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Original Article

Folic Acid Supplementation and the Risk of Small or Large for Gestational Age Births: A Cohort-Based Study from Semnan, Iran

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ABSTRACT

Background: Folic acid supplementation (FAS) has been shown to prevent neural tube abnormalities. However, the association between maternal FAS and fetal development outcomes beyond the twelfth week of pregnancy is still unclear. This cohort study aimed to explore the effects of maternal FA intake prior to and during pregnancy on the incidence of small for gestational age (SGA), large for gestational age (LGA), low birth weight (LBW), and high birth weight (HBW).

Methods: A total of 505 pregnant women who visited Semnan health centers between 2018 and 2021 participated in this prospective Semnan birth cohort study. Data regarding FAS intake were recorded for different periods. Univariate comparisons employed t-tests, one-way analysis of variance (ANOVA), and chi-square tests. To control for confounders, multivariable logistic regression and general linear models (ANCOVA) were used, adjusting for maternal age, BMI, education, employment, and residence. Adjusted odds ratios and mean differences with 95% CIs were calculated.

Results: We found that ongoing FAS (400 µg/day) from 1 month before conception and throughout all three trimesters significantly increased the risk of HBW (adjusted OR = 2.95, 95% CI: 1.02–8.52) without affecting the incidence of SGA, LBW, or LGA.

Conclusion: To verify these results, it is recommended to conduct further studies or large-scale trials along with monitoring folate levels during the entire pregnancy.

Keywords: Birth cohort, Birth weight, Folic acid, Infant

Introduction

Folic acid (FA), the synthetic form of folate, is an essential micronutrient required for DNA synthesis, amino acid formation, and vitamin metabolism. Various health complications in mothers and their offspring have been linked to folate deficiency. It has long been recognized that the risk of neural tube defects (NTDs) in fetuses is diminished by supplementation with FA during the periconceptional period (1). The American College of Obstetrics and Gynecology (ACOG) strongly advises women who intend to conceive and have no known history of any spinal or neural defect in their family to take FA at 0.4 to 0.8 mg/day. The ACOG and most other institutions

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recommend 4 mg/day of FA consumption for women with a history of having had a child with NTDs (2).

Besides its protective role against NTDs, recent research has also focused on the overall effect of maternal FA consumption on pregnancy outcomes, notably fetal growth and birth weight (3). It is well acknowledged that abnormal birth weights, i.e., small for gestational age (SGA) or large for gestational age (LGA), may exert both immediate and long-term impacts on offspring health (4). However, there is inconsistent data in the literature on the impact of FA intake on newborn birth weights (5, 6). The Generation R study demonstrated that periconceptional FAS can enhance birth weight and reduce the risk of SGA (5). Conversely, a meta-analysis that pooled data from the re-analysis of the Aberdeen folate supplementation trial concluded that, while maternal FAS does not affect mean birth weight, supplemental FA at higher dosages can decrease the risk of low birth weight (LBW) in infants (7). Moreover, a large-scale Chinese prospective cohort study indicated that periconceptional FAS was associated with a reduced risk of LGA births, but not SGA births (8).

It has been well documented that FAS prior to and during the first trimester mostly prevents neural tube defects. However, the benefits of ongoing FAS after the twelfth week of pregnancy remain unclear. Accordingly, the aim of the present research was to investigate the effects of ongoing FAS following the first trimester on the incidence of high birth weight (HBW), LBW, SGA, and LGA in pregnant women in the Semnan birth cohort.

Methods

This study was conducted as part of the Iranian Birth Cohort Study (Prospective Epidemiological Research Studies in Iran). This study is ongoing in five regions of Iran (Isfahan, Yazd, Semnan, Sari, and Rafsanjan) and is designed to generate evidence-based data for national health policy-making on the evolutionary origins of health and disease (9). This cohort study examines potential associations between lifestyle, diet, exposure to environmental factors, socioeconomic status, and maternal and child mental and physical health. For the present analysis, we included pregnant women living in Semnan, a city in central Iran. Eligible participants were those who visited Semnan health centers between 2018 and 2021 (10). Data were collected using comprehensive cohort questionnaires that recorded information on maternal characteristics (age, education level, and pre-pregnancy BMI), social factors (employment status and residence), gestational age, and patterns of FAS.

Women of Iranian nationality whose precise information on FAS intake prior to and during pregnancy was available in the Semnan Birth Cohort data, which is a subset of the Persian Birth Cohort, met the inclusion criteria. Patients with diabetes mellitus, obesity, sulfonamide prescriptions, gastrointestinal absorption issues (e.g., ulcerative colitis, Crohn's disease, chronic diarrhea, etc.), FA antagonists, herbal remedies, medicinal plants, and nonsteroidal anti-inflammatory drugs that decrease FA absorption from the gastrointestinal tract were excluded.

Among the 1023 mothers participating in the Semnan Mother and Child Cohort Study, 3 mothers with pre-existing diabetes were excluded. Data from another 27 mothers were also excluded from the analysis due to miscarriage, 11 mothers due to infant death, and 95 mothers due to missing birth weight data. Among the remaining 887 live births, 357 were excluded due to missing data on FAS. In addition, 25 participants who reported FA intake only in the first and third trimesters or the second and third trimesters were excluded due to small sample sizes in these groups. Finally, the ultimate analysis was performed on data from 505 mothers (Figure 1).

Every participant completed an informed consent form after being given an explanation of the study's protocol. Every technique was used in compliance with the Declaration of Helsinki's guidelines. The study procedure was approved by the Research Ethics Committee of Semnan University of Medical Sciences and Health Services (Ethics code: IR.SEMUMS.REC.1401.319).

Gestational age at delivery was measured in completed weeks from the first day of the last menstrual period. Ultrasound scans were performed to confirm all dates of the last menstrual cycle reported by participants during hospital antenatal care. In this project, birth weight less than 2500 g was defined as LBW, while birth weight of 4000 g or more was defined as macrosomia or HBW based on a global reference for fetal weight and birth weight percentiles published in 2011. LGA birth was defined as birth weight above the 90th percentile for a given gestational age (standard). SGA birth was defined as birth weight below the 10th percentile of the standard. Birth weights between the 10th and 90th percentiles for gestational age were considered acceptable (11).

Participants were divided into four groups (Figure 1) based on the duration of their FAS (400 µg/day). Group 1 included 179 women who only received FAS throughout the first three months of pregnancy. Group 2 included 172 women who received FA supplementation beginning one month prior to conception and continuing through the first trimester of pregnancy. Group 3 included 70 mothers who were given FA supplementation throughout both the first and second trimesters of pregnancy. Group 4 included 84 mothers who started FAS one month before conception and sustained it throughout the entire length of pregnancy.

Quantitative variables were described as mean ± standard deviation, and qualitative variables as frequencies (counts and percentages). For univariate analyses, the independent samples t-test was used to compare continuous variables between two FAS conditions, and ANOVA was applied for comparisons across the four FAS groups. The chi-square test was employed to examine associations between FAS groups and categorical birth outcomes (SGA, LGA, LBW, HBW). To control for potential confounding, multivariable logistic regression models were fitted for the binary outcomes (SGA, LGA, LBW, HBW), and general linear models (ANCOVA) were used for continuous outcomes (birth weight, length, head circumference). All adjusted models included maternal age and pre-pregnancy BMI (continuous), educational level (academic vs. non-academic), employment status (employed vs. unemployed), and residence (urban vs. rural) as covariates. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were derived from logistic regression models; adjusted mean differences with 95% CI were obtained from general linear models. SPSS (SPSS Inc., version 22) was used to perform statistical analyses. All statistical tests were two-sided, and a p-value < 0.05 was regarded as statistically significant.

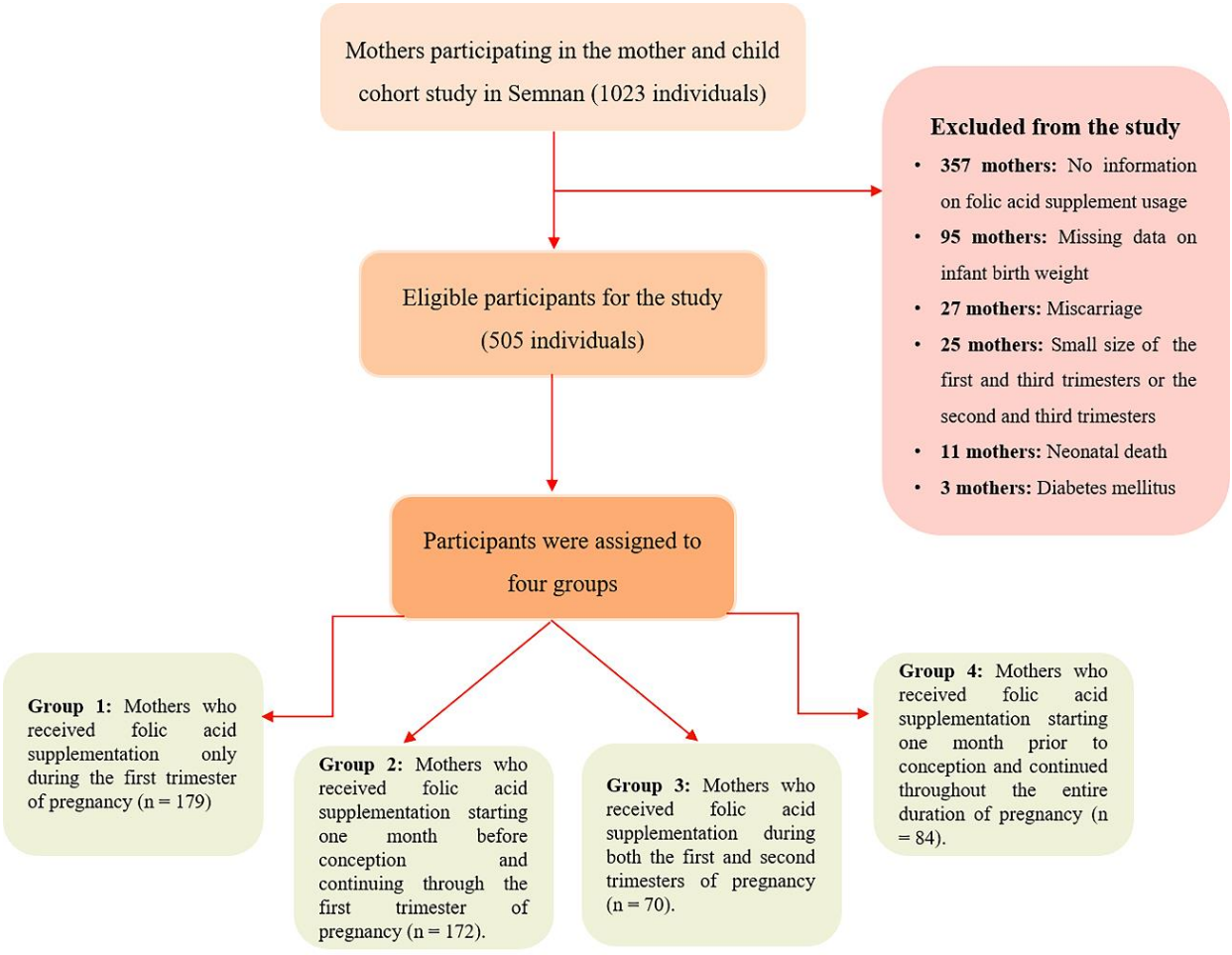

Figure 1. Chart for the selection of participants

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki criteria, and all methods involving research study participants were approved by the Ethics Committee of Semnan University of Medical Sciences (Ethics code: IR.SEMUMS.REC.1401.319). All participants provided written informed consent.

Results

There were no substantial differences among the four groups in terms of mean maternal age, pre-pregnancy BMI, employment status, or area of residence. However, group 2 had a much higher proportion of mothers with an academic education than the other groups (p = 0.03, Table 1).

Based on the patterns of FAS, the mean gestational age at delivery showed no significant differences. For example, the mean gestational age at delivery ranged from 38.08 ± 2.19 weeks in group 3 to 38.57 ± 1.71 weeks in group 1, demonstrating no statistically significant differences (p = 0.131, Table 2). Similarly, individual analysis using the t-test and Mann–Whitney U test for every dietary pattern confirmed that there were no notable connections between the timing of supplementation and the length of gestation (all p > 0.05, Table 2).

The overall occurrence of SGA and LGA was 14.1% and 10.3%, respectively, and the occurrence of LBW (birth weight less than 2500 g) and HBW (birth weight of 4000 g or more) was 9.3% and 3.0%. Unadjusted comparisons of birth outcomes across the four FAS groups are shown in Table 3. Adjusted effect sizes (odds ratios for binary outcomes and mean differences for continuous outcomes) with 95% confidence intervals are summarized in the adjusted associations subsection below. Statistically significant differences were not noted between FAS patterns and the occurrence of SGA or LGA birth frequencies (SGA: 12.8–20.0%, p = 0.409; LGA: 8.1–15.5%, p = 0.314; Table 3). Of particular note, the proportion of HBW infants was significantly greater in group 4 (7.1%; p = 0.047). However, no significant relationship was found between FAS status and the incidence of LBW, SGA, LGA, or mean neonatal birth weight, length, and head circumference among the four groups. In addition, no significant associations were found for other anthropometric measurements of the newborns.

Adjusted multivariable logistic regression for binary outcomes and ANCOVA for continuous outcomes showed no significant associations between FAS patterns and SGA, LGA, LBW, or continuous anthropometric outcomes. All adjusted ORs were non-significant (CIs crossed 1.00) and did not change the univariate interpretation (Table 3); therefore, adjusted data are not shown. Continuous use from preconception through all trimesters was linked to higher HBW odds compared to first-trimester-only use (aOR = 2.95; 95% CI 1.02–8.52), but this borderline result was based on few cases (n = 6; group 4). Adjusted mean differences in birth weight, length, and head circumference were non-significant and consistent with univariate results (Table 3).

Table 1. Maternal baseline characteristics by FAS group. Values are mean ± SD or percentages. P-values are derived from one-way ANOVA for continuous variables and Pearson's chi-square test for categorical variables.

Characteristic	Group 1 (n=179)	Group 2 (n=172)	Group 3 (n=70)	Group 4 (n=84)	P-value*
Age (years, mean ± SD)	29.48 ± 5.33	29.33 ± 4.85	29.53 ± 5.30	30.36 ± 5.03	0.491
Pre-pregnancy BMI (kg/m^2)	25.42 ± 4.79	24.47 ± 4.02	25.33 ± 6.15	25.80 ± 5.10	0.138
Academic education (%)	24.4	35.5	19.1	32.1	0.030
Employed (%)	10.8	17.2	10.3	7.1	0.093
Urban residence (%)	31.3	26.2	20.0	26.2	0.323

Group 1: Mothers took FA supplements in the 1st trimester (n=179); Group 2: Mothers took FA supplements in 1 month before and in the 1st trimester (n=172); Group 3: Mothers took FA supplements in the 1st and 2nd trimesters (n=70); Group 4: Mothers took FA supplements in 1 month before and in all three trimesters (n=84); * One-Way ANOVA or Pearson Chi-Square Tests.

Table 2. FA supplement status and their association with gestational week at labor. For each pattern, the mean gestational age (weeks) is shown for women who followed ('Yes') and did not follow ('No') that pattern. P-values are from Mann-Whitney U tests.

Supplementation Pattern	Yes (mean ± SD, weeks)	No (mean ± SD, weeks)	P-value*
1st trimester	38.57 ± 1.71	38.39 ± 1.90	0.291
1 month before & 1st trimester	38.45 ± 1.92	38.46 ± 1.80	0.900
1st and 2nd trimesters	38.08 ± 2.19	38.52 ± 1.77	0.131
1 month before, 1st-3rd trimesters	38.52 ± 1.58	38.44 ± 1.89	0.908

* Mann-Whitney Test

Table 3. Characteristics of 505 newborns according to FAS use. Birth weight, length, and head circumference are presented as mean ± SD; categorical outcomes are frequencies (%). P-values are from one-way ANOVA (continuous) or chi-square/Kruskal-Wallis tests (categorical).

Outcome	Group 1 (n=179)	Group 2 (n=172)	Group 3 (n=70)	Group 4 (n=84)	P-value*
Birth weight (g, mean ± SD)	3164 ± 501	3124 ± 473	2981 ± 588	3148 ± 533	0.130
Birth length (cm, mean ± SD)	50.77 ± 3.07	50.33 ± 3.08	50.18 ± 3.30	50.79 ± 2.79	0.262
Head circumference (cm, mean ± SD)	34.91 ± 1.47	34.61 ± 1.59	34.52 ± 1.90	34.99 ± 1.74	0.270
SGA (%)	12.8	12.2	20.0	15.5	0.409
LGA (%)	10.6	8.1	8.6	15.5	0.314
LBW (%)	7.3	8.1	15.7	10.7	0.190
HBW (%)	3.4	1.2	1.4	7.1	0.047

Group 1: SGA: small for gestational age; LGA: large for gestational age; LBW: low birth weight (<2500 g); HBW: high birth weight (≥4000 g). Mothers took FA supplements in the 1st trimester (n=179); Group 2: Mothers took FA supplements in 1 month before and in the 1st trimester (n=172); Group 3: Mothers took FA supplements in the 1st and 2nd trimesters (n=70); Group 4: Mothers took FA supplements in 1 month before and in all three trimesters (n=84). LBW births were live-born infants that were <2500 g for birth weight; HBW births were live-born infants that were ≥4000 g for birth weight; *Chi-Square Tests Asymptotic or Exact Significance or Kruskal-Wallis Test.

Discussion

In the present research, group 2 had a higher proportion of mothers with academic education (p = 0.03), likely due to random variation. To manage potential confounding, maternal education was included as a covariate in all multivariable regression models. The adjusted estimates were materially unchanged from the unadjusted results. According to compelling evidence, FAS intake prior to and during the first trimester significantly decreases the risk of NTDs in children. At present, there are conflicting opinions regarding the necessity of maintaining high FA intake levels throughout the entire pregnancy (12).

Our study found that continuing FAS after the twelfth week of pregnancy did not significantly lower the risk of SGA (Table 3: SGA frequencies ranged from 12.8% to 20.0%, p = 0.409; adjusted analyses confirmed non-significance). Research by Wang et al. (2016) and Chen et al. (2015) similarly reported that a higher level of maternal folate concentrations in late pregnancy did not effectively reduce the risk of SGA in a multinational Asian population (12, 13), suggesting that the benefit of FA may only be evident in cases where maternal folate levels are generally low (14). Our finding is not entirely aligned with the results of the 2020 SCOPE international prospective cohort study (conducted on 5606 pregnant women), which indicated that taking FA prior to conception was associated with a significantly lower risk of SGA. However, this association was not statistically significant at 15 weeks' gestation (4). Conversely, a birth cohort analysis performed by Guo and colleagues demonstrated that taking FA was associated with a lower risk of SGA. The findings highlighted that the reduced risk was primarily observed for SGA at 37 weeks or more of gestation. Differences in the formulation of FAS, the initial period and duration of FAS, and various populations may explain these conflicting results (15).

Our study demonstrated that ongoing FA intake after the twelfth week of pregnancy resulted in a higher percentage of LGA; however, this increase was not statistically significant (Table 3: LGA ranged from 8.1% to 15.5%, p = 0.314). Wang et al. (2016) highlighted that ongoing FA intake of 400 µg/day during the second and third trimesters significantly increased the risk of LGA offspring, and this outcome may be influenced by differences in sample size. The number of pregnant mothers included in the statistical analysis of the Chinese cohort study was 2644 (12), whereas our study had a smaller sample size of 505. One potential explanation for the increase in the likelihood of LGA is that nutrients are more crucial during the first trimester for the growth and differentiation of different organs, while later stages of pregnancy are more focused on overall fetal development (16). Further research conducted by Zhang and colleagues recommended that prolonged intake of FAS beyond the first trimester might elevate the likelihood of LGA birth among women with less education and multiparous mothers (17). Consistent with our findings, the SCOPE prospective cohort study conducted in Ireland, Australia, New Zealand, and the United Kingdom did not discover a notable relationship between maternal FA intake during pregnancy and LGA birth. The applicability of their findings to other research may be restricted because they only included samples of primiparous women (18).

Data analysis further indicated that maintaining FAS at a dosage of 400 µg/day into the second and third trimesters (group 4) significantly increased the risk of HBW in offspring (Table 3; adjusted OR = 2.95, 95% CI: 1.02–8.52). Based on only six cases in group 4, this finding has a wide confidence interval and borderline significance, making it preliminary and

in need of confirmation. Currently, there is limited information to confirm that FAS for three months or longer during pregnancy enhances the chances of macrosomia. The findings of this study (adjusted OR = 2.95, 95% CI: 1.02–8.52; Table 3) were contradictory to the outcomes reported by Wang et al. (2016) and Cheng et al. (2019), who did not identify any notable relationship between FA intake for three months or more and HBW (12, 19). A comprehensive review and meta-analysis indicated that increased FA consumption in the first trimester was linked to HBW, with a two-fold increase in FA consumption resulting in a 2% increase in birth weight (20). One possible reason is that FA, as a methyl donor, promotes fetal weight gain by modulating liver gene expression, IGF signaling pathways, and skeletal muscle in Pietrain gilts (21). Multivariable analysis showed that being overweight or obese before pregnancy, having male infants, maternal age of 35 or older, multiparity, and lower educational attainment are all associated with an increased risk of macrosomia (22).

In the current analysis, the incidence of LBW was not significantly different among the four groups of our sample (Table 3: LBW ranged from 7.3% to 15.7%, p = 0.190). The non-significant effects of ongoing FAS following the first trimester on the risk of LBW may be due to the smaller number of women in groups 3 and 4. A previous randomized controlled study consisting of 119 women revealed that ongoing FAS at a daily dosage of 400 μg following the first trimester of pregnancy had no impact on the weight of the newborn. The comparatively small sample size and, consequently, possibly inadequate statistical power to detect a small effect on birth weight could account for this finding (23). Additional research, however, indicated that a 400-μg daily consumption of FA by the mother significantly lowered the likelihood of the infant being born with LBW (24).

Limitations

Using data from a well-established national birth cohort enhances the credibility of our findings, and strict exclusion criteria promote internal validity by preventing confounding issues that can interfere with folate absorption. Also, a thorough examination of the varied range of FA consumption patterns provides results that can be applied more broadly, unlike most previous studies that concentrate only on pre-pregnancy and the first trimester. However, despite the strengths of our research, we acknowledge that it had several limitations. First, this study was conducted in a single center, which limits the ability to apply the findings to different regions. Second, no information on diet was gathered, preventing us from assessing the effects of dietary folate consumption. Third, our sample size was somewhat limited. Fourth, variations in maternal dietary folate intake, certain maternal health conditions (e.g., thyroid disorders and toxoplasmosis), maternal physical activity, detailed dietary intake, and oral hygiene were not assessed, which may have influenced birth outcomes and limited the generalizability of the findings.

Conclusion

In this prospective cohort of expectant mothers, we found that ongoing FAS throughout the second and third trimesters significantly increased the incidence of HBW, without significant effects on the outcomes of LGA, SGA, and LBW. Further research involving a large number of participants and continuous monitoring of folate levels during pregnancy is necessary to corroborate these results.

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None.

Conflicts of interest

The authors declare no conflicts of interest.

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