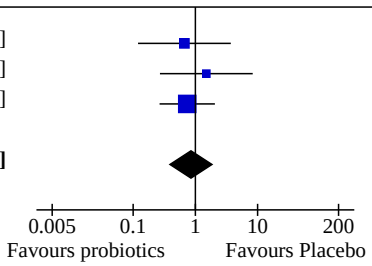
Heterogeneity: Chi² = 0.80, df = 2 (P = 0.67); I² = 0%
Test for overall effect: Z = 1.00 (P = 0.32)
Test for subgroup differences: Not applicable



Analysis 1.18. Comparison 1: Probiotic versus placebo, Outcome 18: Infant hypoglycemia requiring treatment (variously defined)

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	2	30	3	30	25.3%	0.67 [0.12 , 3.71]
Karamali 2018	3	30	2	30	16.8%	1.50 [0.27 , 8.34]
Kijmanawat 2019	5	28	7	29	57.9%	0.74 [0.27 , 2.06]
Total (95% CI)		**88**		**89**	**100.0%**	**0.85 [0.39 , 1.84]**
Total events:	10		12			

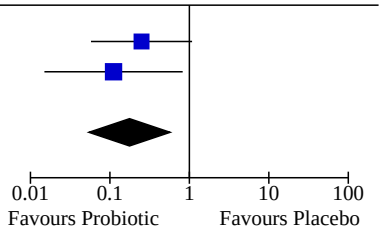
Heterogeneity: Chi² = 0.57, df = 2 (P = 0.75); I² = 0%
Test for overall effect: Z = 0.41 (P = 0.68)
Test for subgroup differences: Not applicable



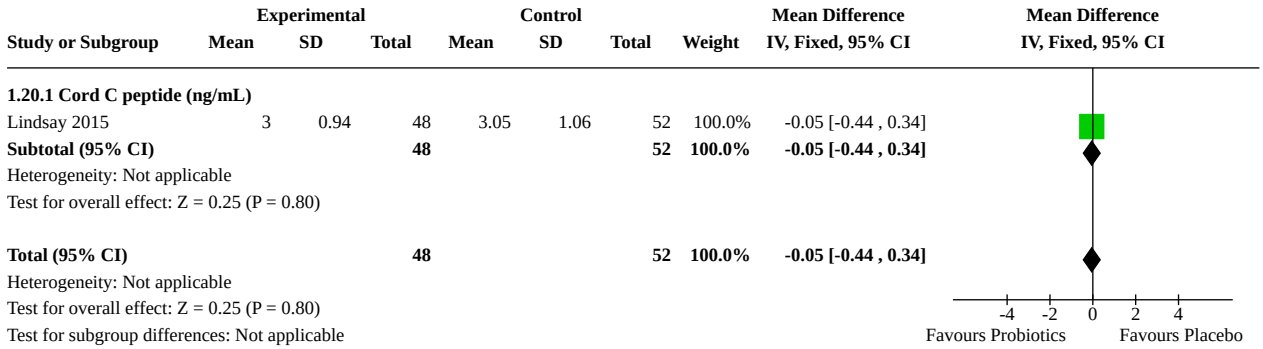
Analysis 1.19. Comparison 1: Probiotic versus placebo, Outcome 19: Hyperbilirubinemia

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Badehnoosh 2018	2	30	8	30	47.1%	0.25 [0.06 , 1.08]
Karamali 2018	1	30	9	30	52.9%	0.11 [0.01 , 0.82]
Total (95% CI)		**60**		**60**	**100.0%**	**0.18 [0.05 , 0.57]**
Total events:	3		17			

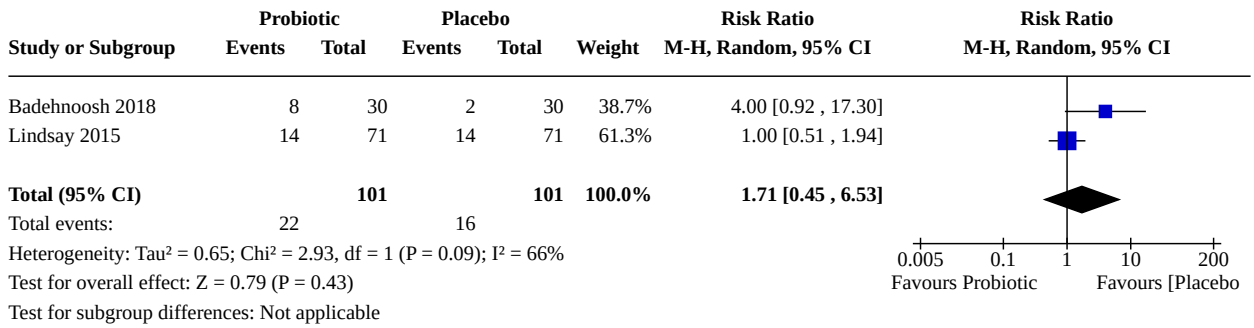
Heterogeneity: Chi² = 0.42, df = 1 (P = 0.52); I² = 0%
Test for overall effect: Z = 2.89 (P = 0.004)
Test for subgroup differences: Not applicable



Analysis 1.20. Comparison 1: Probiotic versus placebo, Outcome 20: Relevant infant biomarker's associated with intervention (cord C peptide, cord insulin)



Analysis 1.21. Comparison 1: Probiotic versus placebo, Outcome 21: Admission to NICU/nursery



APPENDICES

Appendix 1. Keywords for searching trials registries

ICTRP

probiotics AND gestational

probiotics AND diabetes AND pregnancy

ClinicalTrials.gov

Advanced search

Interventional studies | probiotics | gestational diabetes

pregnancy | Interventional Studies | Diabetes | probiotics

HISTORY

Protocol first published: Issue 2, 2018
Review first published: Issue 6, 2020

CONTRIBUTIONS OF AUTHORS

Karaponi Okesene-Gafa prepared the original draft of this review protocol and also carried out the analysis for this review. Abigail Moore assisted with checking data entry. Vanessa Jordan assisted with the analysis and provided technical advice. Caroline Crowther and Lesley McCowan provided input and feedback for this review.

DECLARATIONS OF INTEREST

Karaponi Okesene-Gafa - was recently involved with the Healthy Mums and Babies (HUMBA) randomised controlled demonstration trial, which has now been completed and published. The HUMBA RCT is not be eligible for inclusion in this review. In kind we have been provided with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12) and placebo capsules free of charge from Christian Hansen Denmark (http://www.chr-hansen.com/en) for our HUMBA trial (http://humba.ac.nz/). In this randomised controlled double-blind trial, women were randomised to receive probiotics or placebo capsules with the main aim of reducing pregnancy weight gain and infant birthweight (ANZCTR registration number 12615000400561). In kind: Roche International- equipment and consumables for HBA1c for the HUMBA trial. National Heart Foundation assisted by letting us use some of their resources for the study and development of some of the content in the text messages as part of HUMBA trial. Public interest funding for the Healthy Mums and Babies (HUMBA) trial was received from Cure Kids, Counties Manukau Health, Mercia Barnes Trust Fund, University of Auckland Faculty Research Development Fund and Lottery Health Research Fund. Karaponi Okesene-Gafa received funding from her organisation (Department of Obstetrics and Gynaecology, University of Auckland) for her PhD, with this review forming part of her thesis.

Abigail Moore is a medical student and declares no conflict of interest.

Vanessa Jordan is a Research Fellow in the University of Auckland and declares no conflict of interest.

Lesley McCowan - was also involved with the Healthy Mums and Babies (HUMBA) randomised controlled demonstration trial. The HUMBA RCT is not be eligible for inclusion in this review. In kind we have been provided with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12) and placebo capsules free of charge from Christian Hansen Denmark (http://www.chr-hansen.com/en) for our HUMBA trial (http://humba.ac.nz/). In this randomised controlled double-blind trial, women are randomised to receive probiotics or placebo capsules with the main aim of reducing pregnancy weight gain and infant birthweight (ANZCTR registration number 12615000400561). In kind: Roche International- equipment and consumables for HBA1c for the HUMBA trial. National Heart Foundation assisted by letting us use some of their resources for the study and development of some of the content in the text messages as part of HUMBA trial.

Caroline Crowther - was also involved with the Healthy Mums and Babies (HUMBA) randomised controlled demonstration trial. The HUMBA RCT is not be eligible for inclusion in this review. In kind we have been provided with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12) and placebo capsules free of charge from Christian Hansen Denmark (http://www.chr-hansen.com/en) for our HUMBA trial (http://humba.ac.nz/). In this randomised controlled double-blind trial, women are randomised to receive probiotics or placebo capsules with the main aim of reducing pregnancy weight gain and infant birthweight (ANZCTR registration number 12615000400561). In kind: Roche International- equipment and consumables for HBA1c for the HUMBA trial. National Heart Foundation assisted by letting us use some of their resources for the study and development of some of the content in the text messages as part of HUMBA trial.

SOURCES OF SUPPORT

Internal sources

- Department of Obstetrics and Gynaecology, University of Auckland, New Zealand

  Funding for Dr Okesene-Gafa's PhD with this review as part of her thesis.

External sources

- Liggins Institute, University of Auckland, New Zealand

  Infrastructure support for Cochrane authors has been provided by the Liggins Institute, University of Auckland.
- Cochrane Pregnancy and Chidbirth Australasian Satellite, New Zealand

  We acknowledge support from the Pregnancy and Childbirth Australian Satellite

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The following differences exist between the published protocol for this review (Okesene-Gafa 2018) and the full review.

We changed the title from 'probiotics for treating women with gestational diabetes for improving maternal and fetal health and well-being' to 'probiotic treatment for women with gestational diabetes to improve maternal and infant health and well-being'.

INDEX TERMS

Medical Subject Headings (MeSH)

Confidence Intervals; Diabetes, Gestational [*therapy]; Hypertension, Pregnancy-Induced [epidemiology]; Infant, Postmature; Labor, Induced [statistics & numerical data]; Odds Ratio; Placebos [therapeutic use]; Probiotics [adverse effects] [*therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Adult; Child; Female; Humans; Infant, Newborn; Pregnancy