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Association of spontaneous abortion with dietary folate intake in individuals with different genotypes of FTO gene

Golsa Khalatbari Mohseni[1], Mahsa Shapouri[2], Sheyda Nami[3], Zahra Mousavi[4], Niloufar Pourmalek Lahiji[5], Zahra Mahmoudi[6], Mohadeseh Sadat Mousavi Hoseini[7], Zahra Saeedirad[8], Ali Shamsi-Goushki[9], Soheila Shekari[10], Bojlul Bahar[11], Barbod Alhouei[12], Saeid Doaei[13,14*], Mitra Rezaei[15*] and Maryam Gholamalizadeh[16]

Abstract

Background Research has revealed a possible connection between dietary folate intake and the risk of spontaneous abortion (SA). Interestingly, the FTO gene may play a dual role, influencing both folate needs and SA susceptibility. Therefore, this research sought to investigate the interaction between FTO genotypes, dietary folate intake, and the potential risk of SA.

Methods This case-control study was conducted on 539 adult women, including 192 women with a history of SA and 347 women without a history of abortion. To evaluate FTO gene genotypes for the presence of rs9939609 polymorphism, 5 ml of blood was collected from all participants. A validated semi-quantitative food frequency questionnaire (FFQ) was used to assess the dietary folate intake. Binomial logistic regression was used to investigate the relationship between folate intake and SA in carriers of different FTO genotypes.

Results A negative association was found between dietary folate intake and SA, especially in females with the AA genotype of FTO rs9939609 polymorphism (OR = 0.97, 95% CI 0.95–0.99, $P = 0.04$). The statistical significance of this link persisted even after adjusting for age, body mass index (BMI), drinking alcohol, smoking, and dietary intake.

Conclusions This study indicates that dietary folate intake may protect against SA, particularly in women who have the FTO rs9939609 polymorphism. However, more investigation is required to confirm these findings and elucidate the underlying mechanisms linking SA, the FTO gene, and folate intake.

Keywords Dietary folate intake, Genotypes of FTO gene, Pregnancy, Spontaneous abortion

*Correspondence:
Saeid Doaei
sdoaee@yahoo.com; sdoaei@sbmu.ac.ir
Mitra Rezaei
m.rezaie@sbmu.ac.ir

Full list of author information is available at the end of the article

Quick points

- There was an inverse association between dietary intake of folate and abortion.
- Intake of dietary folate was found to be inversely associated with abortion among carriers of the AA genotype of the FTO rs9939609 polymorphism.

Introduction

Spontaneous abortion (SA), a common health problem, refers to the premature termination of a pregnancy wherein the embryo or fetus cannot survive. It is estimated that roughly 10–20% of clinically recognized pregnancies end in miscarriage by the end of the 12th week [1]. A significant proportion of pregnant women (estimated at 15–25%) could face SA leading to illness and suffering in both the pregnant woman and her family [2]. The World Health Organization (WHO) states that approximately 44 million SA happen every year on a global scale [3]. In developing nations, roughly 358,000 mothers lose their lives worldwide every year resulting from complications of SA [4]. The estimated number of SA in Asia in 2003 and 2008 was 25.9 million and 27.3 million, respectively [5]. Notably, the SA rate was estimated as 5.34 per 100 live births in Iran [6].

The primary causes of SA remain unclear. A number of factors such as hereditary factors increase the chance of developing SA (e.g. embryo chromosome disorders), uterine pathologies and abnormalities in the endometrium, autoimmune diseases, endocrine dysfunctions, and infections caused by bacteria or viruses [7]. Additionally, maternal variables such as the mother's nutritional status may have an impact on the risk of SA [8].

According to reports, a significant quantity of folate is necessary for healthy embryonic development [9]. The primary foods high in folate are beans, whole grains, fruits, liver, and green leafy vegetables [10] and substantial evidence supports that dietary folate intake of pregnant women has a direct impact on pregnancy outcomes. Several studies have pointed out that the risk of SA could potentially be higher when there is a suboptimal folate status [11]. Moreover, it has been noted that folate supplementation protects against neural tube abnormalities [12].

A person's genetic makeup might also influence the level of nutrients which is required to prevent against SA. Certain *FTO* gene polymorphisms, such as rs9939609, have been linked to dietary requirement of folate and altered folate-related metalites (e.g. homocysteine) levels in specific populations. For example, in multiple sclerosis (MS) patients, carriers of the AA genotype for rs9939609 showed elevated homocysteine levels compared to controls, suggesting a direct metabolic effect on how much folate is required to maintain the homocysteine levels in a normal range [13]. This *FTO*'s role in regulating methylation processes, higher methylation levels in *FTO* risk allele carriers (e.g., AA genotype carriers) may correlate with exacerbating obesity-related metabolic disturbances [14].

The FTO gene has been associated with body folate requirement on the one hand and affects the risk of SA on the other hand. There hasn't been any research done to date on the association of the FTO gene polymorphism, folate intake, and SA risk. Motivated by the potential interaction between the FTO gene and dietary folate in influencing SA risk, this study explored the relationship between dietary intake of folate and SA in individuals with different FTO genotypes.

Methods

Study population

This case-control study was initially carried out on 600 Iranian young women, including 200 women with abortion and 400 women without a history of SA in Tehran, Iran. Participants were randomly selected from among women who were referred to Shohadaye Tajrish Hospital for routine medical examination. At the start of the study, the purpose and methodology were clarified to each participant, and a written consent form was obtained. The inclusion criteria for the case group included a history of at least one placental loss before the 20th week of pregnancy, diagnosed within recent 3 months and being between the ages of 20 and 40. The inclusion criteria for the control group were no history of SA and age between 20 and 40 years. Those who were taking folic acid supplements ($n=27$), did not wish to continue participating in the study ($n=21$), or were unable to provide the necessary information ($n=13$), were excluded from the study. Finally, the statistical analysis was performed on 539 adult women in Tehran, Iran, including 192 women who have previously had SA and 347 women without a history of SA.

Data related to age, education level, history of reproductive system diseases, diabetes, hypertension, abortion, pregnancy, smoking, and drinking alcohol were collected using a general questionnaire and through face-to-face interviews. Data on pathological and biochemical factors including right systolic blood pressure (SBP), right diastolic blood pressure (DBP), white blood cell count (WBC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (platelet), lymphocyte count (lymphocyte), monocyte count, blood urea nitrogen (BUN), creatinine, triglycerides (TG), total cholesterol, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP), high-density

lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDL) were extracted from patients' medical records. A 0.5 cm accurate caliper was used to measure the participant's height, and a 0.5 kg accurate digital scale was used to assess their weight.

Examination of FTO gene genotypes

Five ml of blood was collected from all participants to evaluate the genotypes of the FTO gene in terms of the presence of rs9939609 polymorphism. Then, blood cells were separated by centrifugation and DNA was extracted using a standard kit. The extracted DNA samples were amplified using the PCR method and a master mix DNA polymerase (cat. No. A180301; Ampliqon, Denmark). The PCR products were then analyzed to identify the rs9939609 polymorphism using the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method.

Dietary intake of folate

The Food Frequency Questionnaire (FFQ), which was previously validated in Iran [15], was utilized to collect the relevant diet-related data. The FFQ was used to assess data on the intake of different food groups including whole and refined grain, fruits, vegetables, fats, simple sugars, salty snacks, dairy products, and meats over the period of a year. Portion sizes were adjusted to grams after accounting for household measurements. The amount of calories and nutrients was then evaluated using the US Department of Agriculture's food composition table (FCT) (USDA, Release 11, 1994, modified for Iranian cuisine). For local items that were not available in the FCT, the Iranian FCT was taken into account. Total dietary intake of different types of folate was assessed using the Nutritionist IV version 4.1 software (First Databank Division, The Hearst Corporation, San Bruno, CA, USA).

Statistical analysis

The independent t-test and the chi-square test were employed to compare quantitative and qualitative variables, respectively. If the quantitative data distribution was not normal based on the Kolmogorov-Smirnov test, the Mann-Whitney U-test method was used to analyze the data. The binomial logistic regression method was used to investigate the association between dietary intake of folate and SA. Additionally, to examine the impact of FTO gene genotypes on the association between SA and folate intake, regression analyses were conducted separately for individuals with AA, AT and TT genotypes of FTO rs9939609 polymorphism.

In a series of stepwise logistic regression analyses, we examined the relation between the dietary intake of folate and SA and the potential modifying impact of FTO genotypes after adjusting the confounders. The potential confounding variables were selected based on prior literature and were sequentially considered in different models including crude (Model 1), adjusted for age (Model 2), further adjustment for tobacco and alcohol drinking (Model 3), and additional adjustment for BMI and diet (Model 4). All analyses were performed using R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria), considering P value < 0.05 as significant.

Results

The general characteristics of the participants are compiled in Table 1. Alcohol consumption was more frequent in the control group (14.6%) compared to the case group (4.9%), whereas the prevalence of diabetes was higher among the cases (23.3%) than the controls (17.3%) ($P = 0.001$ for both). No significant difference was observed in age, weight, height, body mass index (BMI), age at menarche, pathologic and biochemical factors, PCR results, smoking, and history of hypertension.

The dietary intake of participants is presented in Table 2. There was no significant difference between overall dietary intake of micronutrients and macronutrients of cases and healthy individuals. Dietary intake of folate in the case group was partially lower than the control group (610.29024933 vs. 576.77239898 µg/d, $P = 0.07$).

Also, as shown in Table 3, there was no significant difference in dietary intakes of the case and control groups considering FTO genotypes. A partial lower intake of folate was found in cases with AA genotype of FTO rs9939609 polymorphism (566.63 ± 131.96 vs. 630.53 ± 193.28 µg/d, $P = 0.087$).

A multiple linear regression model was conducted to examine the linear association between SA and folate mean intake, after adjusting for age, energy intake, BMI, and diet. The model revealed that being in the control group was significantly associated with higher folate intake ($\beta = 91.06$, $p < 0.001$) (Table 4).

Table 5 displays the results of a logistic regression examining the relationship between dietary intake of folate and SA. The intake of dietary folate was found to be negatively associated with abortion after adjusting for age (Model 1) (OR: 0.991, 95% CI 0.986–0.996, $P = 0.001$), additional adjustment for drinking alcohol and tobacco (Model 2), and further adjustment for BMI and dietary intake (Model 3).

Logistic regression on the association between SA and dietary intake of folate considering FTO genotypes is illustrated in Table 6. A negative correlation was found between the risk of SA and the intake of folate (OR: 0.97, 95% CI (0.94–0.99), $P = 0.04$), when taking into account the AA genotype of the FTO gene and abortion. The results did not change after adjusting for drinking alcohol

Table 1 Main characteristics of the cases with a history of spontaneous abortion and the controls without a history of spontaneous abortion *

	Cases ($n=192$)	Controls ($n=347$)	*P*
Age (Year)	24.84 ± 10.60	25.60 ± 12.86	0.07
Height (Cm)	156.87 ± 6.19	156.80 ± 5.68	0.88
Weight (Kg)	71.89 ± 10.51	70.35 ± 10.29	0.10
BMI (Kg/m^2)	29.16 ± 4.01	28.59 ± 3.96	0.11
Mens first age (Year)	13.39 ± 1.51	13.27 ± 1.62	0.50
Right DBP (mmHg)	70.52 ± 9.33	70.34 ± 8.80	0.85
Right SBP (mmHg)	109.29 ± 14.03	108.75 ± 14.19	0.73
WBC (K/µL)	6.33 ± 1.52	6.15 ± 1.42	0.28
RBC (M/µL)	4.83 ± 0.40	4.85 ± 0.38	0.59
HGB (gr/dl)	13.35 ± 1.09	13.33 ± 0.96	0.28
HCT (%)	40.53 ± 2.88	40.55 ± 0.2.68	0.94
MCV (fL)	84.16 ± 6.04	83.75 ± 5.53	0.54
MCH (pg)	27.72 ± 2.42	27.55 ± 2.18	0.51
MCHC (gr)	32.91 ± 0.79	27.55 ± 2.18	0.62
Platelets (K/µL)	316.11 ± 66.48	308.20 ± 68.68	0.29
Lymphocyte (10^6/L)	40.97 ± 8.07	41.23 ± 8.92	0.77
Monocyte (10^6/L)	3.25 ± 1.03	3.28 ± 1.06	0.81
BUN (mg/dl)	12.50 ± 3.17	12.62 ± 3.51	0.74
Creatinine (mg/ml)	0.97 ± 0.11	0.96 ± 0.11	0.72
TG (mg/dl)	133.35 ± 79.82	121.55 ± 69.11	0.16
Cholesterol (mg/dl)	195.66 ± 37.24	194.52 ± 39.65	0.79
SGOT (IU/L)	19.04 ± 5.51	18.49 ± 4.87	0.35
SGPT (IU/L)	18.87 ± 7.62	17.70 ± 9.04	0.19
ALP (IU/L)	233.73 ± 76.95	220.18 ± 66.45	0.09
HDLC (mg/dl)	54.39 ± 10.78	55.76 ± 11.44	0.26
LDLC (mg/dl)	114.59 ± 32.52	114.45 ± 33.39	0.97
FTO genotype (%)			
TT	94 (36.96)	40 (28.6)	0.33
AA	22 (8.6)	14 (10.0)	
AT	138 (54.1)	86 (61.4)	
Use alcohol (yes, n, %)	17 (4.9)	28 (14.6)	0.001
Tobacco (yes, n, %)	20 (5.8)	8 (4.2)	0.27
Has diabetes (yes)	81 (23.3)	60 (17.3)	0.03
Has hypertension (yes)	89 (25.6)	52 (27.1)	0.39

*Mean ± SD for the quantitative data and number(%) for the qualititative data. Right SBP: Right Systolic Blood Pressure, Right DBP1: Right Diastolic Blood Pressure, WBC: white blood cell, RBC: red blood cell, HCT: Hematocrit, HGB: Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, MCH: Mean Corpuscular Hemoglobin, MCV: Mean Corpuscular Volume, TG: Triglyceride, BUN: Blood Urea Nitrogen, HDLC: high density lipoprotein cholesterol, LDLC: low density lipoprotein cholesterol, SGPT: Serum Glutamic pyruvic Transaminase, SGOT: Serum Glutamic Oxaloacetic Transaminase, ALP: Alkaline phosphatase

and tobacco (Model 2), and further adjustment for BMI and diet (Model 3).

Discussion

The current study's findings indicated an inverse link between dietary folate and abortion. Intake of dietary folate was found to be inversely linked with abortion among carriers of the AA genotype of the FTO rs9939609 polymorphism (Fig. 1). To the best of our knowledge, this is one of the first studies that evaluated the relation between dietary intake of folate and SA in the presence of FTO polymorphisms in the gene. Although the FTO gene is well known to be involved in obesity, there is newer evidence indicating that it can also affect reproductive health through metabolic and inflammatory pathways [16].

The U.S. Preventive Services Task Force and the Centers for Disease Control and Prevention have advised since 1992 that to avoid neural tube abnormalities, all women who wish to get pregnant should take 400 µg of folic acid every day [17]. In line with the present study, a Hungarian trial found that folic acid increases fertility and the chance of having multiple births [18]. However, the Medical Research Council Vitamin Study, which applied folic acid at a dosage around five times higher than the Hungarian study discovered no negative correlation between folic acid supplementation and miscarriage [19]. Moreover, according to two recent cohort studies, taking multiple vitamins or folic acid during pregnancy was linked to a 50–60% lower incidence of miscarriage [20, 21]. Differences in the obtained results on the association between SA and folate may be caused by differences in the genetic backgrounds of the participants. The present study contributes to the elucidation of the complex interplay between pre-pregnancy dietary folate intake and susceptibility to SA.

Several mechanisms have been put out to explain how folate protects against SA. Reduced cell division, disturbed methylation processes, elevated levels of oxidative stress, apoptosis, and inflammatory cytokine production have all been linked to inadequate folate consumption, and these outcomes may have an impact on the developing fetus [22]. Additionally, a shortage in folate has been proposed as a risk factor for placental abruption and preeclampsia [23]. This suggests that the vascular consequences of a folate shortage may also raise the chance of stillbirth and SA. A second possibility is that babies with neural tube defects are more likely to spontaneously abort and that neural tube defects themselves are associated with low folate levels [24]. Although conceivable, neural tube abnormalities are uncommon illnesses, therefore this explanation would only partially account for the correlation between low folate levels and SA. On the other hand, research indicates an intriguing interaction between specific variations (polymorphisms) within the FTO gene and folate intake, despite no direct link being established. Studies suggest that individuals with certain FTO polymorphisms might experience altered effects of folate intake based on their dietary patterns [25]. For example, adhering to a Mediterranean diet, which is rich in folate, could potentially reduce the risk of type 2 diabetes [26] associated with the FTO polymorphisms [27]. This suggests a complex relationship between

Table 2 Dietary intakes of the cases with a history of spontaneous abortion and the controls without a history of spontaneous abortion

Nutrient	Controls (*n* = 347) (Mean ± SD)	Cases (*n* = 192) (Mean ± SD	*P*-value
Energy (kcal)	2,552.82197017	2,534.83372059	0.74184477
Carbohydrate (g)	363.17265838	359.44437358	0.66462248
Protein (g)	83.79838646	84.71576018	0.71884525
Total fat (g)	94.16683343	92.13989032	0.44124881
PUFA (g)	19.47135463	18.66564207	0.34859953
Saturated fat (g)	28.82789036	28.92774928	0.93139425
Omega-3 (g)	1.22730224	1.23345122	0.93761172
Omega-6 (g)	5.22718557	4.83910679	0.52975299
MUFA (g)	33.48939169	34.24183478	0.62045800
Cholesterol (mg)	258.20086728	268.86228180	0.49893620
Folate total (µg)	610.29024933	576.77239898	0.06997098
Calcium (mg)	1,291.53309482	1,194.42113143	0.07507285
Vitamin E (mg)	18.03692460	16.62924015	0.12274645
Riboflavin (mg)	2.29059655	2.18769068	0.20084437
Vitamin D (µg)	1.83088434	1.97367530	0.32756591
Copper (mg)	1.84698543	1.91618538	0.33874012
Alpha-tocopherol (mg)	12.46370159	11.86087379	0.34835330
Potassium (mg)	5,651.81591482	5,280.69314720	0.37812099
Fluoride (mg)	2,553.71711872	2,707.35310032	0.46722068
Alpha-carotene (µg)	49,050.87094265	39,292.21898017	0.47715824
Chromium (µg)	0.07333447	0.06733109	0.48020079
Vitamin K (µg)	339.84686435	290.37962322	0.50519353
Lutein (µg)	2,182.12987225	2,000.27652194	0.51095470
Thiamine (mg)	2.04273011	2.00264187	0.52646953
Beta-cryptoxanthin (µg)	428.31395597	450.50345653	0.57347966
Magnesium (mg)	419.02290581	410.81995616	0.60152707
Vitamin B12 (µg)	4.56174243	4.42543186	0.62822827
Lycopene (µg)	9,210.39592793	8,799.23408006	0.63815191
Vitamin A (µg)	709.45241783	677.55507194	0.64040360
Zinc (mg)	12.04942632	11.83435813	0.65294176
Beta-carotene (µg)	5,424.87797628	5,091.39285513	0.67211450
Sodium (mg)	6,791.88950619	6,995.05037225	0.68358650
Vitamin B6 (mg)	1.92612184	1.89910977	0.69633531
Iron (mg)	17.94727367	18.15264659	0.73519829
Niacin (mg)	21.18702269	21.38598105	0.80148285
Pantothenic acid (mg)	5.61778072	5.57321215	0.80749892
Phosphorus (mg)	146,208.57598646	143,402.07308171	0.91907609
Biotin (µg)	31.39174251	31.46103683	0.95717395
Vitamin C (mg)	198.86885717	199.75455306	0.96081921
Selenium (µg)	96.10108315	96.22996432	0.97131659
Manganese (mg)	5.67112213	5.67076605	0.99869015

SA, the FTO gene, and dietary folate intake. Additionally, research highlights a potential connection between folate intake and DNA methylation [28], a process that can influence gene expression and potentially impact various metabolic functions through affecting FTO gene [29]. However, the specific mechanisms involved in this interaction and its potential health implications require further investigation. Overall, while the FTO gene may not directly influence folate requirements, its interaction with factors like dietary patterns and potentially DNA methylation indicates a complex interplay that merits further study. Understanding these complex relationships could lead to personalized dietary recommendations to optimize health outcomes [30, 31].

Our findings have significant clinical and public health relevance. Prenatal folic acid supplements are advised by the World Health Organization for women of reproductive age. The majority of American women of reproductive age consume significantly less than the recommended daily intake of 400 µg, notwithstanding this guideline [32]. Furthermore, folic acid dietary fortification has been used in multiple nations and is being explored in others [33, 34]. While the absolute reduction in risk observed in the present study was modest, even modest effects have substantial public health significance if applied across high numbers of individuals. In addition, the stronger association observed in genetically susceptible persons implies promise for individualized nutrition intervention in reproductive health. Our results provide evidence that a higher intake of supplemental folate may be an effective strategy to prevent abortion, especially in people with a genetic risk allele of the FTO gene. This work had key strengths. Firstly, it is one of only a few to investigate the interaction of dietary intake of folate and polymorphisms of the FTO gene in relation to SA and offers an original contribution to reproductive health and nutritional genomics. Secondly, by excluding those who used folate supplements, we were better able to discern the effect of usual dietary intake of folate on the risk for SA free from confounding due to supplementation. Lastly, genotype-stratified analyses allowed us to identify an association in those who had the AA genotype of the FTO rs9939609 polymorphism, supporting an area of potential for tailored nutrition in reproductive healthcare.

Nevertheless, we had some limitations. This study used self-reported data on dietary folate intake, which might introduce measurement errors due to recall bias. Also,

Table 3 Dietary intake among the participants with different FTO genotypes

	TT (Mean ± SD)			AA (Mean ± SD)			AT (Mean ± SD)		
	Controls (*n* = 128)	Cases (*n* = 53)	P	Controls (*n* = 31)	Cases (*n* = 21)	P	Controls (*n* = 188)	Cases (*n* = 118)	P
Energy Intake (kcal/day)	2561.924 ± 545.995	2551.176 ± 373.546	0.910	2648.352 ± 741.060	2430.915 ± 276.966	0.302	2546.192 ± 656.852	2544.269 ± 409.325	0.981
Protein (g/day)	84.169 ± 23.529	83.833 ± 15.797	0.934	88.008 ± 43.431	76.829 ± 14.172	0.360	83.888 ± 31.109	83.353 ± 17.733	0.885
Carbohydrates (g/day)	364.499 ± 92.529	361.340 ± 63.747	0.844	376.133 ± 110.067	345.503 ± 61.471	0.350	362.383 ± 83.439	362.960 ± 56.205	0.955
Total Fat (g/day)	95.011 ± 27.194	93.654 ± 15.081	0.767	96.762 ± 24.023	88.401 ± 11.521	0.234	93.153 ± 31.404	92.940 ± 24.487	0.957
Cholesterol (mg/day)	277.729 ± 127.313	269.082 ± 119.651	0.715	222.451 ± 113.831	272.562 ± 146.698	0.258	258.625 ± 127.925	277.296 ± 152.482	0.325
Saturated Fat (g/day)	28.312 ± 10.536	29.788 ± 8.237	0.432	28.180 ± 9.240	24.050 ± 7.680	0.173	29.198 ± 15.649	29.266 ± 9.572	0.971
Monounsaturated Fatty Acids (g/day)	34.328 ± 12.684	33.061 ± 9.831	0.574	34.186 ± 12.895	33.034 ± 12.236	0.792	32.476 ± 16.223	35.311 ± 14.614	0.188
Polyunsaturated Fatty Acids (g/day)	20.071 ± 7.649	19.883 ± 7.793	0.897	22.519 ± 11.762	21.346 ± 6.497	0.735	18.684 ± 7.105	18.527 ± 7.424	0.874
Omega-3 Fatty Acids (g/day)	1.274 ± 0.669	1.282 ± 0.720	0.947	1.385 ± 1.150	1.179 ± 0.645	0.547	1.195 ± 0.631	1.230 ± 0.561	0.672
Omega-6 Fatty Acids (g/day)	6.186 ± 4.949	5.872 ± 4.138	0.725	5.624 ± 7.216	4.335 ± 3.649	0.541	4.926 ± 3.843	5.090 ± 4.153	0.763
Vitamin A (µg/day)	817.936 ± 1590.676	787.017 ± 420.355	0.904	638.967 ± 287.348	640.046 ± 352.878	0.992	655.429 ± 330.506	659.785 ± 323.197	0.923
Vitamin C (mg/day)	213.006 ± 188.629	214.571 ± 172.604	0.964	189.479 ± 121.897	146.227 ± 69.959	0.237	199.900 ± 119.790	196.999 ± 145.217	0.871
Pantothenic Acid (mg/day)	5.499 ± 1.741	5.610 ± 1.590	0.730	5.879 ± 1.837	5.161 ± 1.715	0.249	5.645 ± 2.109	5.636 ± 1.335	0.973
Vitamin E (mg/day)	19.704 ± 11.113	17.543 ± 7.902	0.267	17.958 ± 11.521	15.244 ± 7.495	0.441	16.861 ± 7.316	17.188 ± 8.139	0.755
Alpha-Tocopherol (mg/day)	13.604 ± 9.727	12.318 ± 4.740	0.428	12.425 ± 7.775	12.031 ± 4.935	0.867	11.553 ± 5.392	12.044 ± 5.234	0.503
Thiamin (mg/day)	2.035 ± 0.625	2.023 ± 0.462	0.914	2.240 ± 0.732	2.047 ± 0.619	0.420	2.070 ± 0.769	2.004 ± 0.469	0.470
Riboflavin (mg/day)	2.315 ± 1.091	2.246 ± 0.508	0.701	2.333 ± 0.855	2.115 ± 0.555	0.405	2.294 ± 1.036	2.212 ± 0.534	0.496
Niacin (mg/day)	21.160 ± 7.127	21.280 ± 7.031	0.929	23.002 ± 8.924	21.250 ± 6.570	0.531	21.380 ± 8.017	21.088 ± 6.227	0.774
Vitamin B6 (mg/day)	1.921 ± 0.880	1.883 ± 0.739	0.810	1.874 ± 0.533	1.548 ± 0.374	0.054	1.935 ± 0.669	1.946 ± 0.495	0.897
Folate (µg/day)	587.039 ± 205.662	595.243 ± 154.792	0.821	630.533 ± 193.283	566.634 ± 131.960	0.087	575.520 ± 175.547	606.708 ± 133.597	0.159
Vitamin B12 (µg/day)	4.838 ± 2.241	4.507 ± 1.907	0.416	4.854 ± 2.663	3.799 ± 2.277	0.229	4.507 ± 3.512	4.384 ± 1.967	0.766
Biotin (µg/day)	31.162 ± 12.750	31.980 ± 12.872	0.735	28.936 ± 7.850	26.785 ± 9.546	0.466	32.064 ± 13.454	31.801 ± 10.011	0.876
Vitamin K (µg/day)	448.089 ± 1658.233	354.107 ± 262.482	0.723	329.603 ± 203.637	282.256 ± 208.805	0.505	289.281 ± 195.366	296.510 ± 196.549	0.788
Calcium (mg/day)	1239.615 ± 525.225	1257.732 ± 363.517	0.843	1351.019 ± 523.503	1222.369 ± 404.473	0.440	1327.624 ± 796.197	1183.812 ± 352.634	0.115
Iron (mg/day)	18.755 ± 8.414	18.821 ± 4.981	0.963	18.648 ± 7.271	18.771 ± 4.866	0.956	17.511 ± 4.942	17.696 ± 4.089	0.771
Vitamin D (µg/day)	1.934 ± 1.242	2.030 ± 1.240	0.684	1.842 ± 1.515	2.330 ± 1.712	0.377	1.840 ± 1.034	1.907 ± 1.055	0.642
Magnesium (mg/day)	422.178 ± 278.087	421.048 ± 109.378	0.980	453.045 ± 185.093	400.605 ± 109.937	0.346	408.071 ± 122.285	413.143 ± 112.472	0.756
Zinc (mg/day)	12.373 ± 4.691	11.780 ± 3.658	0.478	13.085 ± 5.144	10.780 ± 3.452	0.149	11.839 ± 4.975	11.815 ± 3.853	0.969
Copper (mg/day)	1.926 ± 1.020	2.001 ± 0.512	0.660	2.014 ± 0.977	2.052 ± 0.617	0.899	1.751 ± 0.521	1.869 ± 0.617	0.126
Manganese (mg/day)	5.833 ± 2.722	5.602 ± 1.322	0.611	6.398 ± 2.264	5.379 ± 2.061	0.182	5.479 ± 1.894	5.832 ± 1.721	0.162
Selenium (µg/day)	100.417 ± 33.782	91.177 ± 23.000	0.117	99.738 ± 34.370	92.246 ± 30.148	0.509	94.575 ± 36.223	100.515 ± 31.889	0.213

Table 4 Linear regression of the association of spontaneous abortion and folate intake

Predictors	Folate_total		
	Estimates	std. Beta	*P**
(Intercept)	− 49.30	− 0.61	0.587
Group [Control]	91.06	0.46	< 0.001
Age	0.19	0.01	0.810
kcal	0.20	0.60	< 0.001
BMI	0.29	0.01	0.870
UseAlcohol [yes]	83.13	0.42	0.275
dokhaniat [no]	44.52	0.23	0.485
R^2 / R^2 adjusted	0.423 / 0.415		

*Adjusted for age, tobacco and alcohol drinking, BMI, and diet

Table 5 Logistic regression of the association between spontaneous abortion and dietary folate

	OR (95%CI)	*P*
Model 1	0.992 (0.987–0.997)	< 0.01
Model 2	0.992 (0.987–0.997)	< 0.01
Model 3	0.991 (0.986–0.996)	< 0.01

Model 1: Adjusted for age, Model 2: Further adjustment for tobacco and alcohol drinking, Model 3: Further adjusted for BMI and dietary intake

our findings are based on data collected from a specific population and may not be fully generalizable to all global populations due to genetic, environmental, and dietary differences. Furthermore, data on the bioavailability and metabolic pathways of folate are not available and we could not evaluate how the interaction between different genotypes may affect the bioavailability and metabolic pathways of folate. Moreover, this study was carried out on a relatively small sample of women. Also, the study did not take into consideration every possible confounding variable that may have an impact on the association between dietary folate consumption, FTO genotype, and SA risk such as socioeconomic status and degree of physical activity. More investigation is required to confirm these results and elucidate the underlying processes. If the results of the present study are confirmed in future studies, personalized dietary recommendations according to the genotype of SA-related genes may be considered a crucial approach for the prevention of SA.

Table 6 Logistic regression the association between spontaneous abortion and dietary folate considering FTO genotypes

	TT		AA		AT	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Model 1	0.99 (0.98–1.006)	0.43	0.97 (0.94–0.99)	0.02	0.99 (0.99–1.003)	0.30
Model 2	0.99 (0.98–1.006)	0.33	0.97 (0.94–0.99)	0.04	0.99 (0.99–1.003)	0.31
Model 3	0.99 (0.97–1.005)	0.19	0.97 (0.94–0.99)	0.04	0.99 (0.99–1.003)	0.31

Model 1: Adjusted for age, Model 2: Further adjustment for tobacco and alcohol drinking, Model 3: Further adjusted for BMI and dietary intake

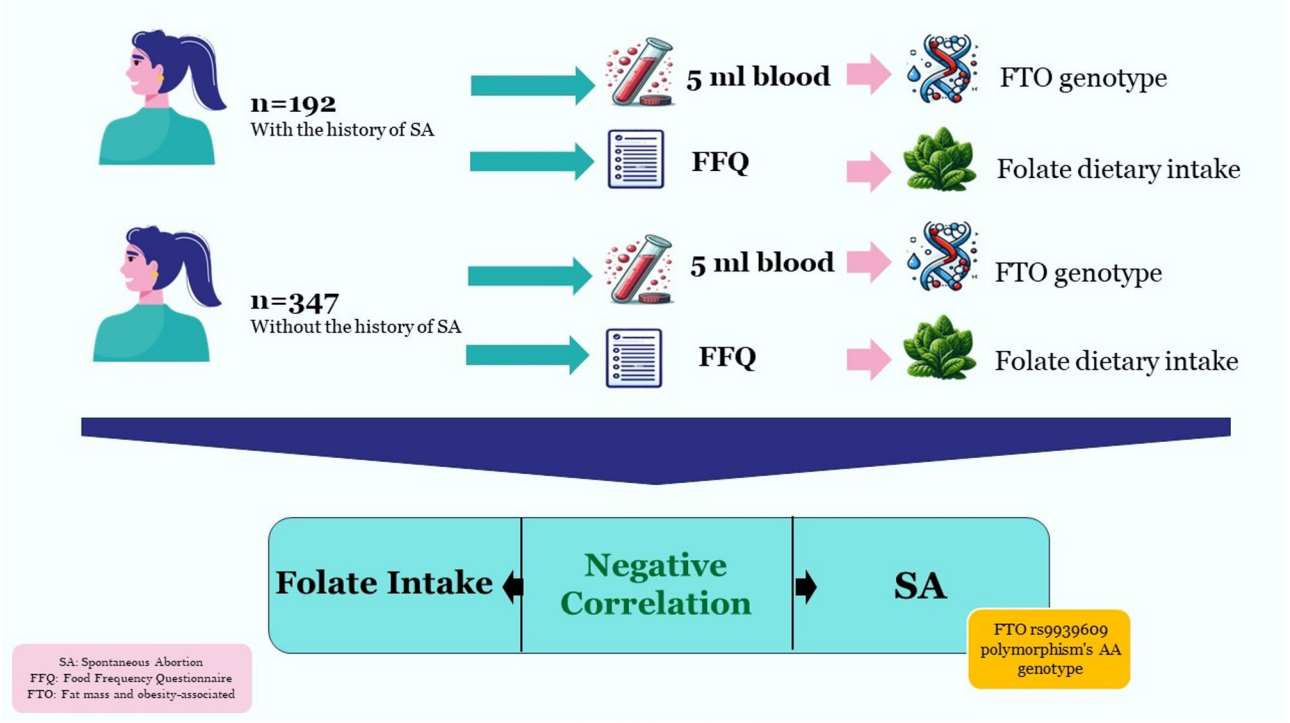

Fig. 1 The association of spontaneous abortion with dietary folate intake in individuals with different genotypes of FTO gene

Conclusion

Our results indicated that a greater intake of dietary folate was related to a reduced risk of SA. This relation persisted after controlling for age, lifestyle, BMI, and total calorie intake. Stratification analysis also revealed that this relation was significant in individuals carrying the AA genotype of the FTO rs9939609 polymorphism. Our study provides valuable insights into the interaction between SA, folate intake, and FTO genotypes—one of the most widely studied obesity-related genes—within a real-world nutritional and cultural context. Given the global prevalence of FTO polymorphisms and the universal importance of folate in reproductive health, our findings contribute to the growing body of evidence on gene-diet interactions in diverse populations. Additional mechanistic studies are mandatory to validate this hypothesis.

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Author contributions
GKHM, MSH, SHN, ZM, NPL,, MSMH, ZM, MSR, ZS, SD were responsible for the study's design, collecting and analyzing data, and drafting of the manuscript. SSH, BB, BA, MGh, and SD contributed to the study's design, conducted data analysis, and provided a critical review of the manuscript. The final manuscript was reviewed and approved by all authors.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate
All methods were carried out in accordance with relevant guidelines and regulations Ethical Committee of the Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.RETECH.REC.1404.130). All procedures of the studies involving human participants were by the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants signed informed consent forms. Informed consent was obtained from participants in the study.

Consent for publication
Institutional consent forms were used in this study.

Competing interests
The authors declare no competing interests.

Author details
[1]Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Clinical Nutrition, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Iran University of medical sciences, Tehran, Iran
[4]Nursing and Midwifery, Shahed University, Tehran, Iran
[5]Department of Medicine, Faculty of Sciences, Guilan University, Rasht, Iran
[6]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
[7]Department of Food Industry Engineering, Padova University, Padova, Italy
[8]Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[9]Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[10]Nutrition and Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[11]Department of Sport Science and Nutrition, Maynooth University, Maynooth, Ireland
[12]Department of Community Nutrition, School of Nutrition and Food Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[13]Department of Obstetrics and Gynecology, Reproductive Health Research Center, Al-Zahra Hospital, Guilan University of Medical Sciences, Rasht, Iran
[14]Department of Community Nutrition, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[15]Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[16]National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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