

Probiotics and Pregnancy

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Abstract Complications of pregnancy are associated with adverse outcomes for mother and baby in the short and long term. The gut microbiome has been identified as a key factor for maintaining health outside of pregnancy and could contribute to pregnancy complications. In addition, the vaginal and the recently revealed placental microbiome are altered in pregnancy and may play a role in pregnancy complications. Probiotic supplementation could help to regulate the unbalanced microflora composition observed in obesity and diabetes. Here, the impact of probiotic supplementation during pregnancy and infancy is reviewed. There are indications for a protective role in preeclampsia, gestational diabetes mellitus, vaginal infections, maternal and infant weight gain and allergic diseases. Large, well-designed randomised controlled clinical trials along with metagenomic analysis are needed to establish the role of probiotics in adverse pregnancy and infancy outcomes.

Keywords Probiotics · Pregnancy · Maternal microbiome · Pregnancy complications · Infant outcomes

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Introduction

Pregnancy is a time of dramatic immune and physiological changes that serve to accommodate the growing fetus. During pregnancy, alterations in the gut and vaginal microbiome populations occur [1•, 2•]. These alterations might influence the maternal metabolic profile and also contribute to the metabolic and immunological health of the offspring [3]. Recently, the role that gut microbiome might play in pregnancy has become the subject of considerable interest. It has been reported that the maternal microbiota from the third trimester of normal pregnancy show signs of inflammation, adiposity and insulin insensitivity similar to those observed in obesity [2•]. Further, it has recently become clear that the placenta has a microbiome. The placental microbiome is associated with preterm birth, indicating that the crosstalk between bacterial communities and the pregnant women may be of great importance [4•].

Current strategies to prevent maternal morbidity and mortality and enhance perinatal health are focused on specific complications of pregnancy. It has been proposed that manipulation of the gut microbiome (the composite of the bacteria present in the gastrointestinal tract) may prevent pregnancy complications [5, 6]. Many factors, including diet, prebiotics, pharmaceutical agents, antibiotics and probiotics, influence the composition of the microbiome [7]. Probiotics can regulate gut and vaginal microflora and thereby promote favourable metabolic activity and produce beneficial metabolites [8]. It has been suggested that supplementation with probiotics can be used as an alternative strategy to influence multiple aspects of pregnancy including intestinal dysbiosis, mucosal immunity, urogenital infections in the mother and alleviation of allergies and atopic diseases in infants [9]. Randomised clinical trials of probiotic supplementation in pregnancy are still relatively few in number but have been directed at conditions such as gestational diabetes mellitus

(GDM), gestational weight gain, preterm delivery and preeclampsia (Table 1). Other trials have investigated the effects of probiotic supplementation in pregnancy on infant health and development including necrotising enterocolitis [10, 11] and neurological disorders such as autism [12, 13]. The increasing rate of maternal obesity and its subsequent health outcomes are a significant public health concern and a major challenge for obstetric practice. Current prevention strategies focus on changes to diet and physical activity and have had limited success. Therefore, there is an urgent need for alternative strategies. In this review, pregnancy-associated changes to the microbiome are briefly summarised followed by a discussion of the current evidence regarding the impact of probiotic supplementation in pregnancy on maternal and infancy outcomes.

Maternal Microbiota

Shifts in the Maternal Gut Microbiome During Pregnancy

The gut microbiome consists of a microbial community of 10^{14} bacteria comprising at least a thousand different species [14]. It is characterised by the collective genomes of these coexisting microbes and can be modulated by host factors such as genetic make-up and diet. The bacteria in the gut microbiome can affect host physiology in health and disease

[15]. For example, the exact composition of the gut microbiome determines the amount of energy that can be harvested from diet [5]. The composition of the gut microbiome is altered in obesity, although it is unclear whether this occurs in response to an obesogenic diet or is a causal factor in the development of obesity [16].

In recent years, numerous studies have identified the existence of interactions between the host and the gut microbiota. In the pregnancy context, Koren et al. performed an elegant clinical study of the gut microbiome of 91 pregnant women. They reported a significant change in microbiome composition from the first to the third trimester of gestation [2•]. In this time period, the mother develops a physiological insulin resistance that serves to ensure an adequate nutrient supply to the growing fetus. When faecal samples from the third trimester were transplanted to germ-free mice, the mice became insulin resistant and increased their fat mass similar to the changes women undergo in pregnancy [2•]. In the third trimester, there was an overall decrease in bacterial diversity within each woman's gut microbiome, although the species lost were highly variable between women. Also, there was an increase in bacteria belonging to *Actinobacteria* and *Proteobacteria*, similar to the gut microbiome in inflammatory bowel disease and obesity [17]. However, other studies [18, 19] did not report the reduction in bacterial diversity in the third trimester of pregnancy. In Jost et al. [19], the maternal gut microbiota remained stable over the perinatal period.

Table 1 Major studies of probiotics in pregnancy and infancy outcomes

	Probiotic strain(s)	Effect	Reference
Pregnancy outcomes			
Gestational weight gain	<i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i>	Decrease the risk of large waist circumference	[40]
GDM	<i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i>	Lower risk of GDM (from 34 to 13 %)	[41•]
Preeclampsia	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i> and <i>Lactobacillus rhamnosus</i>	Lower risk of preeclampsia (20 %) and severe preeclampsia	[51•]
Preterm birth	VSL#3 (<i>Lactobacillus</i> , <i>Bifidobacterium</i> and <i>Streptococcus</i>)	Modulation of the vaginal microbiota and cytokine excretion	[46]
	<i>Lactobacillus rhamnosus</i> GR-1	Modification of the lipopolysaccharide inflammatory response	[48]
Lipid profile	<i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i> Bb12	Reduced triglyceride and LDL concentration postpartum	[55]
Infant outcomes			
Infant anthropometry	<i>Lactobacillus rhamnosus</i> GG	Prevention of excessive weight gain during the first 4 years of life	[56•]
Allergies	<i>Lactobacillus acidophilus</i> LA-5, <i>Bifidobacterium lactis</i> Bb12 and <i>Lactobacillus rhamnosus</i>	Reduction of atopic eczema and rhinoconjunctivitis	[60, 61]
	<i>Lactobacillus rhamnosus</i> HN001	Prevents the development of eczema and possibly atopic sensitization in high-risk infants	[63]
	<i>Lactobacillus reuteri</i>	Decreases allergen responsiveness and IgE-associated eczema	[65]
Necrotising enterocolitis (NEC)	<i>Lactobacilli</i> , <i>Bifidobacterium</i> and <i>Streptococcus thermophilus</i>	Prevents severe NEC and all-cause mortality in preterm infants	[68–72]

However, it only included samples from the third trimester and up to 30 days postpartum. It is not clear if there was a change in the maternal gut microbiome from the first to the third trimester since no samples from the first trimester were obtained. Avershina et al. [18] also reported no major overall changes in the faecal microbiota between early and late pregnancy and attributed this to large differences in the gut composition between pregnant women in the cohort. Furthermore, there is a difference in the analytical methods between the studies with the latter two studies not measuring phylogenetic distances in contrast to the study by Koren et al. [2•]. Different data processing and integration may also affect the validity of the findings, and lastly, the studies amplified different regions of the 16S ribosomal RNA (rRNA) gene which could affect the ability to map the obtained sequences back to the reference databases. A comprehensive analysis of how the enteric microbial variety changes over the course of normal and complicated pregnancy has not been reported yet.

Shifts in the Vaginal Microbiome During Pregnancy

There are few studies of the microbiomes in pregnancy outside of the gastrointestinal tract. However, the vaginal microbiome frequently shows a high proportion of *Lactobacillus* species, which are thought to promote a healthy vaginal microenvironment by producing lactic acid creating an inhospitable milieu for pathogenic bacteria [20, 21]. The composition of the vaginal microbiome in pregnant women has not been linked in great detail to the outcomes of pregnancy [20]. Figure 1 presents the differences in gut and vaginal microbiome from healthy non-pregnant and pregnant women.

Over the course of gestation, both diversity and richness of the vaginal microbiome are reduced. The 16S rRNA gene catalogue exhibits a dominance of *Lactobacillus* species (*Lactobacillus vaginalis*, *Lactobacillus crispatus*, *Lactobacillus gasseri* and *Lactobacillus jensenii*) and lower abundance of 22 other phylotypes in pregnant women [1•, 21], as compared to non-pregnant women where there is a higher predominance of members from the order *Clostridiales* and *Bacteroidales* [1•].

Placental Microbiome

A distinctive microbiome is observed in the placenta. It is mainly composed of non-pathogenic commensal microbiota from the phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Fusobacteria* and the unique presence of *Tenericutes* [4•]. The placental microbiome exhibits similarities to the oral microbiome from non-pregnant subjects [22], including *Prevotella tanneriae* and non-pathogenic *Neisseria* species. It has been suggested that the placental microbiome can be established by the spread of the oral microbiome through the

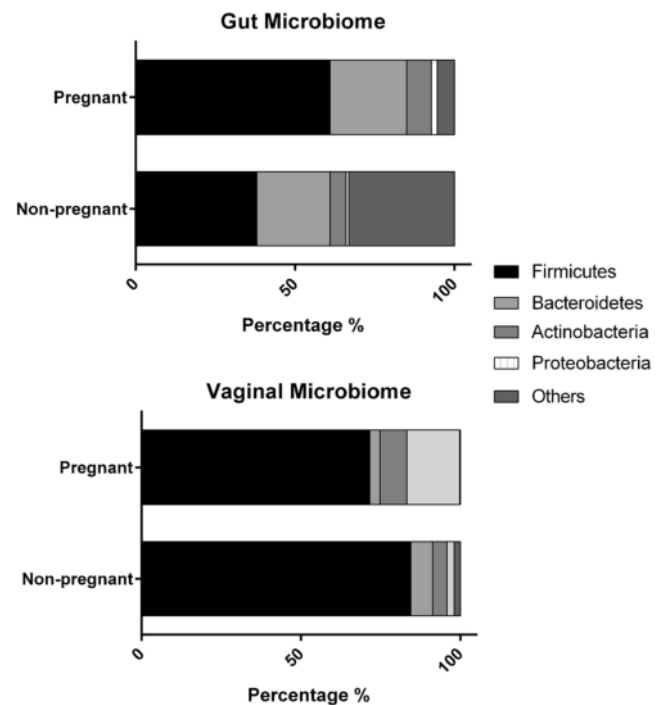


Fig. 1 Phylum-level comparison of the gut and vaginal microbiome, among healthy pregnant and non-pregnant women [2•, 75–77]

circulation. Moreover, differences in the composition of the placental microbiome were associated with preterm birth (<37 weeks): for example, the prevalence of *Paenibacillus* was decreased and *Burkholderia* increased in placentas obtained from preterm deliveries [4•]. The placental microbiome has not been examined in the setting of obesity, diabetes or preeclampsia.

Pregnancy Complications and Infant Outcomes

Obesity and GDM independently contribute to higher risks of complications during gestation, delivery and in the neonatal period. Obesity is a major public health crisis among children and adults, increasing dramatically since 1980 [23]. Obesity during pregnancy is associated with increased risk of gestational diabetes (GDM), hypertension, preeclampsia, caesarean delivery, fetal death and macrosomia (birth weight ≥ 4000 g) [24]. The incidence of GDM is increasing, affecting approximately 7 % of pregnancies and higher in obesity [25]. The adverse outcomes of GDM include preeclampsia, preterm birth and macrosomia, which can lead to shoulder dystocia and higher rates of caesarean delivery. Epidemiological studies have reported that the children of mothers with GDM are more likely to have psychomotor disability and schizophrenia [26, 27]. There is also a higher risk of future type 2 diabetes for both mother and child (Table 2) [28].

Table 2 Worldwide incidence of key pregnancy complications and its subsequent health outcomes

Pregnancy complications	Incidence on pregnancy in developed countries (%)	Health outcomes
Obesity	From 1.8 to 25.3 [78]	GDM Gestational hypertension Preeclampsia Caesarean delivery Preterm birth Anaesthetic risks Miscarriage Fetal macrosomia Congenital malformations Shoulder dystocia Subsequent child obesity
GDM	7 [25]	Type 2 diabetes Fetal macrosomia Caesarean delivery Shoulder dystocia Preeclampsia Prematurity Neonatal metabolic complications
Preeclampsia	10 [79]	Maternal and fetal death Preterm birth Hepatic and renal dysfunction Neonatal thrombocytopenia Pulmonary edema Neurodevelopment risk
Preterm birth	13 [80]	Neonatal morbidity and mortality Long-term neurological damage Pulmonary dysfunction Visual impairment Congenital infections

Prevention of GDM would thus be beneficial. There is limited evidence that lifestyle interventions such as diet and physical activity can prevent GDM [29]. The freshly revealed role of the gut microbiota in humans may propose a new target in the prevention of these and alleviate the undesired maternal and infant outcomes.

Microbiome in Overweight and Obese Pregnant Women

Women who are overweight and obese already have an altered gut microbiome before pregnancy and differences remain in pregnancy. A prospective follow-up study reported that the gut microbiomes of 18 overweight pregnant women exhibit higher counts of *Bacteroidetes*, *Staphylococcus* and *Clostridium* and lower counts of *Bifidobacterium* than those of 36 normal-weight pregnant women, although the difference approached but did not reach statistical significance ($P=0.054$) [30]. Infants of overweight mothers had a higher

prevalence of faecal *Bacteroidetes* and *Staphylococcus* in the first 6 months of life, compared to infants of normal-weight women, who displayed higher levels of *Bifidobacterium* [31]. Moreover, in overweight and obese pregnant women, there is a similar decrease of *Akkermansia muciniphila* when compared to normal-weight pregnant women [32], which may contribute to the higher rate of complications in overweight and obese pregnant women. *A. muciniphila* has been associated with weight loss, improved metabolic control and reduced adipose tissue inflammation [33].

In the Macaque monkey (*Macaca fuscata*), consumption of high-fat maternal or postnatal diet increases the predominance of *Bacteroidetes* in the gut microbiome of mother and offspring [34]. These results suggest that 1) an inherently obesogenic microbiome exists, 2) dietary intake can alter the consumption of the microbial community and 3) high prevalence of *Bacteroidetes* is positively correlated with increased gestational weight gain. These associations may be unique for

pregnancy since a lower prevalence of *Bacteroidetes* was revealed in the obese non-pregnant microbiome [35, 36].

Microbiome in Diabetes

In type 2 diabetes, the gut microbiome is altered with significantly lower proportions of *Firmicutes* and *Clostridia* [37]. A human metagenome-wide association study that is a study of host and bacteria combined revealed a large increase in opportunistic pathogens and sulphate-reducing species in type 2 diabetes [38]. The meconium of newborns whose mothers had type 2 diabetes shows a high prevalence of *Bacteroidetes*, *Parabacteroidetes* and *Lachnospiraceae* [39], whereas in the offspring of normoglycemic mothers, the proportions of *Proteobacteria* is higher. Outside pregnancy, the gut microbiome is altered in the setting of diabetes. This has yet to be further examined in pregnancy. However, the gut microbiome of infants born to women with type 2 diabetes differs from normal, suggesting that the maternal gut microbiome may also be different in the setting of diabetes.

Probiotic Intervention in Pregnancy and Infancy Outcomes

Influence on Maternal Profile

Gestational Weight Gain and GDM

There are only a few clinical trials of probiotic supplementation in pregnancy that have reported on gestational weight gain and GDM. The only randomised placebo-controlled trial that has reported results consisted of 256 women who were stratified for pre-pregnancy BMI. The subjects were randomised to 1) dietary intervention plus probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*) (diet/probiotics), 2) dietary intervention plus placebo (diet/placebo) capsules or 3) standard dietary information plus placebo (control diet/placebo) from early pregnancy up to 6 months of breastfeeding. Probiotic supplementation did not significantly alter gestational weight gain or postpartum weight retention, but the risk of a postpartum waist circumference (≥ 80 cm) was decreased in the diet/probiotics group compared with the control/placebo group (OR=0.30, 95 % CI 0.11–0.85, $P=0.02$) [40]. The incidence of GDM was significantly reduced in the diet/probiotic group from 34 to 13 % ($P=0.003$) [41]. A number of other randomised controlled trials investigating probiotics to prevent GDM are currently underway and will provide more data for a role of probiotics in the prevention of GDM ([42], NCT01436448, ISRCT N97241163).

Preterm Birth

Preterm birth is associated with neonatal death, fetal growth restriction and developmental disorders and is often attributed to maternal infections [43]. Two reviews [44, 45] analysed the use of probiotics to prevent preterm births and conclude that, due to small sample size, there was no decisive evidence from clinical trials confirming the efficacy of probiotics to prevent preterm births in humans. However, probiotics can shift and inhibit pathogens and thereby modulate the inflammatory cascade commonly observed in preterm birth [43]. Furthermore, dietary supplementation with the probiotic VSL#3 (a multistrain mixture of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* strains) resulted in a reduction in the decline of *Bifidobacterium* and a lower increase in *Atopobium vaginae*, both of which are associated with 80 % of the cases of bacterial vaginosis [46, 47]. In vitro animal studies have shown the beneficial effect of the supernatant of *L. rhamnosus* GR1 in attenuating lipopolysaccharide-induced inflammation, conferring a possibility of controlling systemic and intrauterine inflammation [48].

Preeclampsia

Preeclampsia is a condition of pregnancy characterised by hypertension and proteinuria and is associated with increased maternal insulin resistance and low-birth-weight infants [24, 49, 50]. There is some epidemiological evidence suggesting that probiotics might have a role in preventing this serious disorder of human pregnancy. In the Norwegian Mother and Child Cohort, an observational study, high intake of dairy products containing *Lactobacilli* (>200 ml/day) were associated with reduced risk of overall preeclampsia (OR=0.79, 95 % CI 0.66–0.96) and severe preeclampsia (from 1.8 to 1.0 %) even after correcting for BMI, smoking, socioeconomic status, dietary supplementation, maternal age at delivery, education and height [51]. The study did not assess the composition of the vaginal and/or gut microbiome to establish if probiotics alter microbiome composition or confer health benefits by other mechanisms. Furthermore, storage conditions of milk-based products may change microbial counts and thereby affect actual bacterial intake even in women with similar intake of dairy products.

Lipid Profile

Probiotics may alter lipid profiles. In an in vitro environment resembling the gut, *Lactobacilli* strains could remove small amounts of cholesterol, through adherence of cholesterol to the *Lactobacilli* membrane, inhibition of micelle formation and deconjugation of bile salts [52]. Outside of pregnancy, supplementation of *Lactobacillus acidophilus* L1 in men and women over 10 weeks reduced serum cholesterol by 2.4 % compared to that of the placebo group [53]. A randomised

single-blind controlled clinical trial was performed in 70 pregnant women, assigned to probiotic yoghurt containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* or conventional yoghurt. Yoghurt consumption reduced serum total cholesterol ($P=0.001$), HDL ($P=0.002$), LDL ($P=0.006$) and serum triglyceride concentrations ($P=0.03$) over the 9-week study period, with no differences observed between the groups [54]. Consumption of *L. rhamnosus* GG and *B. lactis* Bb12 in capsule in a randomised controlled study ($n=256$) lowered triglyceride concentrations and LDL cholesterol levels postpartum ($P=0.03$) but not during pregnancy [55]. The contradictory results in clinical studies may depend on strain specificity as the depletion of cholesterol can undergo different pathways with different rates of excretion.

Influence on Neonatal Outcomes

Infant Anthropometry

A perinatal probiotic-supplemented dietary counselling follow-up study evaluated fetal and infant growth during the first 24 months of life. Weight gain and growth in length showed no statistically significant difference between the diet/probiotics, diet/placebo and control groups [41]. In addition, a study of mother taking *L. rhamnosus* GG for 4 weeks before expected delivery until 6 months postpartum showed that modulation of the early gut microbiota with probiotics can change the growth pattern of the offspring by limiting excessive weight gain during the first 4 years of life [56].

Allergy and Asthma

Allergic disease including asthma, eczema, hay fever and food allergies are increasing in neonates [57]. Specific immune responses generated by a dietary or environmental factor during pregnancy, breastfeeding and early life may play a role in the development of allergic disease. A longitudinal study revealed that infants with eczema had fewer *Bifidobacterium* and an overall lower diversity in their gut microbiota when compared to control subjects [58, 59]. Moreover, consumption of probiotic milk (*L. acidophilus* LA-5, *B. lactis* BB12 and *L. rhamnosus*) in pregnancy was associated with reduced risk of atopic eczema in infants at 6 months and rhinoconjunctivitis between 18 and 36 months [60]. There was no evidence of reduced asthma [58, 61]. A meta-analysis study revealed that administration of *Lactobacilli* during pregnancy reduced atopic eczema in children aged 2 to 7 years (-5.7% ; $P=0.02$) [62]. In addition, *L. rhamnosus* HN001 was effective against eczema in children between 2 and 4 years, while *Bifidobacterium* had no effect [63]. Other clinical trials reported that *Bifidobacterium longum*, *L. rhamnosus* LPR, *Lactobacillus paracasei* and *B. lactis* do not prevent eczema, suggesting that the effect is strain specific [59, 64].

There is also evidence of immune-modulatory action by probiotics. Pre- and postnatal supplementation of *Lactobacillus reuteri* decreases allergen responsiveness and can increase immunoregulatory capacity in infancy by lowering IgE-associated eczema [65]. Additionally, *L. rhamnosus* stimulates immunological responses such as IgA in breast milk with pregnant women in the probiotic group that were significantly more likely (88 %) to have detectable IgA in early breast milk samples compared to that of the placebo group (60 %) ($P=0.005$) [66]. Asthma has been associated with relative IgA deficiency [67]. But even if probiotics can trigger the production of this immunoglobulin, no relationship was so far reported for reducing the incidence of asthma. Future trials for asthma prevention require extended follow-up trials and could be dependent on the probiotic strain. The results above suggest, however, that probiotic bacteria can modulate neonatal immune programming and can provide effective approaches for allergy prevention.

Emerging Therapeutic Applications

Probiotic therapy in the management of necrotising enterocolitis (NEC) has been intensively studied. A Cochrane meta-analysis of 24 eligible probiotic trials found a significant reduction in the incidence of severe NEC (RR=0.43, 95 % CI 0.33–0.56; 20 studies, 5529 infants) in neonates on probiotics [68]. Moreover, species from *Lactobacillus*, *Bifidobacterium* and *S. thermophilus* have been associated with a reduced risk for NEC and gastrointestinal morbidity, suggesting that early gut colonisation with beneficial bacteria can lower the incidence of NEC [69–72].

Side Effects

Probiotics have been catalogued as generally safe and have been proven to confer health benefits in various diseases. They are routinely employed in food processing and have an excellent safety record. Some *Lactobacillus* species have been associated with endocarditis in adults and sepsis in children although none of these species was a probiotic strain. Also, a vast number of *Lactobacilli* are established in the intestinal microbiota of healthy humans. Fewer reports relating *Bifidobacterium* to sepsis are described, but *Bifidobacteria* exhibit a low grade of pathogenicity, therefore having a better safety profile [73]. In pregnancy, probiotic supplementation is unlikely to negatively affect pregnant and lactating women or to cause an increase in adverse pregnancy outcomes [74]. Caution however is required with immunosuppressed patients or those with an increased risk of sepsis relating to a leaky gut or significant gastrointestinal disease.

Conclusions

The maternal gut microbiome is altered in diabetes and obesity, both of which are linked to adverse pregnancy outcomes. Therefore, changing the composition of the gut microbiome specifically by probiotics before and during pregnancy could become a new strategy for prevention of adverse pregnancy outcomes. There are few studies suggesting that the modification of the gut microbiome by probiotics may serve to prevent pregnancy complications and improve outcomes. Consequently, there is an urgent need for large, well-designed randomised controlled clinical trials to further dissect probiotics as a prevention strategy, in addition to mechanistic studies clarifying potential mechanisms of actions in humans. In order to generalise the benefits of these ‘good bugs’ in pregnancy, metagenomic studies of the human gut microbiota of women living in different geographical regions, of different ethnic background and with different diets must be assessed as these factors may modulate the entire gastrointestinal niche. Isolation of indigenous probiotic strains is necessary in order to expand the range of ‘western strains’.

Additionally, modulation of the maternal gut microbiome composition by the use of probiotics and prebiotics (synbiotics) might enhance probiotic survival and growth, offering a potential advantage over the use of probiotics alone.

Compliance with Ethics Guidelines

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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