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Folic acid supplementation in early pregnancy and the risk of preeclampsia, small for gestational age offspring and preterm delivery

Marit P. MARTINUSSEN, MD, PhD^{1,2}, Michael B. BRACKEN, PhD, MPH³, Elizabeth W. TRICHE, PhD⁴, Geir W. JACOBSEN, MD, PhD⁵, and Kari R. RISNES, MD, PhD^{5,6}

¹Department of Obstetrics and Gynecology, St. Olav's University Hospital, Trondheim, Norway.

²Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

³Yale Center for Perinatal, Pediatric and Environmental Epidemiology, Yale University, New Haven, CT, USA.

⁴ Mandell Center for Multiple Sclerosis and Neuroscience Research, Mount Sinai Rehabilitation Hospital, St. Francis Care, Hartford, CT, USA.

⁵Department of Public Health and General Practice, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

⁶Department of Pediatrics St. Olav's University Hospital, Trondheim Norway.

Abstract

Objective—To assess whether folic acid intake during the first trimester of pregnancy is related to pregnancy outcomes preeclampsia, low birth weight or preterm birth.

Study design—Prospective cohort study of 3,647 women who were followed from the first trimester of pregnancy. Detailed information on quantity of folic acid intake before and during the first three months of pregnancy was recorded. Pregnancy outcome data were abstracted from obstetric records.

Results—Lean mothers who used folic acid supplementation the month before pregnancy had a 40% reduced risk of developing preeclampsia. The adjusted odds ratio (OR) with 95% confidence intervals for (95% CI) preeclampsia in lean mothers (BMI<25) who used folic acid supplements the month before pregnancy was 0.6 (95% CI 0.4-1.0). Obese mothers who used folic acid supplementation in the first trimester had an increased, but not statistically significant risk for

Corresponding Author and request for Reprints: Marit P. Martinussen, Professor, NTNU, Faculty of Medicine, LBK P.O. Box 8905, Medisinsk teknisk forskningssenter NO 7491 Trondheim, Norway. Phone: 004772573806 Phone: 004792247089, marit.martinussen@ntnu.no.

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preterm birth (adjusted OR 1.9 with 95% CI 0.9-4.0). There were no significant associations between folic acid supplementation and low birth weight.

Conclusion—Our study supports a possible protective effect of folate intake in early pregnancy on preeclampsia in lean mothers. There was no support for any beneficial effect of folic acid use on preterm birth or low birth weight, and we found no evidence of any harmful effects of folate use for the outcomes included in our study.

Keywords

folic acid; preeclampsia; low birth weight; preterm labor

INTRODUCTION

Fortification of nutrients with folic acid and folic acid supplementation to women of childbearing age is among the greatest modern advances in the care of pregnant women and their offspring. This implementation has been extremely important in preventing congenital birth defects, especially neural tube defects (NTD).¹ Effects of folic acid supplementation on other adverse pregnancy outcomes have been less well-studied. Low birth weight, whether due to preterm birth or growth restriction, has a negative impact on later health and development in later life.^{2,3} Preeclampsia is a potentially life-threatening condition in pregnancy that has both short- and long-term consequences for mother and baby. Questions remain if deficiencies of micronutrients during pregnancy, including folic acid, may increase risk of preeclampsia, low birth weight, or preterm birth.

Folate is essential for the synthesis of nucleic acids, amino acids, cell division, tissue growth, and DNA synthesis.⁴ Requirements during pregnancy are markedly increased to cover the needs of placental and fetal growth and development. Folate deficiency may impair cellular growth of the placenta. Poor placental perfusion is associated with defective trophoblastic invasion and widespread endothelial dysfunction, as seen in preeclampsia. Following folate deficiency, circulating homocysteine may accumulate,⁵ which may result in endothelial dysfunction.⁶

In a cohort of 3,647 US women with prospective information about folic acid supplementation in the periconceptual period and the first trimester, we assessed the association of folic acid intake and the risk of preeclampsia, low birth weight birth and preterm delivery.

MATERIALS AND METHODS

Study population

The present analyses combine data from two prospective pregnancy cohort studies conducted in New England, USA. Pregnant women were recruited from September 1996 to January 2000 from 56 obstetric practices and 15 clinics associated with 6 hospitals in Connecticut and Massachusetts. Women were eligible if the pregnancy was before 24 weeks' gestational age at enrolment, did not have insulin-dependent diabetes mellitus, spoke English or Spanish, and did not intend to terminate their pregnancy. Of 11,267 women

screened, 9,576 met eligibility criteria. The eligible population was further screened to participate in either the Asthma in Pregnancy Study (AIP) ^{7,8} or the Nutrition in Pregnancy Study (NIP). ⁹

For the AIP study after excluding refusals (n=531), pregnancies that had gone beyond 24 weeks gestational age at home interview (n=389), miscarriages (n=73), and non-participation for other reasons (n=41), 2,379 women were enrolled. After delivery, 171 women were excluded because of multiple births, abortions, stillbirth, or lack of information, leaving 2,208 eligible women who delivered a singleton baby.

For the NIP study a total of 3,631 women were invited of whom 2,478 were enrolled, 639 declined, 424 were lost to follow-up, 72 miscarried prior to enrollment, and 20 were not eligible at enrollment interview. Among the enrolled women, 2,288 delivered a singleton infant.

Data from the two studies were combined for the current analyses. We further excluded participants where complete obstetric information was missing (n=849), leaving 3,647 participants (1,710 from the AIP study and 1,937 from the NIP study).

Data collection in pregnancy

Pregnant women were interviewed, usually at home, before 24 weeks of gestational age. A standardized questionnaire included information on demographic and household characteristics including marital status, family income, health risk factors, medical conditions, and obstetric history. The two study questionnaires were identical for the background information, but differed for the different research focus of each study (asthma and coffee intake, respectively). Information on the use of vitamins and supplements were the same for both studies and could be used for the present analyses.

Information on folic acid, iron and vitamin use was obtained before 24 weeks of gestation from the following questions in the prenatal exposure questionnaire. "Have you used any of the following vitamin or mineral supplements: prenatal vitamins, multivitamin, Vitamin A, Vitamin C, Vitamin E, Iron/Ferrous Sulfate, Folic Acid/Folate Calcium, or Other; specify." If a respondent answered yes, she was specifically asked how often each item had been used (not at all, about once a month, 2-3 times a month, once a week, twice a week, 3-4 times a week, 5-6 times a week, once a day, or two or more times a day). This information was collected by month, from the month before through the third month of pregnancy.

Folic acid exposure

We collected information on folic acid content (micrograms (mcg)) in each of the different vitamin supplements. Using the detailed frequency information from the pregnancy questionnaire, we calculated each respondent's mean daily intake of folic acid supplements for each month from the month before through the third month of pregnancy. Mean folic acid intake in first trimester was defined as the average intake over these four months. For each month and for the first trimester overall, daily folic acid intake was divided into a dichotomous variable of use (no use: <200mcg daily and use: >200mcg average use). To test

for a dose-response relationship, daily intake was also divided into three categories (<200 mcg, 200<600 mcg, >=600mcg).

Outcome information

Pregnancy outcome data, which included prenatal, labor and delivery information, and information about the newborn, were abstracted from obstetric records associated with the delivery hospitalization onto a structured form of pregnancy outcome. Data abstractors were blind to exposure status. Preterm birth was defined as a birth before 37 completed gestational weeks, based on information from the obstetric records. Preference was given to sonography estimates of gestational age (61.2% of pregnancies) and based on the first day of the last menstrual period when this was unavailable.

We defined low birth weight as small for gestational age (SGA), and included infants who had a birth weight less than the 10th percentile for gestational age. As reference we used an external standard of birth weight for gestational age, adjusted for gender and ethnicity that we developed from all singleton births in the United States in 1999, as appropriate for the two cohorts.¹⁰ Birth weights were obtained within 24 hours of delivery.

We defined preeclampsia according to the National Heart, Lung and Blood Institute (NHLBI) guidelines (Working group) Report on high blood pressure in pregnancy.¹¹ Blood pressure and urinary protein readings from the prenatal period through the delivery hospitalization were abstracted from the medical records. A woman was classified as having preeclampsia if she met *both* of the following criteria: 1) de novo hypertension (≥140 mm Hg systolic or ≥90 mm Hg diastolic on two or more occasions at least 6 h apart beginning after the 20th week of gestation; and 2) accompanying proteinuria, defined as urinary protein concentrations of 30 mg/dl or greater (equivalent to a dipstick value of 1+ from two or more specimens collected at least 4 h apart, or one or more urinary dipstick values of 2+ near the end of pregnancy, or one or more catheterized dipstick values of 1+ during delivery hospitalization, or 24-h urine collection with protein of ≥300 mg).

We excluded women for whom pre-existing hypertension could not be ruled out (e.g., no readings available prior to 20 weeks' gestation or physician notes indicating a patient with chronic hypertension) or who met partial criteria for preeclampsia (e.g., pregnancy-induced hypertension or proteinuria with no hypertension).

Study ethics

The Human Investigation Committee of Yale University Medical School (New Haven, Connecticut) approved the study and all respondents provided informed consent prior to participation.

STATISTICAL METHODS

Statistical analyses assessed the association between folic acid supplementation and adverse pregnancy outcomes (preeclampsia, SGA and preterm birth). Information on potential confounding variables was collected from the structured home interview at enrollment.

Group differences in folic acid supplementation according to covariate information were identified by use of the Kruskal-Wallis rank test.

We assessed the effect of folic acid supplementation on adverse outcomes, using logistic regression. We adjusted the analyses for factors known to be associated with health behavior (specifically, vitamin and supplement use) and pregnancy complications; i.e. maternal age, ethnicity, education, marital status, smoking in pregnancy, previous miscarriages, and stillbirths. We adjusted for original study (AIP or NIP). Additional adjustment for use of other vitamins (vitamin C, D and E), and iron and calcium use in the first trimester did not significantly alter the results. Effect estimates are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Effects of folic acid supplementation on each outcome were examined using logistic regression analyses. Folic acid exposure was considered in separate models as: 1) ordinal scale supplementation variable (<200 mcg, 200<600 mcg, >=600mcg), for each month beginning with the month before pregnancy through the 3rd month of pregnancy; and 2) dichotomous supplementation with average daily use across the first trimester of <200 mcg v. ≥200 mcg. In supplementary analyses we stratified according to maternal pre-pregnancy body mass index (BMI) less than and equal to or more than 25 kg/m². We also performed the analyses stratified by maternal smoking status (no smoking versus ever smoked during pregnancy). Statistical analyses were performed with STATA/SE10 (College Station, TX, USA).

RESULTS

Table 1 shows the characteristics of the study population by daily intake of folic acid the month before pregnancy and in the first trimester. Folic acid supplementation was clearly associated with maternal socioeconomic status and health; intake increased with maternal age and was higher among married than single and divorced mothers. Folic acid intake was also higher among white compared with African American, Hispanic, and Asian women. Women with more than 16 years of education had higher intake, while it was lower among those who smoked throughout pregnancy. Women with asthma had a lower intake of folic acid in the month before pregnancy than women without. Moreover, women who had given birth three or more times used less supplementation than primiparae. A history of stillbirth or miscarriages was associated with higher folic supplementation whereas previous pregnancy complications were not related to intake. A pre-pregnancy body mass index equal to or higher than 25 (overweight and obesity) was associated with less folic acid intake in the month before conception.

Table 2 shows associations between use vs. no use of folic acid supplementation one month before conception and throughout first trimester of pregnancy with the risk of preeclampsia, SGA, or preterm delivery. There were no significant associations between use of folic acid supplementation and any of the study outcomes. A supplementary analysis (Supplemental table 1) shows categories of average daily intake by month of early pregnancy. There was no evidence of a dose-response relationship between intake and any of the outcomes.

As shown in Table 3, lean mothers (BMI<25) who used folic acid supplementation the month before pregnancy had a 40% reduced risk of developing preeclampsia after adjusting

for confounders ($OR_{adj}=0.6$, 95% CI: 0.4-1.0). Supplementation in the first trimester was not significantly associated with preeclampsia. Neither pre-pregnancy nor first trimester intake was associated with an SGA birth in any of the BMI groups. Obese mothers who used folic acid supplementation in the first trimester had an increased, but not statistically significant risk for preterm birth ($OR_{adj}=1.9$, 95% CI: 0.9-4.0). Folic acid use the first month before pregnancy was not associated with risk of preterm delivery in any of the BMI groups.

DISCUSSION

In this prospective cohort study, regular supplementary folic acid intake during the month before and during the first trimester of pregnancy was not associated with risk of preeclampsia, SGA offspring, or preterm delivery. However, a forty per cent reduced preeclampsia risk was observed in lean women who used regular folic acid supplements the month before pregnancy. Others have reported that pre-pregnancy overweight modified the effect of multivitamin supplementation since lean users had a reduction in preeclampsia compared with non-users.¹²

The prospective design, where pregnant women were interviewed before 24 weeks gestation, is strength of our study and makes information selection bias unlikely. Using detailed information about folic acid supplementation from our frequency questionnaire, the mean daily intake could be calculated. The design also enabled prospective collection of detailed information on numerous potentially important confounders, including smoking and pre-pregnancy BMI. Since maternal smoking is related to vitamin status of women and to low birth weight of the newborn,¹³ smoking is a potentially important confounder in the relation between vitamin status and pregnancy outcome. Adjustment for smoking did not affect our estimates.

It is a limitation that we had no information about folate intake from nutrients, nor did we have any information about maternal serum folate levels. There is a question whether reported intake corresponds to individual folate levels. Plasma folate levels at 18 weeks did not reflect self-reported supplementation before week 8.¹⁴ However, when reported intake and concentration measures were assessed within a short time interval, a strong and consistent relationship was found.¹⁵ This divergence does not necessarily represent inferior recall since folate in serum reflects recent dietary intake, whereas red blood cell (RBC) folate is considered to more accurately to reflect intake over time.¹⁵

Our main finding that lean women who used folic acid supplements the month before pregnancy had a reduced risk of preeclampsia corresponds with other findings of reduced preeclampsia risk in multivitamin users. Like the current study, use of multivitamins containing folic acid or folic acid before conception decreased the risk of preeclampsia.^{16,17} However, when maternal serum folate or RBC folate were measured, no association between serum folate levels and hypertensive pregnancy disorders was found.¹⁸

Folate may improve placental and systemic endothelial function. It is hypothesized that the most critical time window to improve placental function is late first or early second trimester. Supplementation with multivitamins containing folic acid in the second trimester

resulted in higher serum folate and lower plasma homocysteine levels and was associated with reduced risk of preeclampsia.¹⁹ In an ongoing randomized placebo-controlled trial; The Folic Acid Clinical Trial Study,²⁰ 4000 micrograms folic acid is administered to pregnant women at increased risk of developing preeclampsia, i.e. as a result of potential genetic and metabolic factors,. That order of magnitude is much greater than the highest intakes we observed. Hyperhomocysteinemia is considered a risk for diseases mediated via the placenta, such as preeclampsia,^{21,22,23} pregnancy loss,²¹ and placental abruption and low birth weight.^{23,24} Normally, in healthy pregnant women there is a physiologically induced reduction in tHcy concentration²⁵ which is greater than the decrease from hemodilution and unrelated to the reduction in serum albumin concentrations. Importantly, folic acid supplementation enhances the physiologic reduction in tHcy concentrations.²⁶

Women whose plasma tHcy was in the top decile in early pregnancy were at increased risk of preeclampsia.²¹ However, in the second trimester, folate supplementation reduced both serum homocysteine levels and the preeclampsia risk.²⁷ Furthermore, maternal tHcy levels at delivery were significantly higher in the severe than the mild preeclampsia and control groups.²²

This observation could be explained by reverse causality where preeclampsia potentially increases tHcy levels. Surprisingly, serum homocysteine levels were not associated with a deficiency of folic acid or Vitamin B12, suggesting that these two are not the only factors involved in the occurrence of the high homocysteine levels in preeclampsia.²⁷

Preterm birth and low birth weight

Observational studies suggest that low folate intake or low circulating folate increases the risk for preterm delivery and low birth weight.²⁸ It is reported that RBC folate in the second trimester predicts growth restriction.²⁹ We did not find any association between folic acid supplement intake and preterm delivery or an SGA birth. Smoking is an important confounder since it is related to poor vitamin status, high tHcy and low birth weight. Newborns of smoking mothers, had folate levels significantly lower than infants of non-smoking women.³⁰ Mothers who smoked and did not use folic acid supplements before and in the first trimester had the highest risk of delivering a low birth weight infant.³¹ At birth, plasma folate levels of mother and child were associated with birth weight if the mother smoked.³² Others report that after adjustment for smoking, dietary folate or plasma folate during the second trimester were no risk factors for birth weight.³³ A transfer of folate from the maternal to fetal circulation takes place and the folate concentration in cord blood, erythrocytes and plasma of newborns is 2-3 times higher than in erythrocytes and plasma folate of their mothers.³⁴ Continued supplementation of folic acid after the first trimester resulted in higher cord blood folate status.²⁶ Therefore, it may be speculated whether some pregnant women are prone to deficiency of folic acid, possibly by genetic mechanisms.

We found an increased risk of preterm birth associated with regular first trimester folic acid use in overweight or obese women. A possible explanation for this observation is selection bias due to high-risk women being supplemented with folate. Others have reported that folic acid supplementation could be associated with increased risk of preterm birth.³⁵

Importantly, the results of randomized intervention trials have been equivocal.³⁶ The Cochrane Collaboration concluded in 2013 that the evidence provided so far, did not indicate any benefit of folic acid supplementation during pregnancy on stillbirth/neonatal death or preterm birth, although an increase in birth weight of 140 grams was observed in a meta-analysis representing nearly 18 000 women.³⁷ The majority of studies were heterogeneous as regards the time periods of supplementation studied. Moreover, the respective study populations were quite different, as was folic acid intake including dietary folate and several other biomarkers. When studying the effect of folic acid supplementation, several premises have to be considered. First, it cannot be assumed that the relationship between folate intake and serum red blood cell folate is linear. The MTHFR 677TT genotype affects intracellular folate and is associated with increased tHcy. The genotype occurs with a frequency of 5-10% in the USA. A gene-nutrient interaction has been confirmed and individuals with the TT genotype and low plasma folate concentrations or low folate intake had higher tHcy concentrations.³⁸ However, at high plasma folate concentrations or high folate intake, the difference in tHcy concentrations between the genotypes was small.

Our study supports a possible protective effect of folic acid intake the month before conception on preeclampsia risk in mothers who are not overweight or obese. We found no strong evidence of any harmful effects of folic acid use. In order to establish more conclusive evidence of the role of supplementation on our study outcome, randomized intervention trials that include measures of folate status and tHcy before and during pregnancy in both mother and in the newborn are needed. Later follow up of mothers and their children is necessary to identify possible long-term effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Folic acid supplementation during first trimester of pregnancy by characteristics of study population (n=3,647), AIP and NIP studies, Connecticut, USA.

Characteristics	<i>n</i>	<i>Proportion(%)</i>	<i>Folic acid Intake per day, month before pregnancy Mean (mcg) (SD)</i>	<i>Folic acid Intake per day in first trimester Mean(mcg) (SD)</i>
All Participants	3647	100	293(385)	472(306)
Study				
AIP	1,710	46.9	266(352)	461(300)
NIP	1,937	53.1	317(411)	481(309)
Maternal age (years) (n=3645)				
<20	424	11.6	242(397)	415(321)
20=<25	897	24.6	196(349)	382(307)
25=<30	1,117	30.6	338(402)	505(304)
30=<35	906	24.9	330(375)	510(280)
=>35	302	8.3	373(378) *	579(283) *
Pregnancy Complications (n=3647)				
Preterm birth				
No	3404	93.3	296	472
Yes	243	6.7	279	460
Preeclampsia				
No	3519	96.5	294	472
Yes	128	4.5	279	482
Small for Gestational Age				
No	3378	92.6	293	473
Yes	269	7.4	291	453
Maternal marital status (n=3645)				
Married	2,575	70.6	353(394)	528(292)
Single	924	25.4	149(324)	337(297)
Divorced	149	4.0	138(295) *	345(277) *
Maternal ethnicity (=3641)				
White	2,505	68.8	195(379)	529(291)
African American	290	8.0	172(334)	388(308)
Hispanic	700	19.2	302(399)	319(288)
Asian	67	1.8	328(381)	454(324)
Other	79	2.2	405(371) *	316(278) *
Maternal education (n=3643)				
<12 years	851		179(349)	256(375)
12 years	1,213	33.3	302(400)	474(305)
13-16years	1,205	33.1	328(382)	521(290)

Characteristics	<i>n</i>	<i>Proportion(%)</i>	<i>Folic acid Intake per day, month before pregnancy Mean (mcg) (SD)</i>	<i>Folic acid Intake per day in first trimester Mean(mcg) (SD)</i>
>16years	375	10.3	405(371) *	568(281) *
Smoking in pregnancy (3644)				
Never smoked	2,258	61.9	313(392)	482(309)
Quit before pregnancy	790	21.7	318(387)	497(311)
1st trimester only	357	9.8	171(309)	390(266)
Smoked throughout pregnancy	239	6.6	196(366) *	411(277) *
Mothers diagnosed with asthma (n=1708. Only AIP)				
Yes	1,052	61.6	229(333)	439(288)
No	656	38.4	289(361) *	475(307)
Parity				
0	1,514	41.5	319(394)	488(305)
1	1,347	37.0	293(390)	481(306)
2	561	15.4	255(360)	426(303)
≥3	222	6.1	207(342) *	417(291) *
Previous stillbirths or miscarriages (3647)				
Yes	866	76.2	321(405)	499(322)
No	2,781	23.8	284(379) *	463(299) *
Previous pregnancy complications (n=2,116)				
Yes	685	32.4	284(387)	452(293)
No	1431	67.6	253(359)	463(311)
Body mass index (kg/m²)				
<25	2,431	68.3	310(390)	480(307)
≥25	1,128	31.7	264(372) *	460(296)

* P<0.05 (Kuskal Wallis rank test)

Table 2

Association between use or no use of Folic Acid Supplementation One Month Before Conception and Throughout First Trimester of Pregnancy, and the Pregnancy being Complicated by Preeclampsia SGA (small for gestational age) or Preterm birth (<37 weeks gestation). Results are presented as Odds Ratios (OR) with 95% Confidence Intervals (95% CIs)

Folic Acid Use	n	Preeclampsia				SGA				Preterm			
		Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Month before Conception													
No Use	2,057	Referent		Referent									
Use	1,590	0.9	0.6-1.3	0.8	0.6-1.2	0.9	0.7-1.2	1.1	0.8-1.4	0.7	0.5-0.9	0.9	0.6-1.1
First trimester overall													
No use	346	Referent		Referent									
Use	3,301	1.1	0.6-2.1	1.1	0.6-2.1	1.2	0.8-1.8	1.3	0.8-2.1	1.0	0.7-1.4	1.3	0.9-1.8

^aAdjusted for: study (AIP or NIP), maternal age, maternal ethnicity, maternal education, maternal marital status, parity, maternal smoking in pregnancy and any miscarriages or stillbirths in previous pregnancies

Table 3

Association between use or no use of Folic Acid Supplementation One Month Before Conception and Throughout First Trimester of Pregnancy, and the Pregnancy being Complicated by Preeclampsia SGA (small for gestational age) or Preterm birth (<37 weeks gestation). Results are presented as Odds Ratios (OR) with 95% Confidence Intervals (95% CIs) Analyses are Stratified by Mothers Body Mass Index (BMI).

	Preeclampsia				SGA				Preterm			
	BMI<25 (n=2,420)		BMI>=25 (n=1,128)		BMI<25		BMI>=25		BMI<25		BMI>=25	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Folic Acid Use (n=3,559 with available BMI)												
Month before Conception												
No use	Referent		Referent		Referent		Referent		Referent		Referent	
Use	0.6	0.4-1.0	1.0	0.5-2.0	1.2	0.9-1.6	1.0	0.8-1.8	0.8	0.6-1.2	0.9	0.5-1.5
First trimester overall												
No use	Referent		Referent		Referent		Referent		Referent		Referent	
Use	1.0	0.5-2.0	1.4	0.5-3.5	1.0	0.7-1.5	1.0	0.6-1.6	1.1	0.7-1.7	1.9	0.9-4.0

* Adjusted for: study (AIP or NIP), maternal age, maternal ethnicity, maternal education, maternal marital status, maternal smoking in pregnancy and any miscarriages or stillbirths in previous pregnancies