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Can a probiotic supplement in pregnancy result in transfer to the neonatal gut: A systematic review

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Conflicts of interest

EFM is technical Director at Precision Biotics Ltd. The authors have no other disclosures to declare.

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ABSTRACT

Introduction: The establishment of the neonatal gut microbiome is a crucial step that may have lifelong health implications. We aimed to systematically review evidence on maternal probiotic supplementation during pregnancy and vertical transfer of the corresponding strain to the infant gut. **Material and methods:** Medline, CINAHL, Embase, Web of Science and OVID, from inception to September 2018. Studies of maternal probiotic supplementation for a minimum of 2 weeks' duration and analyses of neonatal stool samples were included. The primary outcome was

presence of the specific probiotic strain in the infant stool. Electronic databases were searched for relevant studies and references were cross-checked. Risk of bias among included studies was assessed and data were extracted independently by two authors. **Results:** Three studies were included in the review. Only one study was identified involving prenatal maternal probiotic supplementation alone. Neonatal colonisation with the maternally administered probiotic was not demonstrated but supplementation with the probiotic influenced levels of a bacterial strain other than that found in the probiotic product. The other two studies identified included both prenatal and postnatal supplementation of either mother or infant. All three studies reported employing strain specific isolation methodology to isolate the supplemented bacterial strain in infant stool but none used whole metagenome shotgun sequencing. **Conclusions:** Low number of studies investigating transfer of a specific probiotic bacterial strain from mother to infant were identified showing inconclusive evidence of vertical transfer.

Keywords

Pregnancy, probiotic, microbiome, neonate, gut colonisation, perinatology

Abbreviations

LGG *Lactobacillus rhamnosus* GG

Key Message

A low number of studies investigating transfer of a specific probiotic bacterial strain from mother to infant identified limited evidence of vertical transfer. However, more research is needed to examine whether vertical transmission of supplemented probiotics occurs.

INTRODUCTION

In recent years our understanding of the intestinal microbiome has expanded considerably as a consequence of the greater insights provided by 16S ribosomal RNA sequencing and, subsequently, whole metagenome sequencing [1-6]. This allows rapid identification of bacteria, and/or other microbes, without the need for laborious and time-consuming culture-based techniques. Gut microorganisms have been involved in aiding in the development of the host

immune system [7] and the disruption of the microbiome is linked with certain disease states, including irritable bowel syndrome [8] and autoimmune disease [9].

The perinatal period is a particular area of interest from a health perspective as this represents a critical window in which one's initial gut microbiome is established, with long-term consequences [10-12]. Emerging research is raising the possibility of trans-placental transfer of microbes as well as evidence of the presence of bacteria in the amniotic fluid [13]. Various factors have been found to influence early microbiome of infants, including gestational age [14], mode of delivery [15], and infant feeding method [16, 17]. Over time the human gut microbiome stabilises and the differences between caesarean versus vaginally delivered infants profiles gradually dissipate [18-20]. Despite this, however, infants born via caesarean section remain at higher risk of, for example, obesity and diabetes in later life, supporting the theory of this 'critical window' in microbial acquisition which may shape an individual's lifelong health [19, 21].

It has been well demonstrated that probiotics are both safe and acceptable to pregnant women [22], however, their specific benefits during pregnancy may be strain-dependent. Some studies suggest beneficial effects for gestational diabetes [23], while others, including work from our own group, have not supported this suggestion [24-26]. We identified a positive effect on maternal lipids and other groups have shown positive metabolic effects of maternal probiotic administration [27], [28]. These findings, combined with the potential lifelong benefits for the infant, if vertical transfer is achieved, makes maternal dietary supplementation with specific probiotics an appealing possibility.

Over half of Irish women take supplements during their pregnancy [29]. Our objective was to review the existing literature to determine if any evidence existed to indicate that maternal supplementation with a probiotic could result in the transfer of the strain to the infant or not.

MATERIAL AND METHODS

Protocol and registration:

Methods of the analysis and inclusion criteria were specified in advance and documented in a protocol registered with PROSPERO, registration number CRD42018106391 and available at <https://www.crd.york.ac.uk/PROSPERO>.

Eligibility Criteria:

Trials that involved prenatal maternal supplementation with a probiotic for at least 2 weeks duration, and for whom infant stool was analysed, were eligible for inclusion. All trial types were included due to the novel nature of the topic. Studies were restricted to include only humans.

Information Sources:

Cochrane and PROSPERO databases were reviewed initially to ensure there were no published or ongoing reviews on the topic. Studies were identified by searching electronic databases and scanning reference lists of articles. No limits were applied for language. This search was applied to Medline (1966 – Present), CINAHL, Embase, Web of Science and OVID electronic databases. The final search was run on 14th September 2018.

Search:

Search terms are as follows: Pregnant women, pregnant woman, pregnancy, pregnant, gravid*, prenatal, perinatal, probiotic, lactobacillales, lactobacillus, Bifidobacterium, infant, newborn, meconium, gastrointestinal microbiome, feces, gut flora. Full search strategy in Supporting Information Appendix S1.

Study selection:

Eligibility assessment was performed independently in an unblinded standardised manner by 2 reviewers. Disagreements between reviewers were resolved on discussion and with the involvement of a third reviewer where necessary.

Data Collection Process:

One reviewing author extracted the following data from included studies and the second author checked the extracted data. Disagreements were resolved by discussion between the two reviewing authors; if no agreement could be reached, a third author resolved it.

Data items:

Information was extracted from each included trial on: (1) characteristics of trial participants (mode of delivery, gestation at delivery, birth weight and infant feeding method), and the trial's inclusion and exclusion criteria; (2) type of intervention (including type, dose, duration and frequency of the probiotic supplementation); (3) method of microbial analysis and the microbial outcome data.

Risk of bias of individual studies:

To ascertain the validity of eligible trials, two reviewers working independently, using the Cochrane Collaboration's tool, examined the adequacy of randomisation, allocation concealment, blinding of participants and outcome reporting.

Summary measures:

The primary outcome was presence of the supplemented bacterial strain in the neonatal stool.

Synthesis of results:

It was not possible to carry out a summary analysis or meta-analysis for this systematic review as the numbers of trials involved were too small and different analyses were used. An overall description of individual results is therefore provided in the results section.

RESULTS

A total of 3 studies involving 3 trials were identified for inclusion in the review. The search of Medline, Embase, Web of Science, and Cinahl databases provided a total of 615 citations. After adjusting for duplicates 477 remained. On review of the titles a further 201 were removed, as not being relevant or being review articles. There were 105 abstracts reviewed, with 18 articles potentially meeting inclusion criteria. On review of the full articles only three met inclusion criteria and were included in the systematic review (Figure 1).

Included studies:

The study characteristics are described in table 1. All three studies were double blind randomised controlled trials [30-32]. All studies were published in English. The intervention period was from 36 weeks' gestation until delivery in the Lahtinen et al. study. Dotterud et al., employed supplementation from 36 weeks' gestation until 3 months postpartum, while Rutten et al., involved supplementation of pregnant women for the last 6 weeks of pregnancy and supplementation of the infant for the first year of life. These included studies involved 660 participants in total. The main inclusion criteria were healthy pregnant women who were not already taking probiotic supplements. Two of the studies involved pregnant women with a personal or family history of atopy [31, 32] and one of the studies specified that the women intended to breastfeed for at least 3 months [30].

In terms of the supplemented probiotic, 2 of 3 studies used *Lactobacillus rhamnosus* GG (LGG) or LGG in combination with other probiotics. Rutten et al., used a combination of *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52 and *Lactococcus lactis* W58 (Ecologic1 Panda, Winclove Probiotics B.V., Amsterdam, the Netherlands) (Table 2). Rutten et al. and Lahtinen et al. used visually matched maltodextrin placebo capsules, while Dotterud et al. delivered the intervention as a probiotic-containing low-fat fermented milk, and the placebo was skimmed fermented milk.

The primary outcome was readily available for 2 of the studies. Dotterud et al. investigated if probiotic administration to pregnant and lactating mothers altered the colonisation pattern and the diversity of the intestinal microbiota of mothers and children. Rutten et al. assessed the long-term effect of pre- and postnatal administration of selected probiotic bacteria on the composition and diversity of gut microbiota, over time, in infants at risk for atopic disease. The primary outcome was unclear for Lahtinen et al.

Overall the 3 randomised controlled trials [30-32] included in the review were deemed to be low risk of bias by two individual reviewers (Table 2). All 3 trials had comparable dropout rates. A study protocol was available for Lahtinen et al. and Rutten et al., however, a pre-specified primary outcome was difficult to obtain for Lahtinen et al. For both, the study protocol pertained

to the original randomised controlled trial with these papers involving a secondary analysis. There was no protocol available for Dotterud et al.

Vertical transfer after prenatal supplementation alone

Lahtinen et al. was the only study that looked at antenatal maternal probiotic supplementation alone. Lahtinen et al. found no evidence of increased *L. rhamnosus* colonisation at any time point in infants whose mothers were assigned to receive LGG probiotic, they did, however, observe a higher prevalence of *Bifidobacterium longum* and *Bifidobacterium breve*. It should be noted that the authors did report a higher vaginal delivery rate (75% versus 66%), a higher breastfeeding rate (54% versus 46%) and higher yoghurt consumption during pregnancy (400g/week versus 300g/week) in the probiotic group. They did not comment on whether or not these differences reached statistical significance and did not control for them in their analyses.

Vertical transfer after pre- and postnatal supplementation

Rutten et al. found the supplemented product present in infant stool, however in this study, pregnant women were supplemented and then the infants themselves were supplemented from birth until 1 year of age. The relative abundance of the putative supplemented strains was highest from 2 weeks to 3 months of age. Dotterud et al. reported that at 10 days and 3 months of age the prevalence and relative abundance of 1 of the supplemented bacteria (LGG) was significantly increased in the intervention group. In this study, mothers continued to receive the probiotic up to 3 months postpartum while breast-feeding, infants were not supplemented.

Each study used different approaches to track the transmission of the probiotic strain in the infant stool. Lahtinen et al. cultured individual *L. rhamnosus* isolates while Dotterud et al. employed both qPCR and 16S amplicon sequencing. Finally, Rutten et al. used a proprietary platform that analyses the intragenic spacer length between the 16s and 23S genes. Details are deficient regarding the statistical analyses used by Rutten et al., and the IS-pro platform used does not appear to be specific enough to identify individual strains [33]. None of the studies used whole metagenome shotgun sequencing. Information is lacking regarding the maternal diet, maternal gut microbiome and the human milk microbiome, all of which are crucially important in terms of monitoring compliance and in understanding methods of mother to infant transfer.

DISCUSSION:

This is the first systematic review to investigate vertical transmission of a particular bacterial strain from mother to infant via probiotic supplementation. Despite a vast array of research carried out on probiotic usage during pregnancy, data in the area of vertical transmission is very limited, as reflected by only 3 papers being eligible for inclusion in this review. Lahtinen et al. is the only study identified that employed an exclusively prenatal supplementation strategy. An increased prevalence of the supplemented strain was not identified in infant stool. An increase in *Bifidobacterium longum* and *Bifidobacterium breve* among the supplemented infants was reported. These species are frequently associated with positive health outcomes and typically found in healthy breast fed infants [34]. Infants with allergy are reported to be less frequently colonised with them [35, 36]. Both Dotterud et al. and Rutten et al. demonstrated presence or increased prevalence of the supplemented strain in the infant stool at some time point in the postnatal period. These two studies included ongoing postnatal supplementation of either mother or infant and thus it is difficult to distil the impact of prenatal maternal supplementation alone. No harmful effects on the microbiome were reported in any of the studies.

Other groups have found that maternal supplementation with probiotics during pregnancy has the potential to impact the infant gut microbiome during this critical window of development. Bisanz et al. found that consumption of a probiotic yoghurt by mothers in pregnancy and for one month postpartum increased the relative abundance of *Bifidobacterium* and decreased *Enterobacteriaceae* in the new born faeces [37]. Guemonde et al., found a higher occurrence of *Bifidobacterium breve* and a lower occurrence of *Bifidobacterium adolescentis* at 5 days of age among infants whose mothers received LGG supplementation compared to those from the placebo group. In addition, they reported that LGG consumption increased the bifidobacterial diversity in infants and reduced the *Bifidobacterium* microbiota similarity between mother and infant [38]. In contrast, in further analysis of the work by Lahtinen et al. (included in this review), Ismail et al. demonstrated that while maternal supplementation with LGG promoted a beneficial bifidobacterial profile, it did not modulate the diversity of the early infant gut microbiome [39].

Unfortunately, there is a lack of high-quality, adequately powered, randomised control trials in this area. The variation in both the strain selection and the administration method of the probiotics makes it difficult to draw true comparisons, along with the different demographics of the participants included and a lack of detail regarding confounding factors such as maternal diet, maternal gut microbiome, mode of delivery and infant feeding methods. It is well established that the microbiome is altered in atopic conditions and two of the three studies looked at this population.

Whole metagenome shotgun sequencing can now provide functional data on microbes and allow for more detailed tracking of microbes from mother to infant [40]. This allows us to more accurately detect the presence of the supplemented probiotic in the infant stool. While Dotterud et al., used a 3-strain probiotic supplement, it was only the LGG in the infant gut that was impacted. Further exploration is needed to see why this is the case. Combining all the above information may allow for more appropriate selection of probiotics which could meaningfully impact on that critical window of colonisation in the neonatal period.

A strength of our review was the strict adherence to the review protocol which was prospectively registered on the PROSPERO website. This ensured a comprehensive search strategy was employed and all existing data was captured.

An important limitation of the review is the low number of studies that met the inclusion criteria meaning that a meta-analysis was not possible. Only one study was identified that involved prenatal maternal supplementation alone and the microbial detection techniques used in all three are outdated. None of the studies truly assessed other contributing factors that influence infant gut colonisation.

Since the publication of these studies there have been further advances in analytical techniques with the development and refinement of whole metagenome shotgun sequencing. This process allows for more accurate tracking of a supplemented strain, once the genome is known. Future studies using this technique are welcomed to further interrogate this concept and the mechanisms by which transfer occurs. Sufficiently powered randomised control trials are needed with detailed data collection to allow thorough investigation of the mode of transfer of microbes from mother to infant. Data collection should include microbial data from multiple body sites (oral cavity, vagina, gut and skin) at multiple time points in the pregnancy along with dietary data.

Postnatal sampling should include assessment of human milk along with infant stool to properly investigate routes of transfer.

CONCLUSION

In a low number of studies investigating transfer of a specific probiotic bacterium from mother to infant we identified inconclusive evidence of vertical transfer. The exact genome of the probiotic strain was not tracked in any study. In addition, only one study was identified involving prenatal maternal supplementation alone. Further research is needed using well designed trials and the most up to date technology to examine whether vertical transmission of supplemented probiotics occurs and to fully explore all routes of transfer of microbes from mother to infant in order to optimise early colonisation of the infant gut.

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Supporting Information legend

Appendix S1. Search strategy.

Figure and table legends

Figure 1. Study Flow Diagram

Table 1. Study characteristics and primary outcomes for all four studies reviewed

Table 2. Results of Cochrane Risk of Bias Assessment Tool

Table 3. Formulation of probiotic/placebo, analyses performed and microbial results for the four studies reviewed

Table 1: Study characteristics and primary outcomes for all three studies reviewed								
Author (year) ^{Ref}	Setting	Trial Registry	Study Design Intervention period	Population Studied: Inclusion/Exclusion Criteria	Participant characteristics Mode of delivery Gestation at delivery Birthweight Infant feeding	Number of drop outs / exclusions / loss to follow up	Number in probiotic group / placebo or control group	Primary outcome
Dotterud et al. (2015) ³⁰	Trondheim, Norway	Clinical Trials.gov NCT00159 523	Double-blind, randomised, placebo- controlled trial 36 weeks gestation-3 months postpartum	<u>Inclusion:</u> Pregnant women who: -were planning to breastfeed for at least 3 months -were in week 36 of pregnancy -liked & tolerated fermented milk -were not at risk of developing pregnancy complications. <u>Exclusion:</u> Pregnant women who were: -taking probiotic supplements during the last 4 weeks -planning to move away from Trondheim < 25 months following randomisation.	Not available Not available Probiotic group: 3671g Placebo group: 3595g Breastfed ≥ 3mo: Probiotic group: 134 Placebo group: 138	53 dropouts from each arm during intervention period <u>2-year follow up:</u> 20 further dropouts from probiotic group 11 further dropouts from placebo group	Probiotic group: n=138 Placebo group: n=140	Alteration of colonisation pattern and diversity of the mothers' and children's intestinal microbiota

Author (year) ^{Ref}	Setting	Trial Registry	Study Design Intervention period	Population Studied: Inclusion/Exclusion Criteria	Participant characteristics Mode of delivery Gestation at delivery Birthweight Infant feeding	Number of drop outs / exclusions / loss to follow up	Number in probiotic group / placebo or control group	Primary outcome
Rutten et al. (2015) ³²	Netherlands	Clinical Trials.gov NCT00200 954	Double-blind, randomised, placebo- controlled trial Maternal supplementation for the last 6 weeks of pregnancy + infant supplementation during the first year of life	<u>Inclusion:</u> Pregnant women with a personal history of allergic disease or a partner PLUS a previous child with allergic disease <u>Exclusion:</u> -Antenatal systemic use of immunomodulatory drugs, like corticosteroids. -Regular use of products containing probiotics.	No details provided	-15 dropouts in the prenatal period + 10 further in the postnatal follow up to age 2. -15 further dropouts at the 6 year follow up Unclear from which arm dropouts were from	<u>At baseline:</u> 60 probiotic 63 placebo Postnatal follow up at: <u>3 months:</u> 52 placebo 50 probiotic <u>2 years:</u> 48 placebo 50 probiotic <u>6 years:</u> 44 placebo 39 probiotic	To assess the long term effects of added probiotics (prenatal + postnatal) on the composition & diversity of gut microbiota over time in infants at risk for atopic disease.
Lahtinen et al. (2009) ³¹	Melbourne, Australia	Original trial No 36 Cochrane Skin Group:	Double-blind, randomised, placebo- controlled trial	<u>Inclusion:</u> Pregnant women with a personal history or a partner or previous child affected by a	<u>Vaginal delivery, n (%)</u> Probiotic group: 43 (75)	Not reported	Probiotic group: n=59 Placebo group: n=57	Unclear

Table 1: Study characteristics and primary outcomes for all three studies reviewed								
Author (year) ^{Ref}	Setting	Trial Registry	Study Design Intervention period	Population Studied: Inclusion/Exclusion Criteria	Participant characteristics Mode of delivery Gestation at delivery Birthweight Infant feeding	Number of drop outs / exclusions / loss to follow up	Number in probiotic group / placebo or control group	Primary outcome
		Ongoing Skin Trials Register	36 weeks' gestation until delivery	doctor-diagnosed allergic disease. <u>Exclusion:</u> -Multiple pregnancies -Known fetal abnormality -Maternal immune deficiency -Women already taking probiotic supplements	Placebo group: 37 (66) <u>Breast-fed</u> <u>exclusively for</u> <u>≥ 3 months, n</u> <u>(%)</u> Probiotic group: 30 (54) Placebo Group: 26 (46)			
LGG: <i>Lactobacillus rhamnosus</i> GG								

Table 2: Results of Cochrane Risk of Bias Assessment Tool [1]			
	Dotterud et al. (2015)	Rutten et al. (2015)	Lahtinen et al. (2009)
Random sequence generation	Low risk	Low risk	Low risk
Allocation concealment	Unclear risk	Unclear risk	Low risk
Blinding of participants and personnel	Low risk	Low risk	Low risk
Blinding of outcome assessment	Low risk	Unclear risk	Low risk
Attrition bias	Low risk	Low risk	Unclear risk
Reporting bias	Unclear risk	Low risk	Unclear risk
Other bias	Unclear risk	Low risk	Unclear risk

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Table 3: Formulation of probiotic/placebo, analyses performed and microbial results for the three studies reviewed				
Author (year)	Formulation of probiotic/placebo Species of bacteria Dosage of bacteria (CFU)	Microbial analysis performed	Presence of probiotic	Alteration in gut microbiome compared to placebo
Dotterud et al. (2015)	<p>250mL probiotic low-fat fermented milk containing:</p> <p>-<i>Lactobacillus rhamnosus</i> GG (LGG) 5×10^{10} CFU</p> <p>-<i>Bifidobacterium animalis</i> subsp <i>lactis</i> Bb-12 (Bb-12) 5×10^{10} CFU</p> <p>-<i>Lacidophilus</i> La-5 (La-5) 5×10^{10} CFU</p> <p>Placebo: 250mL placebo skimmed fermented milk</p>	<p>Quantitative PCR (for detecting the presence of the probiotic strain in all stool samples)</p> <p>16S ribosomal RNA gene deep sequencing on Illumina MiSeq platform (for 3 month old and 2 year old infant stools)</p>	Increased prevalence and relative abundance of LGG in infant stool samples at 10 days and 3 months in the intervention arm but no difference at 1 year or 2 years of age	<p><u>Probiotic group:</u></p> <p>Mothers at 3 months: ↑ prevalence and relative abundance of: -↑ La-5 ($P < 0.005$) -↑ LGG ($P < 0.005$) -↑ Bb-12 ($P < 0.005$)</p> <p>Infants at 10 days -↑ LGG ($P < 0.005$)</p> <p>Infants at 3 months -↑ LGG ($P < 0.005$)</p> <p>Infants: No difference in La-5 or Bb-12 at any time point</p>
Rutten et al. (2015)	<p>Identical individual sachets containing 3g of material:</p> <p>-<i>Bifidobacterium bifidum</i> W23 (1×10^9 CFU)</p> <p>-<i>Bifidobacterium lactis</i> W52 (1×10^9 CFU)</p> <p>-<i>Lactococcus Lactis</i> (Lc Lactis) W58 (1×10^9 CFU)</p>	16S-23S IS profiling	Higher abundance + prevalence of probiotic species in the intervention group*	<p><u>Probiotic group:</u></p> <p>↑Bifidobacteria at 1 month ↑Lc Lactis at 2 weeks + 1 month</p> <p><u>Placebo group:</u></p> <p>↑ diversity of Bacteroidetes in at 2 weeks</p>

	(Ecologic®) Panda Placebo: Identical sachet containing rice starch and maltodextran			
Lahtinen et al. (2009)	Capsule containing: <i>Lactobacillus rhamnosus</i> GG (LGG) 1.8 x 10 ¹⁰ CFU Placebo: Identical capsule of maltodextrin	Quantitative PCR + T-RFLP (for analyzing Bifidobacteria in stool samples) Culture isolation and strain-specific PCR (for detecting the presence of the probiotic strain)	<i>Lactobacillus rhamnosus</i> present in both groups with no statistical difference	<u>Probiotic group:</u> ↑ <i>Bifidobacterium longum</i> in infants
CFU: Colony Forming Units, PCR: Polymerase Chain Reaction, T-RFLP: Terminal restriction fragment length polymorphism *ongoing postnatal supplementation to infants up to 1 year of life

Figure 1: Study flow diagram

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2007). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *BMC Med* 6(7): a1000007. doi:10.1371/journal.pmed.1000007

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