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Preconception folate status and reproductive outcomes among a prospective cohort of folate-replete women

Elizabeth A. DE VILBISS, PhD, Sunni L. MUMFORD, PhD, Lindsey A. SJAARDA, PhD, Matthew T. CONNELL, DO, Keewan KIM, PhD, James L. MILLS, MD, Robert M. SILVER, MD, and Enrique F. SCHISTERMAN, PhD

Epidemiology Branch, Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda (EAD, SLM, LAS, MTC, KK, JLM, and EFS); Department of Obstetrics and Gynecology, University of Utah, Salt Lake City (RMS)

Abstract

Background: Most studies of folate metabolism and reproduction have been conducted after pregnancy and in folate-deficient populations. However, measurement of maternal folate status pre-conceptionally may be most relevant to certain folate-linked early processes preceding a successful pregnancy, and there has been a major increase in folate concentrations in women of childbearing age in high resource settings.

Objective: Examine associations between pre-conceptional biomarkers of maternal folate status (folate and homocysteine) and reproductive outcomes in folate-replete women.

Design: Cohort nested within the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a block-randomized, double-blind, placebo-controlled trial whereby women were randomized to daily low-dose aspirin (81 mg/day) or placebo and all women received folic acid (400 µg/day). 1,228 women with 1–2 previous pregnancy losses and no documented infertility were recruited from 4 clinical sites in the US (2006–12) and were attempting pregnancy for up to 6 menstrual cycles. Log-binomial regression models were used to estimate relative risks (RRs) and 95%

Corresponding author: Enrique F. Schisterman, PhD, Epidemiology Branch, Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, 6710B Rockledge Dr., MSC 7004, Bethesda, MD, 20892-7004, United States (301-435-6893, schistee@mail.nih.gov).

Author Contributions:

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Ethical approval: Written informed consent was provided by all participants. The data coordinating center and each site obtained Institutional Review Board approvals.

Clinical Trial Registration: [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT0046736) #NCT0046736

confidence intervals between preconception serum folate and plasma homocysteine for anovulation, pregnancy, and pregnancy loss.

Results: Higher plasma homocysteine was non-linearly associated with higher risks of pregnancy loss only among women with two prior losses: a relative risk of 1.43 (95% CI: 1.08, 1.89) was found for plasma homocysteine concentrations at the study median of 8.0 $\mu\text{mol/L}$ compared to a U.S population median of 6.0 $\mu\text{mol/L}$. No meaningful relationships were found between serum folate and any reproductive outcome or between plasma homocysteine and anovulation or conception.

Conclusions: These data justify further study of the role of folate and homocysteine metabolism in normal and abnormal early pregnancy.

Keywords

homocysteine; folic acid; pregnancy loss; miscarriage

INTRODUCTION

Insufficient folate status has been associated with many reproductive complications including anovulation¹, sub-fertility², and early pregnancy loss¹. Homocysteine is commonly used as an indicator of folate status. An amino acid involved in one-carbon metabolism of folate, higher homocysteine levels have been associated with hormonal changes across the menstrual cycle¹. It is hypothesized that elevated homocysteine may be related to vascular changes associated with abnormal placentation^{3–6}, which may increase the risk of pregnancy loss.

Measurement of maternal folate status pre-conceptionally may be most relevant to certain folate-linked early processes preceding a successful pregnancy, such as ovulation, or implantation whereby the placenta begins to differentiate. However, most studies of folate metabolism and reproduction have been conducted either during or after pregnancy^{5, 7–11}, subsequent to placental differentiation and early pregnancy losses. While historical studies on this topic have been conducted in relatively folate-deficient populations, examination of folate-replete women would yield findings more generalizable to the U.S. population of women actively attempting natural conception. We therefore aim to investigate how biomarkers of maternal folate status, the result of B vitamin intake and folate metabolism, are prospectively related to reproductive outcomes among folate-replete women with a history of pregnancy loss.

SUBJECTS and METHODS

Study sample

Data were drawn from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a block-randomized, double-blind, placebo-controlled trial designed to study the effects of preconception low-dose aspirin on live birth in women with 1–2 prior pregnancy losses¹². Study design, methods, inclusion, and exclusion criteria have previously been described^{12, 13}. Participant flow chart is provided in Figure 1. Briefly, 1,228 women 18–40

years of age with no known major medical conditions or documented infertility were recruited from 4 clinical sites in the US (2006–12). Inclusion was limited to women having regular menstrual cycles and attempting to conceive naturally. Women were randomized to daily low-dose aspirin (81 mg/day) or placebo and all women received folic acid (400 µg/day). Women were followed up to six menstrual cycles, and through pregnancy for those who became pregnant; study treatment continued through week 36 of pregnancy. The trial was registered on [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00467363) (#NCT00467363).

Human and nonhuman experimentation

The Institutional Review Board at each study site and data coordinating center approved the trial protocol and obtained approvals. All participants provided written informed consent prior to enrolling.

Serum folate and plasma homocysteine measurement

Serum folate and plasma homocysteine levels were measured at baseline, prior to consumption of study medication. Preconception serum sample collection coincided with days 2–4 of each participant's menstrual cycle and stored at –80°C until assay. Serum folate was measured by the Folate III reagent/competitive immunoassay method/electrochemiluminescence using the Roche COBAS 6000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). The inter-assay laboratory coefficients of variation (CVs) were 9.9% and 6.3% at 8.63 nmol/L and 26.73 nmol/L, respectively. Total plasma homocysteine was measured by liquid chromatography/tandem mass spectrometry using Waters' 2795 Liquid Chromatography equipped with an autosampler and column oven. Data were obtained using the MassLynx software with automated data processing by QuanLynx software. The inter-assay laboratory CVs are 4.6 and 6.8% at mean concentrations of 1.0 and 22.6 µmol/L, respectively.

Outcome ascertainment

Reproductive outcomes included anovulation, pregnancy, and pregnancy loss^{13–15}.

Anovulation—Fertility monitors used for intercourse timing (Clearblue® Easy Fertility Monitor, Alere, Waltham, MA, USA) measured luteinizing hormone (LH) and estrone-3-glucuronide concentrations in first-morning urine¹⁶. The test stick measures urinary LH by a classical sandwich assay, and the monitor optically reads the intensity of the line. Hormone values were downloaded from the internal memory chip at the end of each cycle.

Ovulation was detected using fertility monitors for up to six cycles of preconception follow-up, and urinary luteal pregnanediol-3-glucuronide (PdG; a progesterone metabolite) measurements were used to improve the sensitivity of ovulation detection in the first two cycles of study participation. Cycles without a *peak* reading on the monitor or daily LH concentrations <2.5 times the average of the previous 5 days were considered anovulatory, as well as cycles with PdG <5 µg/mL^{16–18}. For fertility monitor criteria to apply, data must have been available on the 15th day before the end of the cycle to classify a lack of *peak*, and for the moving average method, tests on at least 2 of the previous 5 days with at least 1 in the immediate 2 previous days for a valid average were required; otherwise the cycle was

unable to be categorized by the fertility monitor criteria. Cycles with insufficient data for all methods were classified as missing (n = 397). Reasons for missing anovulation data were infrequent fertility monitor testing (10 testing days), missing tests on or around the anticipated day of LH surge (15 days before the start of menses¹⁹), non-use of the fertility monitor, or testing malfunctions. Cycles resulting in conception were considered ovulatory.

Pregnancy—Pregnancies were identified by hCG in three ways: 1) a positive urine pregnancy test using a home pregnancy kit (Quidel Quickvue One step hCG test, Quidel Corporation, San Diego, CA), 2) an in-clinic urine test among women reporting a missed menses, or 3) by free beta hCG measured in spot urine samples from visits occurring between cycle days 2 and 4, and in daily first-morning urine samples from the last 10 cycle-days of participants' first two menstrual cycles (initial test: catalogue no. RIS0011R, BioVendor, Asheville, NC; confirmatory test: catalogue no. 4221-16, Diagnostic Automation Inc., Calabasas, CA). Following a positive urine pregnancy test, ultrasound-confirmed pregnancy was a secondary outcome defined per study protocol by the presence of a gestational sac via ultrasound at approximately 6.5 weeks gestation.

Pregnancy loss—Pregnancy loss was identified by the absence of clinical signs of pregnancy following a positive urine pregnancy test or positive free beta hCG in stored urine samples, or by the participant or clinician after ultrasound confirmation.

Covariates

At baseline, participants completed questionnaires on demographics, lifestyle habits, socioeconomic status, and reproductive history. In the baseline questionnaire, women were asked whether they were currently taking a supplement containing folic acid, but dose was not assessed. Height and weight were measured at baseline to calculate body mass index (BMI; kg/m²), which was not normally distributed. As the reciprocal of BMI more closely approximated normality, this transformation was used to account for BMI in adjusted models.

Statistical analyses

Serum folate, approximately normally distributed, was summarized as means and standard deviations with t-tests or ANOVAs by categorical baseline characteristics. Plasma homocysteine, approximately log-normally distributed, was summarized as medians and interquartile ranges with Wilcoxon Mann Whitney tests by categorical baseline characteristics. Relationships between exposures and continuous maternal characteristics were examined using linear regression (homocysteine was log-transformed) and restricted cubic splines to examine potential non-linearities.

All analyses utilized multiple imputation and weighting. Multiple imputation was used to address missing data for women with missing folate (n = 195; 15.9%), homocysteine (n = 22; 1.8%), BMI (n = 20; 1.6%), and anovulation (n = 397; 13% of cycles). Chained equations with all indicated covariates generated 50 datasets to analyze pregnancy, pregnancy loss, and live birth. Twenty datasets were separately generated to analyze anovulation due to multiple cycles per woman²⁰.

Given that pregnancy loss is conditional upon becoming pregnant, these analyses were conducted only among women who became pregnant, with inverse probability weights used to account for the potential that women who became pregnant may have had different characteristics than those who did not. Similarly, as participants who withdrew may have also differed than those who completed the study, inverse probability weights were separately constructed for study withdrawals. Pregnancy and withdrawal weights were based on factors associated with becoming pregnant and study withdrawal, respectively. Analytic details on calculation of weights are included in the Methods Supplement (Methods Supplement - Online Supplemental Material).

Log-binomial regression models were used to estimate relative risks (RRs) and 95% confidence intervals between serum folate and plasma homocysteine for anovulation, pregnancy, and pregnancy loss. An ad hoc analysis examined time to pregnancy. Estimates were calculated for an increase of 15 nmol/L serum folate or 2 μ mol/L plasma homocysteine, each approximately 1 standard deviation of exposure. Ad hoc analyses for homocysteine were stratified by prior loss number. Unadjusted models and models adjusted for age, reciprocal of BMI, prior number of pregnancy losses loss (1, 2), and parity (0, 1+) were examined. Since there were no relationships between loss and marital status, race, smoking, education, income, or alcohol intake in this population, these covariates were not included in adjusted models. Restricted cubic splines were used to examine potential non-linearities (Online Supplemental Material).

Analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Study sample characteristics

Among 1,228 study participants, there were 797 (64.9%) pregnancies and 188 (23.6%) pregnancy losses. One hundred and sixty-nine ($n = 169$) of 188 (90%) of pregnancy losses occurred within 12 weeks gestation. There were 1,222 women who contributed 4266 total cycles; 6 women withdrew from the study prior to contributing any cycles. Among cycles with sufficient data ($n = 3869$), 486 (12.6%) were classified as anovulatory. Three hundred and thirty-five ($n = 335$) of 1228 (27.3%) women were classified as having at least one anovulatory cycle.

Approximately 80% of women reported taking a supplement containing folic acid at baseline (Table 1). Serum folate measurements ranged from 14.5 to 90.1 nmol/L (median: 58.2 nmol/L). Plasma homocysteine ranged from 2.9 to 31.6 μ mol/L (median: 8.0 μ mol/L). Preconception serum folate and log plasma homocysteine were positively associated with maternal age (Table 1). Serum folate was negatively, and plasma homocysteine was positively, associated with BMI. Higher mean folate values were observed among women who were married, white, had beyond a high school education, had higher incomes, and did not smoke or drink alcohol, while higher median homocysteine values were observed among women who were not-married, non-white, and who reported smoking daily and drinking more often in the previous year.

Folate, homocysteine, and reproductive outcomes

There was no evidence of meaningful relationships between preconception serum folate and any outcome or between preconception plasma homocysteine and anovulation, pregnancy, or time to pregnancy in linear models (Table 2, Table 3, Supplemental Table 1); restricted cubic splines did not indicate meaningful deviations from linearity.

Plasma homocysteine was associated with a higher risk of pregnancy loss in a linear model (RR = 1.12 [1.00 to 1.23] per 2 $\mu\text{mol/L}$ increase). When stratified by prior loss number, this association was only evident among women with 2 prior losses. The RR for each 2 $\mu\text{mol/L}$ increase in plasma homocysteine was 1.15 (1.00, 1.29) for two prior losses, compared to 1.03 (0.84, 1.23) for one prior loss. Restricted cubic splines indicated deviation from linearity only among women with two prior losses (Figure 2). Compared to 6.0 $\mu\text{mol/L}$ (congruent with NHANES U.S. population estimates²¹), at 8.0 $\mu\text{mol/L}$ (the study population median), the unadjusted nonlinear model for pregnancy loss produced an estimate of RR = 1.43 (1.08, 1.89), compared to 1.11 (0.98, 1.25) for the unadjusted linear model.

COMMENT

Principal findings

In this prospective cohort of healthy folate-replete women attempting pregnancy after 1–2 prior pregnancy losses, serum folate was not associated with anovulation, pregnancy, or pregnancy loss. While plasma homocysteine was not associated with anovulation, becoming pregnant, higher preconception plasma homocysteine was associated with a higher risk of pregnancy loss only among women with 2 prior losses.

Strengths and limitations of study

This is the first study to examine pre-conceptional biomarkers of folate status and reproductive outcomes among healthy folate-replete women. Measuring circulating folate biomarkers accounts for genetic variants that affect one-carbon metabolism. Measurement on days 2–4 of each woman's cycle likely reduced biomarker variability since fluctuations in one-carbon metabolism are influenced by changes in cyclical hormones^{22–24}. This study overcomes limitations of previous work conducted in pregnant⁵ and non-pregnant^{6–11, 25} women, which occur after placental differentiation and miss early losses.

Analytic methods were utilized to reduce the potential impact of bias. To utilize all study observations and improve efficiency, multiple imputation was used primarily to account for missing folate and anovulation data. While rarely realized in practice, complete-case analysis assumes that the missing data are not related to missing or observed data. This assumption is relaxed in multiple imputation, which assumes that missing data can be related to observed data. Inverse probability weighting accounted for potential selection bias incurred by restricting pregnancy and loss analyses to women who did not withdraw, and pregnancy loss analyses to women who became pregnant. In contrast to prior studies, the exposure in the current study precedes pregnancy, so pregnancy is an intermediate variable on the path to pregnancy loss. Since there may be factors associated with becoming pregnant that are also associated with pregnancy loss after becoming pregnant, unless these factors are

taken into account through inverse probability weighting, selecting on the intermediate variable (e.g., pregnancy) in pregnancy loss analyses can induce bias through attenuation of calculated risks²⁶.

The study also has several limitations. We are unable to discern baseline folic acid supplement formulation, dosage, duration, or frequency, which was unrelated to baseline serum folate or plasma homocysteine measurements. It is possible that serum folate is not a sufficiently sensitive marker of chronic maternal folate status as compared to red blood cell folate, as serum folate is sensitive to recent ingestion of folic acid or folate-rich food²⁷. Since homocysteine is less volatile, it is a better measure of long-term folate status. Given the high serum folate concentrations observed in this study, it is unlikely that this alone explains the discordant findings for folate and homocysteine. Homocysteine is influenced by B vitamins other than folate (vitamins B₁₂, B₆, and B₂) and by the *MTHFR* 677C→T variant, which were not measured in this study. Study generalizability is limited to women actively attempting to conceive following 1–2 prior losses and predominantly to white women of higher socioeconomic status living in the United States.

Comparison with other studies

This is the first study to examine pre-conceptional folate and homocysteine in relation to multiple reproductive outcomes in a folate-replete population of women attempting pregnancy. Our finding that higher preconception plasma homocysteine was associated with higher risks of pregnancy loss among folate-replete women with 2 prior losses is concordant with prior cross-sectional and retrospective case-control studies of recurrent pregnancy loss^{7–11, 25}. However, these prior studies were conducted among more folate-deplete populations of non-pregnant women. While a more recent study of pregnant women unselected by loss history also supports these findings⁵, none of the prior studies were conducted pre-conception, a critical exposure window.

While a previous study of healthy women with regular menses found that higher homocysteine was associated with an elevated risk of anovulation at the time of expected ovulation, this association was attenuated by approximately 50% during the mid-follicular phase¹ (a more comparable time point to the present study). Indeed, the hormonal milieu at different phases of the menstrual cycle may influence the observed relationships. Similar to our findings, neither folate measured at expected ovulation nor in the mid-follicular phase was associated with anovulation risk in the former study. Interestingly, the population examined in the former study was also folate-replete, so the pattern of identifying relationships with homocysteine, but not for folate, further support that homocysteine may be associated with adverse reproductive outcomes by mechanisms independent of folate.

Notably, there was an absence of an association between levels of serum folate and any of the reproductive outcomes evaluated in the current study. Prior studies^{11, 28} that reported associations between low serum folate and early pregnancy loss in women with prior history of pregnancy loss were conducted in non-folate-replete populations. In contrast, the mean serum folate in the current study was 56.6 nmol/L and no individuals had a negative folate balance (<7 nmol/L) or subclinical deficiency (< 10 nmol/L)²⁷.

Meaning of the study

High homocysteine is a risk factor for vascular disease, which acts on blood vessel walls to elicit changes to the endothelium²⁹. This could cause premature damage^{30, 31} to decidual or chorionic vasculature^{32, 33}, which could disrupt placentation³⁴. Abnormal placental vasculature has been noted among women with elevated homocysteine levels measured early in pregnancy⁵ and following pregnancy loss⁶. Among women with at least two consecutive early pregnancy losses, elevated total homocysteine was associated with smaller median areas and perimeters of chorionic villous vasculature, and fasting homocysteine was negatively correlated with vascular element perimeter⁶. Defective placentation has been observed in about two-thirds of pregnancy losses occurring in the first trimester³⁵. Plasma homocysteine could also indirectly contribute to pregnancy loss. For instance, since higher plasma homocysteine was associated with lower luteal phase progesterone in a recent study of healthy regularly menstruating women¹, this could contribute to luteal phase deficiency whereby the corpus luteum does not produce sufficient progesterone to maintain the early embryo.

High homocysteine is a marker for abnormal folate metabolism and/or deficiencies in B vitamins (e.g., active folate, B₁₂, or B₆)⁵, which can be the result of diminished homocysteine remethylation. Given that women in the current study were folate-replete and folate was not inversely associated with adverse outcomes in this study, high homocysteine in this population may have been the result of deficiency in enzymes involved in remethylation, such as 5,10-methylenetetrahydrofolate reductase (MTHFR)⁷. In particular, a mutation in *MTHFR677* has been previously been associated with elevated risk of pregnancy loss in some, but not all, studies^{36, 37}.

Unanswered questions and proposals for future research

This is the first study to examine pre-conceptional biological indicators of folate status and multiple reproductive outcomes among healthy folate-replete women. In this population, higher preconception plasma homocysteine, but not folate, was associated with higher risk of pregnancy loss among women with 2 prior losses. These findings provide valuable new evidence on the relationships between markers of one-carbon metabolism and pregnancy loss risk in folate-replete women attempting to conceive after a history of pregnancy loss. These data justify further evaluation to clarify the role of folate and homocysteine metabolism in normal and abnormal reproduction and early pregnancy. Since safe treatment options are available, this pathway is an attractive therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a glance:

- A.** Most studies of folate metabolism and reproduction have been conducted in folate deficient populations and among non-pregnant women.
- B.** Our findings suggest that preconception homocysteine, but not folate, is linked to pregnancy loss among folate-replete women with a history of 2 pregnancy losses.
- C.** These data justify further study of the role of folate and homocysteine metabolism in normal and abnormal early pregnancy.

Condensation:

Preconception homocysteine was linked to pregnancy loss among folate-replete women with two prior losses, justifying further study of folate metabolism in normal and abnormal pregnancy.

Figure 1.

Effects of Aspirin in Gestation and Reproduction recruitment stages: Number of women who were screened, eligible, and completed baseline and randomization visits. *Reproduced with permission*¹².

Figure 2.

Restricted cubic spline of weighted^a and adjusted^b relative risks and 95% confidence intervals between preconception **plasma homocysteine** and **pregnancy loss** by prior loss number, relative to 6.0 μmol/L^c ^a Anovulation models weighted for missing anovulation (n = 403; 9.4% cycles) and homocysteine (n = 22; 1.8% women); pregnancy models weighted for withdrawal before pregnancy (n = 128; 10.4%) and missing homocysteine, pregnancy loss models weighted for withdrawal (n = 140; 11.4% women), non-pregnancy (n = 431, 35.1% women), and missing homocysteine.

^b Adjusted for age, reciprocal of BMI, previous loss number (1 vs. 2), and parity (0, 1+)

^c Relative risks defined relative to median values for homocysteine (6.0 μmol/L)^{12, 13} among U.S. reproductive-aged women

Table 1.

Preconception folate and homocysteine by covariates

Covariate		Folate ^a (nmol/L)		Log homocysteine ^a (μmol/L)	
Continuous	n	β (SE)	n	β (SE)	
Age	1033	0.16 (0.098)	1206	0.0045 (0.0013)	
BMI	1015	−0.52 (0.069)	1186	0.0016 (0.00093)	
Missing	20		20		
		Folate ^b (nmol/L)		Homocysteine ^c (μmol/L)	
Categorical	n	mean (SD)	n	median (IQR)	
Marital status					
Married	935	57.8 (14.6)	1106	7.9 (7.0–9.1)	
Not married	98	45.8 (14.7)	100	8.4 (7.3–9.9)	
Parity					
0	480	57.7 (14.0)	564	8.1 (7.1–9.3)	
1+	553	55.8 (15.7)	642	7.8 (6.9–9.0)	
Loss number					
1	681	57.2 (14.8)	807	7.9 (7.0–9.1)	
2	352	55.6 (15.3)	399	8.0 (6.9–9.3)	
Race					
White	973	57.2 (14.7)	1142	7.9 (6.9–9.1)	
Non-white	60	47.0 (16.5)	64	8.4 (7.7–9.5)	
Education					
Up to high school	152	51.5 (14.7)	163	8.1 (7.0–9.4)	
Beyond high school	880	57.6 (14.9)	1042	7.9 (7.0–9.1)	
Missing	1	34.3 (n/a)	1	8.2 (n/a)	
Annual income (USD)					
19,999	85	52.0 (14.9)	92	7.9 (6.9–9.1)	
20–39,999	280	55.9 (15.5)	308	8.0 (7.0–9.1)	
40–74,999	145	59.3 (13.9)	178	8.0 (7.0–9.1)	
75–99,999	114	59.0 (15.0)	148	8.0 (7.2–9.5)	
100,000	408	56.5 (14.8)	479	7.9 (6.9–9.2)	
Missing	1	54.2 (n/a)	1	7.4 (n/a)	
Smoking status					
Any	135	51.8 (15.1)	147	8.5 (7.3–9.9)	
None	888	58.0 (14.6)	1049	7.9 (6.9–9.1)	
Missing	10	53.3 (13.1)	10	8.3 (8.0–9.2)	
Alcohol consumption (past yr)					
Any	336	52.8 (15.4)	398	8.2 (7.1–9.5)	
None	683	58.6 (14.6)	793	7.8 (6.9–9.0)	

Missing	14	54.7 (7.7)	15	8.1 (7.5–8.4)
<hr/>				
Taking folic acid (baseline)				
Yes	816	56.6 (14.9)	957	8.0 (7.0–9.2)
No	202	57.4 (15.3)	234	7.8 (7.0–9.0)
Missing	15	50.2 (14.1)	15	8.1 (6.9–9.1)

^aLinear regression; linearity confirmed through restricted cubic splines; homocysteine log transformed to attain approximate normality

^bT-test and ANOVA for approximate normal distribution of serum folate

^cWilcoxon Mann Whitney test for non-normal distribution of plasma homocysteine

Table 2.

Weighted^a and pooled^b relative risks and 95% confidence intervals between preconception serum folate^c and anovulation, pregnancy, and pregnancy loss

Outcome by exposure	Unadjusted RR (95% CI)	Fully adjusted RR (95% CI)
Serum folate		
Anovulation	0.91 (0.80, 1.02)	0.98 (0.86, 1.10)
Pregnancy	1.01 (0.98, 1.05)	1.00 (0.98, 1.03)
Pregnancy loss	1.06 (0.90, 1.21)	1.07 (0.91, 1.22)

RR – relative risk

^a Anovulation models (n = 4266 cycles, 1222 women) weighted by the inverse of the cluster size; pregnancy models (n = 1100) weighted for withdrawal before pregnancy (n = 128; 10.4%); pregnancy loss models (n = 657) weighted for withdrawal (n = 140; 11.4% women) and non-pregnancy (n = 431, 35.1% women)

^b Estimates individually calculated for each of 20 imputations for anovulation and 50 imputations for pregnancy and pregnancy loss; imputation-specific estimates pooled into a single summary measure estimate for each outcome

^c Calculated for an increase of 15 nmol/L serum folate, approximately 1 standard deviation

Weighted^a and pooled^b relative risks and 95% confidence intervals between preconception plasma homocysteine^c and pregnancy loss

Table 3.

Outcome by exposure	1 or 2 prior losses			1 prior loss			2 prior losses		
	Unadjusted	Fully adjusted	RR (95% CI)	Unadjusted	Fully adjusted	RR (95% CI)	Unadjusted	Fully adjusted	RR (95% CI)
Plasma homocysteine									
Anovulation	1.01 (0.90, 1.11)	1.00 (0.89, 1.11)	1.02 (0.86, 1.18)	1.02 (0.86, 1.18)	0.99 (0.84, 1.15)	0.99 (0.86, 1.12)	0.99 (0.84, 1.13)		
Pregnancy	0.95 (0.91, 1.00)	0.99 (0.96, 1.02)	0.93 (0.87, 0.99)	0.93 (0.87, 0.99)	0.98 (0.92, 1.03)	0.99 (0.92, 1.05)	0.99 (0.94, 1.04)		
Pregnancy loss	1.12 (1.01, 1.24)	1.12 (1.00, 1.23)	1.05 (0.86, 1.25)	1.05 (0.86, 1.25)	1.03 (0.84, 1.23)	1.11 (0.98, 1.25)	1.15 (1.00, 1.29)		

RR – relative risk

^aAnovulation models (n = 4266 cycles, 1222 women) weighted by the inverse of the cluster size; pregnancy models (n = 1100) weighted for withdrawal before pregnancy (n = 128; 10.4%); pregnancy loss models (n = 657) weighted for withdrawal (n = 140; 11.4% women) and non-pregnancy (n = 431; 35.1% women)

^bEstimates individually calculated for each of 20 imputations for anovulation and 50 imputations for pregnancy and pregnancy loss; imputation-specific estimates pooled into a single summary measure estimate for each outcome

^cCalculated for an increase of 2 µmol/L plasma homocysteine, approximately 1 standard deviation