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ORIGINAL RESEARCH ARTICLE

The effect of *Lactiplantibacillus plantarum* 299v together with a low dose of iron on iron status in healthy pregnant women: A randomized clinical trial

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Abstract

Introduction: Iron deficiency during pregnancy is a global health problem and is associated with adverse pregnancy outcomes. The aim of this randomized, double-blind, placebo-controlled study was to evaluate the effect of *Lactiplantibacillus plantarum* 299v (Lp299v, 10^{10} colony forming units), 4.2 mg iron, 12 mg ascorbic acid and 30 µg folic acid (Lp) on iron status in healthy, non-anemic, pregnant Swedish women.

Material and methods: A total of 326 women were randomized to receive Lp (n = 161) or placebo (n = 165) twice daily from gestational week 10–12 until end of pregnancy or until the potential start of iron therapy. The primary endpoint was serum ferritin at week 28.

Results: Intake of Lp attenuated the decrease in serum ferritin from baseline to week 28 (p = 0.003) and week 35 (p < 0.001) and resulted in reduced prevalence of iron deficiency (59% vs 78%, p = 0.017) and iron deficiency anemia (7.4% vs 21%, p = 0.023) at week 35. Intake of Lp also resulted in beneficial effects on the soluble transferrin receptor (p = 0.011) and total body iron (p < 0.001) at week 35. Gestational length and birthweight were comparable between groups. The proportion of women reporting adverse events during the study was comparable between groups.

Conclusions: Intake of Lp from early pregnancy was safe, attenuated the loss of iron stores and improved iron status in healthy pregnant women.

KEYWORDS
DSM 9843, ferritin, iron absorption, iron deficiency, iron status, *Lactiplantibacillus plantarum* 299v, pregnancy, probiotics

1 | INTRODUCTION

Iron deficiency during pregnancy is a major public health problem leading to the development of iron deficiency anemia.[1] Global estimates indicate that about 40% of pregnant women suffer from anemia.[2] Iron deficiency anemia during pregnancy is associated with increased risk of preterm birth, low birthweight, perinatal and neonatal mortality[3] as well as offspring neurodevelopmental disorders.[4] The iron requirements increase from around 0.8 mg/day to about 7.5 mg/day in the third trimester.[5,6] For many pregnant women, body iron stores and the dietary iron

Abbreviations: AE, adverse events; FAS, full analyses set; Hb, hemoglobin; Lp, denotes the group receiving active study product; Lp299v, *Lactiplantibacillus plantarum* 299v; MCV, mean corpuscular volume; PPS, per protocol set; SAE, serious adverse event; sTfR, soluble transferrin receptor.

intake are not sufficient to meet the iron demands in the second and third trimester of pregnancy. The standard treatment for iron deficiency consists of iron supplementation and most countries have programs for routine screening and iron supplementation during pregnancy. However, a large proportion of high-dose iron supplements remains unabsorbed in the intestine, commonly causing adverse gastrointestinal events, reduced compliance and inefficient repletion of iron stores.[7] Emerging data also suggest that non-absorbed iron could be harmful through negative modifications of the gut microbiota.[8] Furthermore, excess iron supplementation has been associated with increased risk of reproductive and pregnancy-related disorders.[9,10] The association between iron overload and these diseases has been suggested to be mediated by iron-dependent lipid peroxidation within cell membranes leading to programmed cell death, so-called ferroptosis.[11] For women who are initially iron replete and non-anemic, the need for prophylactic iron during pregnancy is uncertain,[12] prompting the consideration of potential adverse side effects of indiscriminate iron supplementation. Thus, new strategies for a more physiological improvement of the iron status during pregnancy are warranted. A probiotic supplement with the potential to increase iron absorption while reducing the risk of gastrointestinal side effects would be beneficial for prevention of iron deficiency and iron deficiency anemia during pregnancy.

It has previously been shown that the probiotic strain *Lactiplantibacillus plantarum* 299v® (Lp299v, DSM 9843) significantly increases iron absorption from several food matrices,[13-15] as recently examined in a systematic review and meta-analysis.[16] Also, intake of Lp299v has previously been shown to increase the hemoglobin content and hematocrit values in anemic rats.[17] In addition, a recent randomized clinical trial in iron deficient athletes indicated that intake of Lp299v together with 20 mg of iron could result in a more substantial and rapid improvement in iron status compared with 20 mg of iron alone.[18] The mechanism behind the effect is not known but Lp299v has been shown to increase the amount of ferric iron (Fe^{3+}) in in vitro digested meals and drinks. This, in combination with an increased level of a ferric reductase (duodenal cytochrome B, DcytB) in human intestinal co-cultures of enterocytes and goblet cells (Caco-2/HT29-MTX cells) in the presence of Lp299v, may be part of the positive effect on iron absorption.[19]

The aim of the present study was to evaluate further the effects of Lp299v in combination with a low amount of iron, ascorbic acid and folic acid on iron status in healthy pregnancies. There is a gradual reduction in serum ferritin in pregnancy and the nadir concentrations are reached at gestational weeks 35–38, followed by a moderate increase towards delivery.[6] In the present study, ferritin levels were followed until week 35 and the levels at week 28 were selected as the primary endpoint.

Key message

Iron deficiency during pregnancy is a global health problem. Intake of the probiotic strain *Lactiplantibacillus plantarum* 299v together with a low dose of iron, folic acid and ascorbic acid is safe and improves iron status in healthy pregnant women.

2 | MATERIAL AND METHODS

2.1 | Study design

This was a randomized, double-blind, placebo-controlled, parallel study. The women were randomized at a ratio of 1:1 to receive Lp or placebo. The study was a multi-center study including five sites in Sweden. The randomization was stratified for center with one randomization list per center. The generation of the randomization lists was delegated to an independent biostatistician. The allocation of study product was not disclosed to the investigators, other medical staff or the sponsor until clean file was declared and the database was locked. The blinding was maintained throughout the study. The women were enrolled and assigned to the intervention by the midwife based on the randomization list. The women were enrolled in the study at gestational weeks 10–12 (baseline visit) and instructed to consume the study product until delivery or until the potential start of iron therapy as advised by the midwife. Apart from the baseline visit, the study included four additional visits to the midwife clinic; at gestational weeks 25, 28, 35 and a follow-up visit 8 weeks after delivery. In addition, the study included a phone call at week 15 to remind the women about study procedures, to assess adverse events and concomitant medications as well as answer any questions.

2.2 | Study participants

A total of 340 women women were recruited at antenatal midwife clinics between September 2016 and March 2018. Healthy, non-anemic (hemoglobin ≥110 g/L) pregnant women, 18–42 years, with a singleton gestation were eligible for the study. The women had to be iron replete (serum ferritin ≥20 μg/L) and have a body mass index between 18 and 30 kg/m^2 at the baseline visit. Exclusion criteria were chronic disease associated with anemia, known thalassemia, hyperemesis gravidarum, chronic gastrointestinal disease, use of antibiotics within 4 weeks prior to the baseline visit, smoking or use of nicotine-containing products after positive pregnancy test, history of alcohol abuse or excess intake of alcohol or blood or plasma donation within 3 months prior to the baseline visit. The women were instructed not to consume any other probiotic products during the study. Furthermore, the women were instructed not to consume any iron supplements including multimineral/vitamin supplement containing iron during the study, unless iron therapy was recommended by the midwife. In that case, the women were instructed to terminate the consumption of the study product but remained in the study. The women otherwise followed the standardized antenatal guidelines.

2.3 | Study product

The Lp capsule contained freeze-dried *Lactiplantibacillus plantarum* 299v® (10^{10} colony forming units), 4.2 mg iron (ferrous fumarate), 12 mg ascorbic acid and 30 μg folic acid per capsule, along with maize starch (bulking agent), maltodextrin (bulking agent), magnesium stearate (processing aid), cellulose fiber and cellulose derivatives (coating of vitamins and iron). The placebo capsules were of identical appearance, taste and texture and contained maize starch and magnesium stearate only. The women were instructed to consume the study product twice daily in connection with the two main meals of the day. The amount of iron, ascorbic acid and folic acid in the Lp product could be considered low. The iron in the study product (4.2 mg per capsule) was mainly included to ensure a baseline dietary iron intake. It constitutes approximately 30% of the recommended daily intake for women in Sweden and corresponds to an average amount of iron in a regular meal. The amount of ascorbic acid and folic acid constitutes approximately 15% and 7.5%, respectively, of the daily recommended intake for women in Sweden.

2.4 | Outcomes

Blood samples were collected for analysis of iron status (serum ferritin, hemoglobin [Hb]), soluble transferrin receptor (sTfR), total iron binding capacity, plasma iron, transferrin saturation, mean corpuscular volume (MCV) and C-reactive protein at certified local hospital laboratories using standardized and validated procedures. Serum ferritin is a sensitive marker of iron status[6] and was therefore chosen as the primary endpoint.

Total body iron was calculated according to Cook et al.[20] The prevalence of iron deficiency, anemia and iron deficiency anemia were examined at gestational weeks 25, 28 and 35 and at the 8-week follow-up visit. Iron deficiency was defined as serum ferritin <15 μg/L and anemia as Hb < 110 g/L. Iron deficiency anemia was defined as serum ferritin <15 μg/L and Hb < 110 g/L.[2] The women were instructed to fill out a dietary questionnaire at baseline, week 28 and week 35. The dietary questionnaire included questions on whether the subject had a vegetarian or vegan diet (yes/no), the average amount of dairy products, vegetables and meat consumed per week, and one question regarding active intake of food items rich in iron (yes/no) as well as one question regarding intake of food items naturally rich in lactic acid bacteria such as yogurt, sour milk and lactic acid fermented vegetables (yes/no). Birthweight and gestational length were recorded at the 8-week follow-up visit. The prevalence of low birthweight (<2500 g) and preterm birth (birth before 37 completed weeks of gestation) were assessed. Safety was evaluated for all women with intake of at least one dose of the study product by recording the occurrence of adverse events throughout the study period.

Iron status and other efficacy measurements were assessed in both the full analysis set (FAS) and the per protocol set (PPS). The FAS was defined as all randomized women with an intake of at least one dose of the study product and at least one post-baseline efficacy assessment. The PPS was defined as all randomized women with no major protocol deviations, a compliance above 80% and no intake of iron supplements. Major protocol deviation included violation of inclusion and exclusion criteria, visit window deviations, deviations related to the study product or deviations related to study assessments. The women who were advised to start iron therapy by their midwife, according to regular clinical guidelines, were instructed to stop intake of the study product but remained in the study. Compliance was calculated based on the total number of capsules returned related to the total number of administered capsules and intervention length.

2.5 | Statistical analyses

The primary endpoint was the difference in serum ferritin at gestational week 28 after intake of Lp in comparison with placebo. To achieve a difference in serum ferritin of 4.2 μg/L between Lp and placebo with an estimated standard deviation of 10 and a power of 80% (alpha = 0.05), at least 89 women per group had to complete the visit at week 28 without any iron therapy. To allow for dropouts and for women withdrawn due to concomitant iron therapy, 326 women were included.

The primary endpoint (absolute change in log-transformed serum ferritin at week 28) was analyzed using Student's t-test. The log-transformation was done because the distribution of ferritin levels was non-normal; however, for ease of interpretation, untransformed ferritin data are presented. Further analysis of the primary endpoint was performed by analysis of covariance with treatment, center, the interaction effect (treatment × center) and baseline log serum ferritin as covariates for the absolute change from baseline (if the interaction effect and center were non-significant, these were removed from the model). For the remaining continuous endpoints, between-treatment change over time was analyzed using the non-parametric Wilcoxon Rank Sum test and the within-treatment change over time was analyzed using the Wilcoxon Signed Rank Sum test. For categorical outcomes the Chi-square test without continuity correction was used to analyze change over time. No adjustments for multiple comparisons or imputation of missing data were performed. All p-values are two-tailed and are considered to be significant if $p < 0.05$. Data are presented as mean with standard deviation (SD). Statistical analysis was performed using SAS Version 9.4 (SAS Institute).

2.6 | Ethical approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee in Lund, Sweden (Dnr 2016/418), date of approval 16 August 2016. Written informed consent was obtained from all women. Participation was voluntary and

could be discontinued at any time without explanation. The trial is registered at clinicaltrials.gov (NCT02912416).

3 | RESULTS

3.1 | Subject disposition and characteristics

The subject disposition is shown in Figure 1. At week 28, the FAS was comprised of 101 women allocated to Lp and 107 women allocated to placebo and the PPS of 88 women in the Lp group and 90 in the placebo group. The PPS included only those women that did not receive concomitant iron therapy as recommended by the midwife. If not otherwise specified, all presented efficacy results are for the PPS.

The demographics and baseline characteristics of the women included in the FAS are summarized in Table 1. Demographics and baseline characteristics were comparable between the two groups. There were no significant differences in birthweight or gestational length between the two groups (Table 2).

3.2 | Iron status

Iron status at gestational weeks 25, 28 and 35 can be found in Table 3. The primary outcome was ferritin levels at week 28; at this timepoint, the decrease was −44 µg/L for the Lp group and −49 µg/L for the placebo group (p = 0.003). Further analysis of the primary endpoint using analysis of covariance (ANCOVA) showed that there was no site effect or site by treatment interaction, but baseline log serum ferritin had an effect and including this as a covariate resulted in a p value of 0.005. The decrease from baseline was also significantly smaller in the Lp group than in the placebo group at gestational week 25 (p = 0.015) and week 35 (p < 0.001). The ferritin

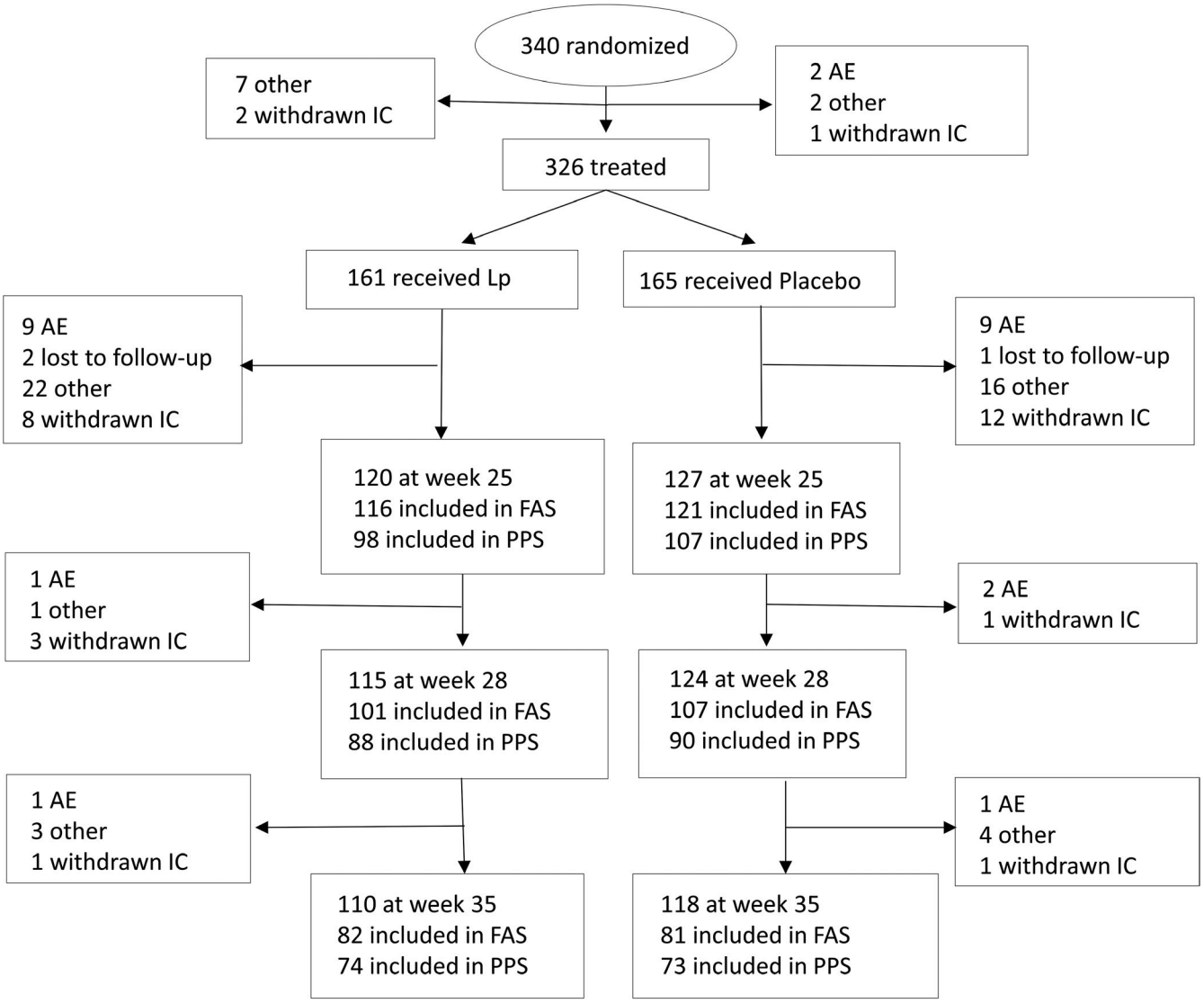

FIGURE 1 Flow chart of subject disposition and allocation to datasets. AE, adverse events; FAS, full analyses set; IC, informed consent; Lp, denotes the group receiving active study product; PPS, per protocol set

TABLE 1 Demographics and baseline characteristics

	Lp			Placebo			Total		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Age (years)	30.2	4.3	120	30.6	4.4	128	30.4	4.3	248
BMI (kg/m^2)	23.9	3.1	120	23.6	2.9	128	23.8	2.9	248
Previous pregnancy, *n* (%)	71 (59)	-	120	77 (60)	-	128	148 (60)	-	248
Ferritin (µg/L)	57.4	43.1	120	62.2	38.3	128	59.9	40.7	248
Hb (g/L)	128.6	9.9	120	128.5	8.2	128	128.5	9.0	248
sTfR (mg/L)	0.94	0.23	119	0.97	0.24	128	0.95	0.23	247
Total body iron (mg/kg)	12.8	2.5	119	13.0	2.5	128	12.9	2.5	247
TIBC (µmol/L)	66.4	8.8	120	65.5	9.4	128	65.9	9.1	248
Plasma iron (µmol/L)	19.8	6.5	120	19.4	6.5	128	19.6	6.5	248
Transferrin saturation	0.33	0.16	120	0.30	0.11	128	0.32	0.14	248
MCV (fL)	86.9	3.4	120	87.2	3.6	128	87.0	3.5	248
CRP (mg/L)	3.6	4.9	120	5.1	9.4	128	4.4	7.6	248

Note: Data presented for the full analyses set (FAS) and as mean with SD.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; Hb, hemoglobin; Lp denotes the group receiving the active study product; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor; TIBC, total iron binding capacity.

TABLE 2 Birthweight, gestational length, prevalence of low birthweight and preterm birth in the two treatment groups

	Lp (*n* = 112)		Placebo (*n* = 116)		
	Mean	SD	Mean	SD	*p* value
Birthweight (g)	3569	464	3557	483	0.908
Gestational length (days)	280	11	280	11	0.650
Birthweight/gestational length	12.8	1.5	12.7	1.6	
Low birthweight	1/112 (0.89%)	–	1/116 (0.86%)	–	0.980
Preterm birth	4/112 (3.6%)	–	6/116 (5.2%)	–	0.555

Note: Data presented for the full analyses set (FAS). No significant differences between the groups were found.

Abbreviation: Lp, denotes the group receiving the active study product .

levels 8 weeks after delivery were still lower than at baseline, with a total mean of 47.8 µg/L. Consumption of Lp throughout pregnancy resulted in a ferritin value of 49.4 µg/L as compared with 40.2 µg/L for the placebo at the 8-week follow-up visit (−14 µg/L vs −25 µg/L, $p = 0.034$). The prevalence of iron deficiency at week 35 was significantly lower in the Lp group (59%) than in the placebo group (78%, $p = 0.017$) but no significant differences between the groups were found earlier during the pregnancy (Figure 2A).

The Hb levels decreased in both groups during the gestational period. The decrease from baseline was significantly smaller in the Lp group at gestational week 25 ($p = 0.030$) and at week 35 ($p = 0.002$). At week 28, the difference did not reach statistical significance ($p = 0.065$). At the follow-up visit, the Hb levels were close to those at baseline and with no differences between the groups. The prevalence of anemia was significantly lower in the Lp group than in placebo group at week 28 (14% vs 26%, $p = 0.050$) and at week 35 (7.4% vs 21%, $p = 0.023$), but not at week 25. The prevalence of iron deficiency anemia was significantly lower in the Lp group than in the placebo group at week 35 (7.4% vs 21%, $p = 0.023$), that is, at week 35 all cases with anemia were defined as iron deficiency anemia (Figure 2B).

To further investigate the effect of Lp, additional markers of iron status were analyzed. The soluble transferrin receptor (sTfR) increased in both groups over time but the increase in soluble transferrin receptor was significantly smaller in the Lp group at week 28 ($p = 0.011$) and at week 35 ($p = 0.011$); no differences were detected at week 25. Total body iron decreased in both groups but the decrease from the baseline visit was significantly smaller in the Lp group than in the placebo group at week 25 ($p = 0.041$), week 28 ($p = 0.002$) and week 35 ($p < 0.001$).

An increase in total iron binding capacity (TIBC) was observed in both groups but the increase was significantly smaller in the Lp group at week 35 ($p = 0.019$); no differences were detected at week 25 or 28. At the 8-week follow-up visit, the increase was still significantly smaller in the Lp group than in the placebo group in women taking the study product until delivery (−1.2 vs 3.2 µmol/L, $p = 0.005$). There were no significant differences between the groups in plasma iron, transferrin saturation or mean corpuscular

TABLE 3 Iron status at gestational weeks 25, 28 and 35 in the treatment groups

	Baseline				Week 25					Week 28					Week 35				
	Lp (*n* = 85–87)		Placebo (*n* = 89–90)		Lp (*n* = 94–98)		Placebo (*n* = 105–106)			Lp (*n* = 85–87)		Placebo (*n* = 89–90)			Lp (*n* = 68–71)		Placebo (*n* = 71–72)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p*-value	Mean	SD	Mean	SD	*p*-value	Mean	SD	SD	SD	*p*-value
Ferritin abs (µg/L)	61.2	46.8	64.0	39.1	22.2	20.6	21.1	19.8	0.015	17.3	14.8	15.5	13.6	0.003	16.0	10.4	13.8	11.9	<0.001
Hb (g/L)	130.3	10.1	130.5	7.4	120.0	11.3	117.5	8.3	0.030	118.7	7.9	116.5	8.7	0.065	122.5	8.0	117.4	8.8	0.002
sTfR (mg/L)	0.93	0.23	0.96	0.19	1.1	0.3	1.2	0.3	0.556	1.2	0.3	1.3	0.3	0.011	1.5	0.5	1.7	0.5	0.011
Total body iron	13.1	2.5	13.2	2.2	8.7	2.8	8.1	2.8	0.041	7.5	2.6	6.7	2.8	0.002	6.7	2.5	5.7	2.6	<0.001
TIBC (µmol/L)	66.2	9.0	64.8	9.6	87.4	13.8	87.8	12.6	0.443	91.8	11.9	92.8	13.4	0.074	102.0	14.4	103.7	13.8	0.019
Plasma iron (µmol/L)	20.1	6.5	19.8	6.9	16.2	5.8	14.6	5.9	0.707	13.6	5.0	12.1	5.0	0.591	15.1	6.0	12.1	5.9	0.323
Transferrin saturation	0.33	0.14	0.31	0.11	0.19	0.07	0.19	0.14	0.705	0.15	0.06	0.14	0.07	0.659	0.17	0.14	0.12	0.07	0.268
MCV (fL)	87.0	3.7	87.3	3.0	89.0	3.5	89.2	3.8	0.675	89.5	3.5	89.7	3.6	0.802	87.9	4.3	87.4	4.3	0.136

Note: The statistical analyses were based on changes from baseline to each timepoint, between treatments. For ferritin, Student's *t*-test using log-transformed values was used. Otherwise, Wilcoxon Rank Sum Test was used.

Abbreviations: Hb, hemoglobin; Lp denotes the group receiving the active study product; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor; TIBC, total iron binding capacity.

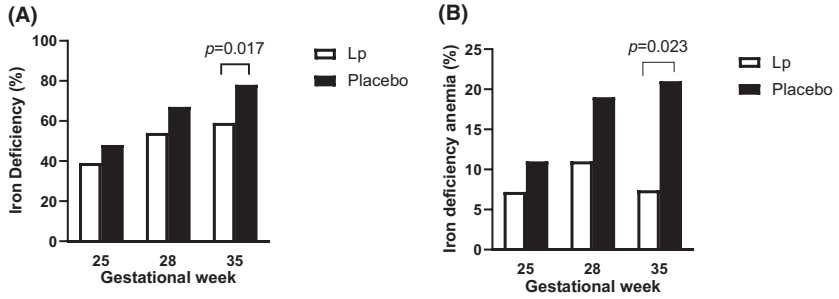

FIGURE 2 Prevalence of iron deficiency (A) and iron deficiency anemia (B) at gestational weeks 25, 28 and 35. The Chi-square test was used for statistical analyses. *n* = 97 and 106, respectively, for the Lp and placebo group at week 25. *n* = 87 and 89, respectively, for the Lp and placebo group at week 28. *n* = 68 and 72, respectively, for the Lp and placebo group at week 35

volume at any measured timepoint during the study (Table 3). A total of 131 women completed the study without iron therapy, 70 in the Lp group and 61 in the placebo group. At partus, 28% of the women in the Lp group and 39% in the placebo group consumed iron supplements ($p = 0.075$).

To evaluate whether changes in dietary habits could have influenced the observed effects on iron status between the groups, food intake questionnaires were filled in at the baseline visit and at weeks 28 and 35. Very few changes in dietary habits over time were found and no significant correlations between change in ferritin and hemoglobin values and changes in dietary habits during the study were detected (data not shown).

3.3 | Compliance with study product and safety

Compliance with the study product was similar between the groups (Appendix S1). The proportion of women reporting adverse events and serious adverse events (SAE) and the number, severity and causality of the adverse events and serious adverse events reported were comparable between treatment groups (Appendix S1).

4 | DISCUSSION

In this randomized controlled trial, we were able to show that intake of *L. plantarum* 299v and a low dose of iron, folic acid and ascorbic acid from the first trimester resulted in a significantly smaller decrease in ferritin during pregnancy compared with placebo. This finding indicates a smaller reduction of iron stores and thus an improved iron status. Furthermore, a significantly lower prevalence of iron deficiency was evident in the women taking Lp compared with placebo (59% vs 78% at week 35). Importantly, intake of Lp also resulted in a significantly smaller decrease in hemoglobin levels during pregnancy as compared with placebo together with a lower prevalence of iron deficiency anemia, especially in late pregnancy. A consensus on a specific threshold of serum ferritin to define iron deficiency in pregnancy is lacking and there is currently a variation in the threshold used. A systematic review by Daru et al. showed that the most commonly used thresholds for serum ferritin to define iron deficiency were <12 and <15 μg/L.[21] Here we defined iron deficiency as a ferritin level <15 μg/L, which according to the WHO, corresponds to depleted iron stores. According to the WHO, mild anemia in pregnancy is defined as having a hemoglobin value between 100 and 109 g/L.

Interestingly, even when comparing the prevalence of iron deficiency at week 28 in the whole study population, that is, also including women who stopped treatment and started traditional iron therapy at any time point before week 28, the Lp group had a lower prevalence of iron deficiency compared with the placebo group (50% vs 63%, $p = 0.049$). This indicates that intake of Lp starting from early pregnancy, no matter the duration, could protect against iron deficiency in late pregnancy. Furthermore, the women who consumed Lp until gestational week 35 or longer had a significantly lower total iron binding capacity after delivery, compared with placebo. Also, the women taking study product until delivery displayed significantly higher ferritin levels at the follow-up visit (49.4 vs 40.2 μg/L, $p = 0.034$). This could indicate that intake of Lp during pregnancy also improves iron status after delivery, which would be beneficial for maternal postpartum recovery as well as for future pregnancies. Overall, the number and severity of the adverse events reported in the study was comparable between the groups. In addition, the compliance was similar between Lp and placebo (95% vs 94%). Together this clearly indicates that intake of Lp is safe and well tolerated during pregnancy.

To the best of our knowledge, this is the first study which evaluates a new, more physiological approach to improve iron status during pregnancy using probiotics. The effect of iron supplementation during pregnancy has been studied extensively and, as an example, 60 randomized or quasi-randomized trials were included in a Cochrane review in 2012.[12] It is not straightforward to compare the results from these studies with the present study but a study with a similar design by Zhao et al. administered 60 mg of iron per day to pregnant women in China and measured iron status near term.[22] When comparing their data with the iron status in week 35 for the Lp group, similar values were found for hemoglobin (122 g/L for both studies), serum ferritin (15.3 vs 16.0 μg/L), iron deficiency (57% vs 59%) and iron deficiency anemia (10.6% vs 7.4%). Cogswell et al. studied supplementation with 30 mg of iron daily from early pregnancy in two different randomized trials in the USA.[23,24] They measured the iron status at the same time

point as in the present study (gestational week 28), but in contrast to the present study, did not observe any significant differences in iron status including iron deficiency anemia compared with placebo. However, the iron supplementation led to a significantly higher birthweight compared with placebo in both studies. Considering that no significant effect on iron status was obtained using 30 mg of iron alone, one could hypothesize that Lp offers a benefit that iron alone does not, in improving iron status during pregnancy.

A strength of the present study was that it was powered to detect also small changes in iron status and that several markers of iron status were evaluated. This was important since relatively few women developed iron deficiency anemia compared with the global prevalence of about 40%,[2] which could be regarded as a limitation of the study. It could be speculated that the effect of Lp would be more pronounced in a more iron-deficient population. Furthermore, the number of women in need of iron therapy according to standard guidelines was relatively low in this population. Thirty-one women (28%) in the Lp group and 45 (39%) in the placebo group were recommended iron therapy at some time before delivery ($p = 0.075$).

Another limitation of the study was the fact that the placebo product did not contain the corresponding amount of iron, folic acid and ascorbic acid as the intervention product. Therefore, conclusions can only be drawn for the combination of components and not for any single one. However, the amounts of iron, folic acid and ascorbic acid were rather low and can be obtained in an ordinary meal. Also, an earlier pilot trial indicated that intake of Lp299v with 20 mg iron resulted in improved iron status compared with 20 mg alone in iron-deficient women.[18] It can therefore be hypothesized that Lp299v was of major importance for the effect on iron status in the present study, but further studies are needed to confirm this hypothesis.

The mechanism underlying the observed improved iron status after intake of Lp is not fully known but may be related to the presence of Lp299v in the first section of the small intestine, where iron absorption takes place, and the increase in available Fe^{3+} species and DcytB.[19,25] The absorption of the iron included in the capsule as well as that of dietary iron could be influenced by Lp299v and therefore the women were advised to consume the capsules in connection with a meal.

As expected, serum ferritin decreased during pregnancy in both groups. Women taking Lp had a mean ferritin level of 17.3 µg/L at week 28 as compared with 15.5 µg/L for the women in the placebo group, that is, the difference in absolute numbers is relatively small and indicates, as expected in this later stage of pregnancy, relatively low iron stores in both groups. Many women enter pregnancy with insufficient iron stores and it could be speculated that initiating the consumption of Lp even prior of becoming pregnant could have an additional beneficial impact on iron status during pregnancy. However, this remains to be investigated.

5 | CONCLUSION

The results from this randomized controlled trial show that intake of *Lactiplantibacillus plantarum* 299v together with a low dose of iron, folic acid and ascorbic acid twice daily from early pregnancy is safe and improves iron status during pregnancy.

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CONFLICT OF INTEREST

UA, GÖ, TN and NL are employed by Probi AB. The other authors (SRH and LH) declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

GÖ, TN, NL and LH designed the research project. UA and GÖ analyzed data. UA, GÖ, SRH and LH wrote the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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