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# Timing of probiotic milk consumption during pregnancy and effects on the incidence of preeclampsia and preterm delivery: a prospective observational cohort study in Norway

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## ABSTRACT

**Objectives** To investigate whether the timing of probiotic milk intake before, during early or late pregnancy influences associations with preeclampsia and preterm delivery.

**Design** Population based prospective cohort study.

**Setting** Norway, between 1999 and 2008.

**Participants** 70 149 singleton pregnancies resulting in live-born babies from the Norwegian Mother and Child Cohort Study (no chronic disease, answered questionnaires, no placenta previa/cerclage/serious malformation of fetus, first enrolment pregnancy). Only nulliparous women (n=37 050) were included in the preeclampsia analysis. Both iatrogenic and spontaneous preterm delivery (between gestational weeks 22+0 and 36+6) with spontaneous term controls (between gestational weeks 39+0 and 40+6) were included in the preterm delivery analysis resulting in 34 458 cases.

**Main outcome measures** Adjusted OR for preeclampsia and preterm delivery according to consumption of probiotic milk at three different time periods (before pregnancy, during early and late pregnancy).

**Results** Probiotic milk intake in late pregnancy (but not before or in early pregnancy) was significantly associated with lower preeclampsia risk (adjusted OR: 0.80 (95% CI 0.68 to 0.94) p-value: 0.007). Probiotic intake during early (but not before or during late pregnancy) was significantly associated with lower risk of preterm delivery (adjusted OR: 0.79 (0.64 to 0.97) p-value: 0.03).

**Conclusions** In this observational study, we found an association between timing of probiotic milk consumption during pregnancy and the incidence of the adverse pregnancy outcomes preeclampsia and preterm delivery. If future randomised controlled trials could establish a causal association between probiotics consumption and reduced risk of preeclampsia and preterm delivery, recommending probiotics would be a promising public health measure to reduce these adverse pregnancy outcomes.

## Strengths and limitations of this study

- Large sample size and participants from both urban and rural regions, of different ages and socioeconomic groups.
- Link to the medical birth registry of Norway.
- Self-selection bias has been investigated, and no bias was found in eight selected exposure-outcome associations.
- The questionnaires were filled in before delivery, avoiding confounding by retrospective responses.
- Limitations include self-reported dietary data and that unmeasured confounding can not be ruled out.

## BACKGROUND

Preeclampsia and preterm delivery are two major challenges in modern obstetrics. Preeclampsia is a multisystem disorder affecting 2%–8% of all pregnancies.[1] Preterm delivery, defined by the WHO as birth occurring before 37 weeks of gestation, is the leading cause of perinatal mortality and a major cause of paediatric morbidity and disability.[2] The rates of preterm delivery in Scandinavia, 5.6% to 6.4%, are among the lowest in the world.[3] In the United States, however, the rate is as high as 9.6%.[4]

Maternal inflammatory response is a crucial part in the pathophysiology of these conditions.[5–16] The pathogenesis of preeclampsia involves activation of the coagulation system and endothelial cell dysfunction.[17] It is believed that this endothelial dysfunction is part of a wider maternal inflammatory response, present in normal pregnancy but exaggerated in preeclampsia.[5] Increased inflammatory response seems to play a greater role especially in early-onset[6 7] and severe[8–10] preeclampsia. Preterm delivery is clinically divided into spontaneous preterm delivery, including preterm labour and preterm prelabor rupture of membranes, and iatrogenic preterm delivery. As with preeclampsia,

an increased inflammatory response seems to play a role in spontaneous preterm delivery. This is partly explained by microbial invasion of the amniotic cavity leading to activation of the innate immune system and increased production of prostaglandins, leading to intrauterine contractility and increased risk of preterm labour. Additional inflammatory mediators influence the degradation of extracellular matrix in the fetal membranes, contributing to processes leading toward preterm prelabor rupture of membranes.[11 12 14 16]

Modern obstetrics has not yet developed reliable methods to prevent or treat either of these conditions. Medical intervention in preeclampsia remains limited,[18] although prophylactic aspirin treatment might be of importance.[19 20] Interventions aimed at predicting and preventing spontaneous preterm delivery have also yielded limited success, although cervical length measurement and prophylactic progesterone treatment are promising.[21 22]

Growing evidence suggests that maternal diet influences pregnancy outcome, for example dietary pattern characterised by high intake of vegetables, fruits, and vegetable oils, which is associated with reduced risk of preeclampsia and preterm delivery.[23–26] Probiotics are defined as 'live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host'.[27] It has been shown that orally ingested probiotics have the potential to colonise the vagina[28] and normalise the bacterial flora in the lower genital tract.[29] Probiotics may have an anti-inflammatory effect on lipopolysaccharide inflammatory response in human placental trophoblast cells.[30 31] The anti-inflammatory effect of orally ingested probiotics has also been shown in vivo.[32 33] Two previous studies in the Norwegian Mother and Child Cohort Study (MoBa) showed associations between intake of milk containing probiotics during the first half of pregnancy and reduced risk of preeclampsia and spontaneous preterm delivery.[34 35] Since pregnancy is a time of rapid development and differentiation, the aim of this study was specifically to investigate whether there might be a certain time window before, during early or late pregnancy for a probiotic exposure effect on these two adverse pregnancy outcomes.

## METHODS

### Population and study design

MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health.[36 37] Participants were recruited from all over Norway in 1999–2008. The women consented to participation in 41% of the pregnancies. The cohort now includes 114 500 children, 95 200 mothers and 75 200 fathers. This study is based on version 6 of the quality-assured data files released for research in 2011. Informed consent was obtained from each MoBa participant on recruitment.

The women were asked to answer three questionnaires during pregnancy, at gestational week 15 (Questionnaire 1 (Q1)), 22 (Questionnaire 2 (Q2)) and 30 (Questionnaire 3 (Q3)). Q2 is a food frequency questionnaire (FFQ), whereas Q1 and Q3 are more general questionnaires covering health, exposures, lifestyle and other background factors. The records of the participating women are linked to the Medical Birth Registry of Norway (MBRN).[38] This study is based on data from MoBa Q1, Q3 and the MBRN, in contrast to our previous studies where the exposure data was from Q2.[34 35]

In MoBa, 98 725 women gave birth to live singleton babies. Of these 91 038 had answered both Q1 and Q3 and were eligible for inclusion in the study. After exclusion of women with chronic diseases or pregnancy complications (rheumatoid arthritis, chronic kidney disease, chronic hypertension, chronic heart disease, diabetes mellitus types 1 and 2 and gestational diabetes, immunosuppression, epilepsy, asthma, placenta previa, cerclage, serious malformation), 70 149 pregnancies were included in the study. (figure 1) Only a woman's first enrolled pregnancy in MoBa was included.

### Exposure

Q1[39] and Q3[40] contain questions about intake of two different milk products containing probiotic lactobacilli, in which consumption is defined in terms of 'cups/glasses per day', specified as 1 mug=2 cups, 0.5 litres=4 cups and 1.5 litres=12 cups. In Q1, the women were asked to report their consumption both prior to becoming pregnant and during pregnancy up until the time that the questionnaire was completed (on average around week 17), while Q3 (answered around week 30) asked about consumption from week 13 and until answering the questionnaire. The participant is defined as a probiotic consumer if she has written any number larger than zero, with no cut-off. The probiotic milk products were product A (Biola, all types, manufactured by Tine SA, Oslo.), containing *Lactobacillus acidophilus (LA-5), Bifidobacterium lactis (Bb12),* and *Lactobacillus rhamnosus GG (LGG);* and product B (Cultura, all types, manufactured by Tine SA, Oslo.), containing *Lactobacillus acidophilus (LA-5)* and *Bifidobacterium lactis (Bb12).* These were the only probiotic food items commonly available in Norwegian stores at the time of the study. The content of probiotic bacteria in these beverages is $10^8$ probiotic bacteria/mL according to the manufacturer. We did not include information regarding the use of probiotic supplements, as detailed assessment of dietary supplement use in MoBa[41] showed that less than 0.5% of the women reported use of supplements containing probiotic substances. The contribution of probiotics from dietary supplements was therefore considered negligible. Consumption prior to pregnancy reported in Q1 was defined as consumption before pregnancy, consumption reported during pregnancy in Q1 was defined as consumption during 'early pregnancy', while consumption reported in Q3 was defined as consumption during 'late pregnancy'.

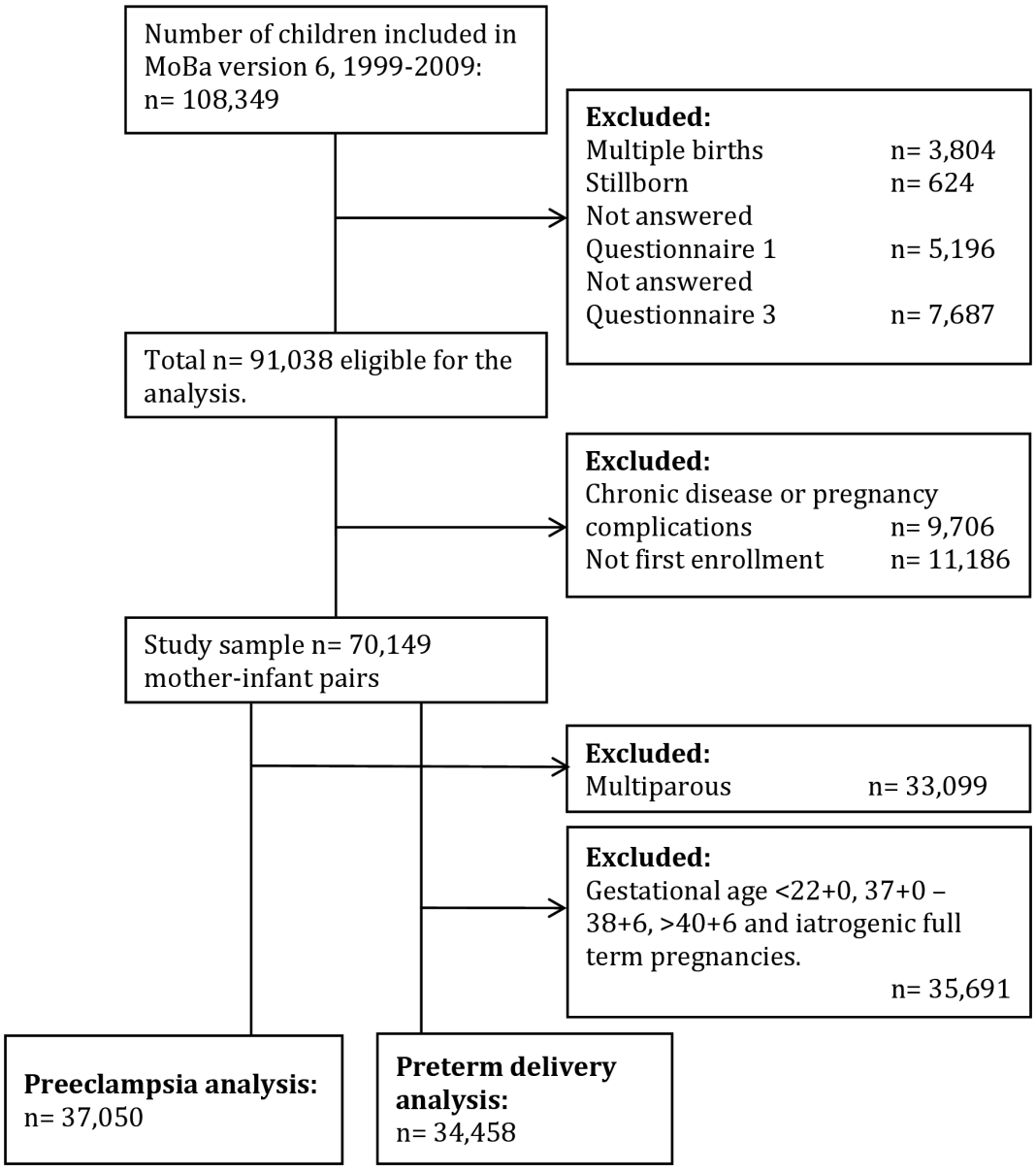



**Figure 1** Flow chart showing selection of study participants from the Norwegian Mother and Child cohort study (MoBa).

## Outcome

The main outcomes were preeclampsia and preterm delivery in the current pregnancy, as registered in the MBRN. MBRN data contain information from the pregnancy and birth records and are based on forms filled in by doctors or midwives after birth. There are five boxes that can be ticked in the form connected to preeclampsia: hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome; eclampsia; early onset preeclampsia (diagnosed before 34 weeks); mild preeclampsia and severe preeclampsia. In this study, preeclampsia was diagnosed if any of these boxes were ticked. All pregnant women in Norway receive free prenatal care, including blood pressure measurement and urine analysis for protein at each visit. According to the Norwegian Society of Obstetrics and Gynaecology, the diagnostic criteria for preeclampsia are systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks' gestation, combined with proteinuria ≥0.3 grams per 24 hours or total protein/creatinine ratio >0.3 (or ≥+1 on urine dipstick on at least two occasions). Severe preeclampsia is defined as blood pressure ≥160/110, clinical signs such as epigastric pain, headache or other cerebral symptoms, rapidly developing oedema, lung oedema, eclampsia, proteinuria ≥3 g/24 hours, oliguria <500 mL/24 hours, or HELLP.[42] Severe preeclampsia in this study includes even HELLP, early onset preeclampsia and eclampsia. The registered preeclampsia diagnoses in the MBRN have recently been validated.[43] As in our previous publication, only nulliparous women (n=37 050) were included in the preeclampsia analysis.[35]

Preterm delivery was defined as delivery between 22+0 and 36+6 weeks of gestation and spontaneous preterm delivery as delivery after either preterm labour or preterm prelabor rupture of membranes (PPROM). Gestational age was based on second-trimester ultrasound

in 97.9% of the pregnancies and on last menstrual period in the remaining. Early (22+0–33+6) and late (34+0–36+6) spontaneous preterm delivery subgroups were analysed separately. As in our previous study, the comparison group in the preterm delivery analysis consisted of spontaneous term pregnancies delivered at gestational week 39+0–40+6 (35), resulting in a total of 34 458 women included in the preterm delivery analysis.

### Covariates

The logistic regression models were adjusted for known risk factors for preeclampsia and preterm delivery. The maternal characteristics and lifestyle variables initially examined as potential confounding variables were as follows: maternal age, height and educational level; parity; history of late miscarriage (after gestational week 12); history of preterm delivery (only for multiparous women in the preterm delivery analysis); pre-pregnant body mass index (BMI); marital status; smoking and alcohol intake during current pregnancy; household income; fetal sex; in vitro fertilisation (IVF); intake of non-probiotic milk (ie, regular milk and sour milk, including kefir and yoghurt); and use of dietary supplements as a marker of health-conscious behaviour.[25] Dietary supplement use was divided into three categories: 'no supplement use', 'use of supplements without vitamin D' and 'use of supplements with vitamin D', since it has been shown that supplementary vitamin D is associated with reduced risk of preeclampsia in previous studies.[44] Self-reported pre-pregnancy weight and height were used to calculate BMI (weight (kg)/height ($m^2$)). The variables BMI, maternal height, and history of late miscarriage were used as continuous variables. Maternal age at delivery was used as a continuous variable, except in table 1, where it was divided into six categories (<19 years, 20–24 years, 25–29 years, 30–34 years, 35–39 years, >40 years). Smoking (categorised yes/no) was self-reported in Q1 and has been validated with plasma cotinine as the reference measure.[45] Parity (categorised in 0, 1, 2, and 3+para) was based on combined information from the MBRN and MoBa. Self-reported maternal educational level was divided into three categories (≤12 years, 13–16 years, ≥17 years.) Self-reported maternal and paternal annual incomes were divided into three categories (both have income <300 000 Norwegian crowns (NOK), one of them has income ≥300 000 NOK, both have income ≥300 000 NOK). Fetal sex was adjusted for due to findings, reported by Yeganegi *et al*, of a significant sex difference in inflammatory response to probiotics.[30] First-trimester smoking, dietary supplement use, alcohol consumption, non-probiotic milk/yoghurt consumption and IVF were registered as yes or no. History of preterm delivery was categorised into yes, no, and nulliparous. Maternal age at delivery, history of preterm delivery, IVF, fetal sex and history of late miscarriage (12–24 weeks) were based on MBRN information. Maternal intake of non-probiotic milk (ie, regular milk and sour milk, including kefir and yoghurt) and alcohol consumption were reported in Q1 and Q3. Dietary supplement use was based on Q1.

### Statistical methods

Intake of probiotic milk in relation to maternal characteristics was examined using Pearson´s $\chi^2$, while mean intake of probiotic milk according to maternal characteristics was examined using the Kruskal Wallis test. We estimated adjusted odds ratios (aOR) for the association between intake of probiotic milk (as categorised variables) and preeclampsia and preterm delivery using multiple logistic regression models with exposure at all three periods and covariates in the model. In categorical variables, missing data were given a category of their own. In a sensitivity analysis, missing values regarding food/beverage frequencies were classified as non consumers. The significance of exposure at each period as well as an overall significance of the null hypothesis of no impact of intake at any of the three periods was analysed. Preeclampsia and preterm delivery, including subtypes of both, were examined as separate outcome variables. Confounding variables were included in the final model if the covariate was associated with the exposure at P<0.05 (see table 1) or a priori (if there was a strong theoretical or clinical reason for keeping them in the model). The significance level was set at 5% (2 tailed) and all analyses were performed with SPSS version 24.

### Patient involvement

Patients were not involved in the design and conduct of this study.

## RESULTS

### Probiotic milk consumption in the study population

Consumption of probiotic milk in the whole study population (n=70 149) before pregnancy was reported by 6502 (23.3%) of the women, (mean 1.56 cups/day among consumers), during early pregnancy by 11 221 (37.6%) women, (mean 1.60 cups/day among consumers), and during late pregnancy by 12 784 (32.2%) women, (mean 1.51 cups/day among consumers). As illustrated in figure 2, a substantial part of the study population consumed probiotic milk during more than one time period.

Intake of probiotic milk according to maternal characteristics is presented in table 1. Intake of probiotic milk was more common in women who were older, primiparous, had BMI <25, did not smoke, used dietary supplements, consumed non-probiotic yoghurt, and had higher educational levels and family income. Alcohol intake during pregnancy was more common among probiotic consumers.

### Probiotic milk consumption and preeclampsia

Among the 37 050 nulliparous women included in the preeclampsia analysis, preeclampsia was diagnosed in 1851 (5.0%), including 550 cases of severe preeclampsia. Intake of probiotic milk during late pregnancy was significantly associated with a lower risk of preeclampsia (aOR: 0.80 (95% CI: 0.64 to 0.94)), while there was no significant

**Table 1** Intake of probiotics in relation to maternal characteristics in 70 149 pregnancies

| | Non consumers | Consumers* | | Mean intake of probiotics among probiotic consumers‡ | |
|---|---|---|---|---|---|
| | n (%) | n (%) | P value† | cups/day (SD) | P value§ |
| All ¶ | 29 770 | 17 493 | | 1.50 (1.1) | |
| Maternal age | | | | | |
| <19 y | 368 (1.2) | 93 (0.5) | <0.001 | 2.25 (2.0) | <0.001 |
| 20–24 y | 3445 (11.6) | 1530 (8.7) | | 1.66 (1.4) | |
| 25–29 y | 10 603 (35.6) | 6056 (34.6) | | 1.54 (1.2) | |
| 30–34 y | 11 013 (37.0) | 6730 (38.5) | | 1.50 (1.1) | |
| 35–39 y | 3900 (13.1) | 2711 (15.5) | | 1.49 (1.0) | |
| >40 y | 441 (1.5) | 373 (2.1) | | 1.53 (1.2) | |
| Parity | | | | | |
| 0 | 15 588 (52.4) | 10 713 (61.2) | < 0.001 | 1.53 (1.1) | 0.20 |
| 1 | 9231 (31.0) | 4458 (25.5) | | 1.53 (1.3) | |
| 2 | 4042 (13.6) | 1893 (10.8) | | 1.53 (1.0) | |
| ≥3 | 888 (3.0) | 417 (2.4) | | 1.59 (1.1) | |
| Missing data | 21 (0.1) | 12 (0.1) | | 1.67 (1.6) | |
| Previous spontaneous abortion > 12 – 21 (+ 6 d) wks | | | | | |
| No history | 24 270 (81.5) | 14 033 (80.2) | <0.001 | 1.53 (1.2) | 0.96 |
| Yes history | 702 (2.4) | 386 (2.2) | | 1.52 (1.0) | |
| Missing data | 4798 (16.1) | 3074 (17.6) | | 1.53 (1.1) | |
| History of preterm delivery | | | | | |
| No history | 13 179 (44.3) | 6286 (35.9) | <0.001 | 1.53 (1.3) | 0.21 |
| Nulliparous | 15 586 (52.4) | 10 712 (61.2) | | 1.53 (1.1) | |
| Yes history | 1005 (3.4) | 495 (2.8) | | 1.47 (0.8) | |
| BMI before pregnancy | | | | | |
| <18.5 kg/m² | 866 (2.9) | 535 (3.1) | <0.001 | 1.59 (1.1) | 0.30 |
| ≥18.5 to<25 kg/m² | 18 694 (62.8) | 12 233 (69.9) | | 1.52 (1.1) | |
| ≥25 to<30 kg/m² | 6515 (21.9) | 3227 (18.4) | | 1.56 (1.1) | |
| ≥30 to<35 kg/m² | 2135 (7.2) | 843 (4.8) | | 1.54 (1.1) | |
| ≥35 kg/m² | 832 (2.8) | 257 (1.5) | | 1.64 (1.6) | |
| Missing data | 728 (2.4) | 398 (2.3) | | 1.50 (1.0) | |
| Marital status | | | | | |
| Married/cohabiting | 28 650 (96.2) | 16 873 (96.5) | <0.001 | 1.52 (1.1) | <0.001 |
| Not married/cohabiting | 1120 (3.8) | 620 (3.5) | | 1.75 (1.2) | |
| Smoking during pregnancy | | | | | |
| No | 26 753 (89.9) | 16 214 (92.7) | <0.001 | 1.52 (1.1) | <0.001 |
| Yes | 2686 (9.0) | 1027 (5.9) | | 1.73 (1.4) | |
| Missing data | 331 (1.1) | 252 (1.4) | | 1.61 (1.0) | |
| Alcohol intake during pregnancy | | | | | |
| No | 24 907 (83.7) | 13 965 (79.8) | <0.001 | 1.53 (1.2) | 0.92 |
| Yes | 2694 (9.0) | 2031 (11.6) | | 1.47 (0.9) | |
| Missing data | 2169 (7.3) | 21 497 (8.6) | | 1.65 (1.2) | |
| Educational level | | | | | |
| ≤12 y | 9263 (31.1) | 4089 (23.4) | <0.001 | 1.73 (1.5) | <0.001 |

Continued

Table 1 Continued

| | Non consumers | Consumers* | | Mean intake of probiotics among probiotic consumers‡ | |
|---|---|---|---|---|---|
| | n (%) | n (%) | P value† | cups/day (SD) | P value§ |
| 13–16 y | 12 806 (43.0) | 7407 (42.3) | | 1.50 (1.0) | |
| ≥17 y | 7117 (23.9) | 5629 (32.2) | | 1.43 (0.9) | |
| Missing data | 584 (2.0) | 368 (2.1) | | 1.59 (1.7) | |
| Annual income for participant and partner | | | | | |
| Both have incomes<NOK 300 000 | 8892 (29.9) | 4181 (23.9) | <0.001 | 1.65 (1.4) | <0.001 |
| One has income≥NOK 300 000 | 12 248 (41.1) | 6870 (39.3) | | 1.52 (1.1) | |
| Both have incomes≥NOK 300 000 | 7855 (26.4) | 5993 (34.3) | | 1.46 (1.0) | |
| Missing data | 775 (2.6) | 449 (2.6) | | 1.57 (1.0) | |
| Dietary supplements | | | | | |
| Yes, without vitamin D | 1795 (6.0) | 1301 (7.4) | <0.001 | 1.55 (1.1) | 0.10 |
| Yes, with vitamin D | 2782 (9.3) | 2249 (12.9) | | 1.52 (1.1) | |
| No | 25 193 (84.6) | 13 943 (79.7) | | 1.53 (1.1) | |
| Non probiotic milk intake | | | | | |
| Yes | 23 585 (79.2) | 13 660 (78.1) | <0.001 | 1.52 (1.1) | 0.04 |
| No | 6158 (20.7) | 2229 (12.7) | | 1.55 (1.1) | |
| Missing data | 27 (0.1) | 1604 (9.2) | | 1.57 (1.3) | |
| Non probiotic yoghurt intake | | | | | |
| Yes | 7507 (25.2) | 5794 (33.1) | <0.001 | 1.60 (1.2) | <0.001 |
| No | 22 159 (74.4) | 6916 (39.5) | | 1.51 (1.1) | |
| Missing data | 104 (0.3) | 4783 (27.3) | | 1.47 (1.1) | |

*Consumer is defined as a woman with consumption at any time point (before, during early and/or late pregnancy).
†Pearson's $\chi^2$ asymptotic 2-sided test of intake frequencies in groups.
‡Probiotic intake during early pregnancy.
§Kruskal-Wallis test.
¶Missing data regarding probiotic consumption n(%): 22 886 (32.6)
NOK, Norwegian Krone.

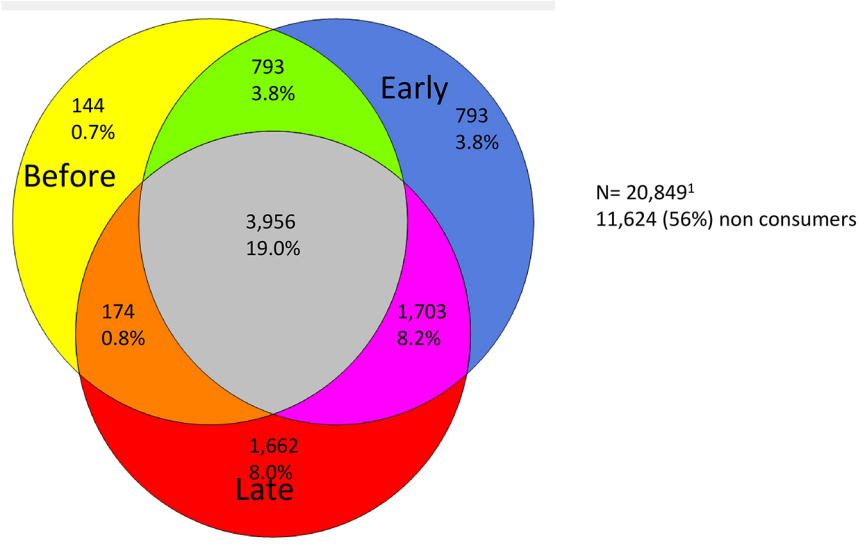
association with pre-pregnancy probiotic milk consumption and consumption during early pregnancy. (table 2)

When the subtypes mild and severe preeclampsia were examined separately, a statistically significant association was only found for probiotic milk consumption during late pregnancy and severe preeclampsia (aOR: 0.68 (0.50 to 0.92)). (table 2) We found no significant dose response effect when consumption was divided into 0 cups/day, one cup/day and ≥2 cups/day.

When the two products where analysed in the same logistic regression model, no statistical difference could be found: Product A: late pregnancy: aOR: 0.80 (0.67 to 0.97); Product B: late pregnancy: aOR: 0.80 (0.63 to 0.99). However, there was a substantial consumption overlap between the two products. Probiotic milk products A and B were the only probiotic products widely available in Norway until 2007, when other probiotic products were introduced on the Norwegian market. Sensitivity analyses excluding births after 2007 resulted in similar results as for the full sample (late pregnancy: aOR: 0.82 (0.68 to 0.98)). Sensitivity analysis, with missing values in variables regarding food/beverage frequency recoded as non consumers, resulted in similar results as when missing values were coded as a separate category: preeclampisa and probiotic consumption in late pregnancy: aOR: 0.79 (0.69 to 0.92).

### Probiotic consumption and preterm delivery

In the 34 458 women included in the preterm delivery analysis, preterm delivery occurred in 2858 cases, of which 1795 were spontaneous preterm deliveries and 1063 were iatrogenic preterm deliveries. There was a significant association between consumption of probiotic milk during early pregnancy and reduced risk of preterm delivery (aOR: 0.79 (0.64 to 0.97)), while there was no significant association with pre-pregnancy probiotic milk consumption and consumption during late pregnancy (table 3). In subgroup analysis for early (gestational weeks 22+0–33+6) and late preterm delivery (gestational weeks 34+0–36+6), a significant association was found for probiotic consumption during early pregnancy and lower risk of late preterm delivery (aOR: 0.73 (0.57 to 0.92)) (table 3).




1. Individuals with missing data at any of the three time points were excluded, n= 49,300.

**Figure 2** Venn-diagram illustrating the probiotic consumption pattern among the study population during the three time periods.

When preterm delivery was divided into spontaneous and iatrogenic preterm delivery, association was only found for probiotic consumption during early pregnancy and lower risk of spontaneous preterm delivery (aOR: 0.74 (0.57 to 0.96)) (table 3). Four hundred and thirty of the 1063 cases of iatrogenic preterm delivery had a preeclampsia diagnosis. We found similar results as for the whole group when the group of spontaneous preterm delivery was divided into early and late preterm delivery. (see online supplemental table 1)

We found no significant dose response effect when consumption was divided into 0 cups/day, one cup/day and ≥2 cups/day. No significant difference was found between the two products when analysed in the same logistic regression model (data not shown), however there was a substantial consumption overlap between the two products.

Sensitivity analyses excluding births after 2007 resulted in similar results as for the full sample (early pregnancy: aOR: 0.82 (0.68 to 0.98)). Sensitivity analysis with missing values in variables regarding food/beverage frequency recoded as non consumers, resulted in similar results as when missing values were coded as a separate category: preterm delivery and probiotic consumption in early pregnancy: aOR: 0.79 (0.66 to 0.93).

**Table 2** Intake of probiotics before and during pregnancy and risk of preeclampsia in nulliparous women, n=37 050

| | Cases (controls)* | Adjusted OR (CI)† | P value |
|---|---|---|---|
| Preeclampsia all | 1836 (34 941) | | |
| Overall | | – | 0.02 |
| Before | | 1.06 (0.83 to 1.35) | 0.65 |
| Early | | 0.90 (0.72 to 1.12) | 0.35 |
| Late | | 0.80 (0.68 to 0.94) | 0.007 |
| Preeclampsia mild | 1066 (34 941) | | |
| Overall | | – | 0.62 |
| Before | | 1.05 (0.77 to 1.43) | 0.77 |
| Early | | 1.0 (0.75 to 1.32) | 0.98 |
| Late | | 0.86 (0.70 to 1.06) | 0.15 |
| Preeclampsia severe | 545 (34 941) | | |
| Overall | | – | 0.02 |
| Before | | 1.00 (0.63 to 1.60) | 0.99 |
| Early | | 0.82 (0.54 to 1.25) | 0.36 |
| Late | | 0.68 (0.50 to 0.92) | 0.01 |

*The sum of mild and severe does not add up to all due to the fact that some were unspecified.
†Adjusted for maternal age, height and educational level; pre-pregnant body mass index (BMI); marital status; smoking, alcohol and intake of non-probiotic milk products during current pregnancy; family income; fetal sex; in vitro fertilisation (IVF) and intake of dietary supplements (with and without vitamin D).

## DISCUSSION

We investigated the association between timing (before, early and late pregnancy) of probiotic milk consumption and the risk of developing preeclampsia or preterm delivery. Our results showed that probiotic milk intake during late pregnancy (but not before or in early pregnancy) was associated with reduced risk of preeclampsia, and that intake during early pregnancy (but not before or in late pregnancy) was associated with reduced risk of preterm delivery. No dose response effect was found. In this study we were not able to separate the impact of the specific probiotic products. When analysing subgroups,

Table 3 Intake of probiotics before and during pregnancy and risk of preterm delivery in singleton pregnancies, n=34 458

| | Preterm delivery (full term) | Adjusted OR (CI)* | P value |
|---|---|---|---|
| Preterm delivery all | 2455 (26 910) | | |
| Overall | | – | 0.08 |
| Before | | 1.16 (0.92 to 1.45) | 0.21 |
| Early | | 0.79 (0.64 to 0.97) | 0.03 |
| Late | | 0.99 (0.85 to 1.14) | 0.85 |
| Preterm delivery early | 498 (26 910) | | |
| Overall | | – | 0.76 |
| Before | | 0.86 (0.55 to 1.37) | 0.52 |
| Early | | 1.07 (0.70 to 1.62) | 0.76 |
| Late | | 0.94 (0.69 to 1.28) | 0.69 |
| Preterm delivery late | 1957 (26 910) | | |
| Overall | | – | 0.05 |
| Before | | 1.25 (0.97 to 1.60) | 0.08 |
| Early | | 0.73 (0.57 to 0.92) | 0.008 |
| Late | | 0.98 (0.85 to 1.13) | 0.78 |
| Spontaneous Preterm delivery | 1536 (26 910) | | |
| Overall | | – | 0.03 |
| Before | | 1.23 (0.94 to 1.62) | 0.14 |
| Early | | 0.74 (0.57 to 0.96) | 0.02 |
| Late | | 0.97 (0.67 to 1.40) | 0.85 |
| Iatrogenic Preterm delivery | 919 (26 910) | | |
| Overall | | – | 0.76 |
| Before | | 1.02 (0.71 to 1.47) | 0.90 |
| Early | | 0.90 (0.64 to 1.25) | 0.52 |
| Late | | 1.06 (0.85 to 1.33) | 0.60 |

*Adjusted for maternal age, height and educational level; parity; history of late miscarriage; history of preterm delivery (for multiparous only); pre-pregnant body mass index (BMI); marital status; smoking, alcohol, and intake of non-probiotic milk products during current pregnancy; family income; fetal sex; in vitro fertilisation (IVF) and intake of dietary supplements (with and without vitamin D).

we found a stronger association between probiotic milk intake and reduced risk of spontaneous and late preterm delivery.

We were surprised to find a lack of association between probiotic intake and iatrogenic preterm delivery, since an important reason for selecting pregnancies for induction in preterm pregnancy is severe preeclampsia and intrauterine growth restriction, which can be a cause of placental dysfunction in preeclampsia. A substantial part (430 (40%)) of cases with iatrogenic preterm delivery had in fact a preeclampsia diagnosis. However, looking at the number of cases of iatrogenic preterm delivery included in the regression (n=919), a lack of power could explain the lack of association.

The biological hypothesis behind this work was that probiotics might have an effect on the inflammatory cascade following a possible infection, leading to preterm delivery.[13 15] Therefore, we would have expected to find a stronger association with early preterm delivery since infection is thought to be the major cause in early preterm delivery.[46 47] The none-significant finding could however be due to lack of power since there were only 498 cases of early preterm delivery. Further, it must be recognised that the preterm parturition syndrome has different aetiologies and the potential role of probiotic bacteria in preventing preterm delivery could therefore not be expected to be the same for all cases.[13]

The two previous MoBa studies indicating a protective effect of probiotic milk on preeclampsia and spontaneous preterm delivery were based on the FFQ answered in mid pregnancy.[34 35] As specific environmental factors such as infection (eg, congenital rubella syndrome after infection during the first trimester[48]) or medication (eg, specific birth defects after thalidomid medication in certain pregnancy weeks[49]) might influence pregnancy outcome during a certain time period, with no effect during other periods of pregnancy, it was pertinent to follow-up the results to investigate a potential importance of timing. Investigating whether probiotics have their effect already during placentation or later during pregnancy, for example after onset of preeclampsia symptoms, might also be a helpful guide when investigating the mechanisms behind these adverse outcomes. Previous publications in the MoBa cohort and other cohorts demonstrate that women tend to adopt a more health-conscious diet when they become pregnant, that is, reduced intake of coffee, alcohol and sweet beverages, and increased intake of fruit and vegetables.[50–53] The same pattern was seen for probiotics consumption, which increased during early pregnancy but decreased slightly during late pregnancy, but still remained higher than before pregnancy. This additionally highlights the need to study consumption at different time-points.

There is evidence that a wider maternal inflammatory response plays a role in the pathogenesis of preeclampsia.[4] Steinborn *et al* showed that preeclampsia and preterm delivery are characterised by changes in the composition of regulatory T cell, decreasing their suppressive activity.[54] Increased inflammatory response seems to play a greater role especially in severe preeclampsia,[8–10] which might explain our finding of a significant association only between probiotic intake and severe preeclampsia. These results are also in alignment with our previous study.[34] Our biological hypothesis included both a local probiotics-mediated effect and an effect on the systemic

inflammatory response. In vitro studies have shown that probiotics (*Lactobacillus rhamnosus GR-1* and *LGG*) may have an anti-inflammatory effect on LPS inflammatory response in human placental trophoblast cells.[30 31] It has been shown that daily prophylactic intake of probiotics ($0.5\times10^8$ *Bifidobacterium longum*, $0.5\times10^7$ *Lactobacillus bulgaricus*, and $0.5\times10^7$ *Streptococcus thermophilus*) could reduce the deviated T-helper cell T-helper 1 (Th1)/T-helper 2 (Th2) response induced by severe traumatic brain injury resulting in a lower rate of nosocomial infections.[55] These results are very interesting since it is known that during pregnancy, the mother's immune system changes from Th1-dominated cellular immunity to Th2-dominated humoral immunity in order to accept the fetal 'semi-allograft'.[56 57] In a randomised, double blind, placebo-controlled trial it was shown that maternal probiotics supplementation ($10^9$ *Bifidobacterium lactis* alone or in combination with $10^9$ *Lactobacillus rhamnosus* GG) significantly modulated the expression of toll-like receptor (TLR)-related genes both in the placenta and in the gut of babies delivered by elective caesarean section at term. These findings suggest a link between the maternal gut and that of the developing fetus and that microbial contact at the feto-placental interface may be considered a physiological phenomenon.[58] The characterisation of the unique microbiome of the placenta, which resembles the oral more than the vaginal microbiome, is another interesting finding in this regard.[59] Further, periodontal disease and the oral microbiome have been shown to be linked to preterm birth and preeclampsia.[60–63]

Probiotics have the potential to impact the pathophysiological processes involved in hypertension, inflammation, renal function and diabetes.[64] Various microorganisms have been found to possess such properties, although *Lactobacillus* and *Bifidobacterium* are the most common probiotic food adjuvants. It has been shown that probiotics ($1.68\times10^{10}$ *L. rhamnosus* CFUs per ml, $3.2\times10^{10}$ *L. casei*, and $5.2\times10^{10}$ *L. acidophilus*) modulate human gene expression in the gut lining, acting similarly to drugs that target high blood pressure.[32] Clinical intervention trials have shown a reduction of blood pressure in non-pregnant individuals with probiotics intake (two strains of *Streptococcus thermophilus* (CFU $10\times10^7$) and two strains of *Lactobacillus acidophilus* (CFU $2\times10^7$) in one of the products and one strain of *Enterococcus faecium* (human species) (CFU $6\times10^7$) and two strains of *Streptococcus thermophilus* (CFU $1\times10^9$) in the other product).[65 66] It could thus be hypothesised that probiotics might reduce the risk of preeclampsia by modulating blood pressure. This might explain our finding of a stronger association between probiotics consumption and preeclampsia, when probiotics are consumed during late pregnancy.

## Strengths and limitations

The main strength is the large sample size and the link to the MBRN. MoBa is a pregnancy cohort with participants from both urban and rural regions, of different ages and socioeconomic groups and with a wide range of probiotics intake frequencies. The participation rate is 40.6%. Self-selection bias has been investigated, showing that single women under the age of 25 are underrepresented in MoBa. However, differences regarding preterm delivery incidence were minor and no differences in preeclampsia incidence were found. No bias was found in eight selected exposure-outcome associations.[67] The questionnaires were filled in before delivery, avoiding confounding by retrospective responses. It is, however, difficult to obtain a true picture of dietary intake from a questionnaire. Questions about food answered in early pregnancy are especially challenging since many women experience changes in appetite. Another strength in the current study is that we have information about 'typical' consumption both before pregnancy and during early and late pregnancy, in contrast to a randomised controlled trial setting where the consumption is strictly defined by the study protocol. Probiotics are freely available and becoming more and more common as adjuvants on the food market. As we have shown, women continue to consume probiotics during pregnancy, emphasising the importance to investigate possible pregnancy-effects of the 'typical' consumption at any timing before or during pregnancy. Furthermore, we had access to information about lifestyle, allowing adjustment for potential health-conscious behaviour. However, although we adjusted for a number of confounding variables, including education and income which are the most important markers of socioeconomic position in Norway,[68 69] unmeasured confounding cannot be ruled out.

Another strength is that the women reported whether their supplement contained vitamin D or not, which allowed us to adjust for intake of vitamin D, since studies have shown that vitamin D may be related to a lower risk for preeclampsia.[44] None of the two probiotic milk products contained any added vitamin D during 1999–2008, ruling out that associations with the probiotic products could in fact be due to vitamin D.

Until 2007, the two Norwegian-produced probiotic milk products assessed in the MoBa questionnaires were the only probiotic food products commonly available on the Norwegian market, which is why we performed a sensitivity analysis excluding births after 2007. This did however not change the results. Still, women recruited to the study during years 2005–2008 may have consumed probiotic products other than the two assessed in these questionnaires. It is possible that some probiotic consumers have been classified as non-consumers in case they consumed probiotics from other sources. Another source of misclassification is that sour milk was included in"non-probiotic milk'. This misclassification of the exposure would most likely contribute to attenuation of a potential association with the outcomes studied.

Previous studies on probiotics have evaluated the health effect of different products containing various probiotic strains. Our study setting did not allow to evaluate certain strains of probiotics who may have an effect on these two adverse pregnancy outcomes, or at which daily

dose and dose regime. The main difference between the two probiotic products in this study was the presence of *LGG*. However, due to a substantial consumption overlap, we were not able to separate the impact of the specific probiotic products in this study. These results should however only imply the need for further studies, especially since there are earlier studies, showing the potential anti-inflammatory effect of *Lactobacillus rhamnosus* both in vitro and in vivo.[30 31 33 70] Yang *et al* have indeed shown promising results where supernatant (bacteriocin) of *Lactobacillus rhamnosus GR-1* attenuated the LPS-induced inflammation and preterm birth in the mouse model.[70] In a recent prospective randomised trial in women with PPROM, vaginal probiotics ($1\times10^8$ *Lactobacillus rhamnosus* and *L. gasseri*) as an adjunct to antibiotic prophylaxis were shown to prolong the latency period until delivery and improved the perinatal outcome.[71] The probiotic consumers in our study consumed a mean dose of around 1.5 cups per day. This is equivalent to a consumption of at least 188 mL per day, and a probiotic dose of $1.9\times10^{10}$ (*Bb12*, *LA-5* and *LGG* in product A and *Bb12* and *LA-5* in product B) according to the manufacturer. Strain viability during the product shelf life and its survival and pharmacokinetics in the gastrointestinal tract are important aspects that could not be analysed within the limits of our study. However, probiotic bacteria in commercial milk products have been shown to exhibit a high survival rate in the whole gastrointestinal tract.[72 73]

## CONCLUSION

Probiotics might have a protective effect against adverse pregnancy outcomes and timing of probiotic intake might be relevant. Intake of probiotic milk during late pregnancy was associated with decreased risk of preeclampsia, and intake during early pregnancy was associated with decreased risk of preterm delivery. However, this observational study cannot establish causality and the pathophysiological effect of probiotics on the maternal inflammatory response must be studied in more detail, including strain-/species-specific randomised controlled trials. If future randomised controlled studies support a protective effect of probiotics consumption on reduced risk of preeclampsia and preterm delivery, recommending probiotics consumption would be a promising public health measure to prevent these adverse pregnancy outcomes.

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**Competing interests** None declared.

**Ethics approval** The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Committee for Ethics in Medical Research in South-Eastern Norway (S-95113 and S-97045) and the Norwegian Data Inspectorate. This specific project was approved by The Regional Committee for Medical and Health Research Ethics South East (REK/S-06075a (2010/2683)). All MoBa participants provided written informed consent before enrolment into the study.

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