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Maternal nutritional status in first-trimester pregnant women – A pilot study

Bogdan Ioan Ștefănescu [1,2], George Țocu [1,2*], Corina Manole [1,2], Georgiana Bianca Constantin [1], Valerii Luțenco [1,2], Tiberiu Ioan Mihalache [1,2], Andrei Alin Ionescu [1,2], Miruna Maria Ștefănescu [3], Laurențiu Tony Hangan [4], Sergiu Ioachim Chirilă [4]

[1] Faculty of Medicine and Pharmacy, Research Center in the Medical-Pharmaceutical Field, "Dunărea de Jos" University of Galati, Galati, ROMANIA
[2] "Sf. Apostol Andrei" County Emergency Clinical Hospital, Galati, ROMANIA
[3] Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, ROMANIA
[4] Faculty of Medicine, "Ovidius" University of Constanta, Constanta, ROMANIA
***Corresponding Author:** george.tocu@ugal.ro

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ABSTRACT

Background/objectives: Spontaneous abortion (SAb) is the most frequent first-trimester complication, often with unclear etiology. This study compared hematological and biochemical markers of maternal nutritional status at clinical presentation in women with ongoing pregnancy and first-trimester SAb.

Methods: A prospective analytical observational comparative study included 127 women at 6 to 11 gestational weeks admitted between September and December 2025. The ongoing pregnancy group included 105 women, and the SAb group included 22 women. Hematological indices, serum proteins, vitamin B12, iron, ferritin, folate, vitamin D, calcium, and magnesium were assessed.

Results: SAb was associated with lower hemoglobin, hematocrit, iron, folate, and vitamin D levels, while vitamin B12 was higher. Ferritin, calcium, and magnesium did not differ significantly.

Conclusions: Serum folate and vitamin D remain associated with SAb, without proving causality or predictive value.

Keywords: first trimester pregnancy, folate deficiency, vitamin D deficiency, iron homeostasis, pregnancy loss

INTRODUCTION

According to the World Health Organization (WHO), spontaneous abortion (SAb) is defined as the spontaneous termination of pregnancy before 20 weeks of gestation or the expulsion of a fetus weighing less than 500 grams [1, 2].

Although SAb is the most common unfavorable pregnancy outcome, reaching almost 15% of all recognized pregnancies, the exact cause remains unknown in many cases. Besides chromosomal abnormalities, significant malformations of the embryonic structures, significant maternal comorbidities and external toxic factors, many recent animal and human studies suggest that maternal nutritional status may also play a role in the etiology of SAb [3, 4]. In addition, maternal malnutrition is also associated with impaired embryonic and fetal development and growth [5].

Iron requirements increase during pregnancy as a consequence of maternal blood volume expansion, increased erythropoiesis, placental development, and fetal growth [6]. Serum iron and ferritin are frequently used to evaluate iron availability and iron stores in pregnant women. Disturbances in iron homeostasis have also been reported in relation to adverse reproductive outcomes, including recurrent pregnancy loss [7].

Alterations in selected trace elements and minerals, including copper, magnesium, zinc, calcium, and the calcium/magnesium ratio, have also been described in pregnancies complicated by SAb [8, 9].

Moreover, iron, folate, and vitamin B12 are mandatory for deoxyribonucleic acid (DNA), RNA, and protein-synthesis, which is essential for fetal growth. The demand for folate significantly escalates during pregnancy. It was indicated that a diminished serum folate level may contribute to repeated pregnancy losses [10].

Alongside folate insufficiency, impaired folate metabolism, including hyperhomocysteinemia and vitamin B12 deficiency, is recognized to elevate the risk of neural tube defects, congenital anomalies, placental abruption, and cardiac problems [11-16].

Vitamin D deficiency, while historically linked to maternal and newborn bone disorders, is more prevalent in women experiencing significant reproductive and pregnancy-associated problems, including preeclampsia, gestational diabetes, and premature birth [11].

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Vitamin D has been increasingly studied in reproductive medicine because of its potential involvement in immune regulation, implantation, placental development, and maternal-fetal tolerance. Low vitamin D status has been reported in association with several adverse pregnancy outcomes, including preeclampsia, gestational diabetes, preterm birth, and pregnancy loss, although the strength of this association may vary according to study design and timing of assessment [11, 17].

The purpose of this study was to compare selected hematological and biochemical markers of maternal nutritional status measured at clinical presentation between women with ongoing pregnancy in the first trimester with a control group of patients with SAb admitted in emergency.

MATERIALS AND METHODS

Study Design and Setting

We conducted a prospective analytical observational comparative study in the 2nd Clinic of Obstetrics and Gynecology, Clinical Emergency County Hospital "Sf. Ap. Andrei", Galaţi, between September 1, 2025, and December 31, 2025. A total of 127 pregnant women between 6 and 11 gestational weeks were included after admission and clinical and laboratory assessment at presentation. The total number of patients was divided into two groups: a study arm consisting of 105 patients with ongoing pregnancy and a control arm including 22 patients with spontaneous pregnancy loss. Recurrent spontaneous abortion (RSAb), defined as a history of at least three spontaneous pregnancy losses, was not identified in the analyzed population.

Study Population and Data Collection

A structured dataset was created for each patient. Recorded variables included maternal demographic characteristics, namely age, gestational age at admission, area of residence (urban or rural), educational level, and preconception folic acid intake. Obstetrical history variables included parity and number of previous abortions. Associated maternal pathology and laboratory parameters relevant to maternal nutritional status in early pregnancy were recorded.

All variables were entered at the individual patient level, with one row per pregnancy and one column for each clinical or laboratory variable, allowing direct comparison between the two groups and subsequent statistical analysis of the relationship between maternal nutritional biomarkers and pregnancy outcome.

Inclusion and Exclusion Criteria

The study included pregnant women with a gestational age between 6 and 11 weeks who were admitted during the study period and underwent both clinical and laboratory evaluation at admission. Patients were excluded if complete clinical or laboratory data were not available for analysis.

Blood Collection, Sample Transport, and Processing

Maternal venous blood samples were collected at the time of admission to the department of obstetrics and gynecology. Specimens were transferred to the hospital laboratory according to the standard institutional transport procedure by authorized hospital personnel.

Hematological analyses were performed on whole blood collected in vacuum tubes containing ethylenediaminetetraacetic acid, which is the standard anticoagulant for complete blood count testing. Biochemical and immunological analyses were performed on blood collected in serum tubes, allowing clot formation followed by centrifugation and serum separation.

Samples for hematological testing were processed by direct automated analysis within a maximum of 1 hour after arrival in the laboratory. For biochemical and immunological testing, samples were allowed to clot and then centrifuged; serum was obtained and processed within a maximum of 2 hours after collection. All specimens were handled according to the routine standard operating procedures of the hospital laboratory.

Laboratory Assessment and Analytical Methods

The evaluated laboratory parameters included hemoglobin concentration, hematocrit, serum iron, ferritin, serum folate, vitamin B12, vitamin D, total serum proteins, calcium, and magnesium.

Hematological parameters, namely hemoglobin concentration and hematocrit, were measured using the Mindray BC-6800 Plus automated hematology analyzer. This platform provides automated complete blood count analysis with 5-part leukocyte differential and uses multidimensional cellular analysis technology based on laser scatter and fluorescence signals for white blood cell differentiation.

Biochemical parameters, namely serum iron, ferritin, total serum proteins, calcium, and magnesium, were measured using the Mindray BS-800M clinical chemistry analyzer. According to the manufacturer's technical specifications, this platform performs clinical chemistry testing using automated photometric methodology.

Immunological parameters, namely serum folate, vitamin B12, and vitamin D, were measured using the Atellica IM 1600 analyzer. According to the manufacturer's specifications, this platform performs immunoassay testing using chemiluminescent detection methodology based on advanced acridinium ester technology.

The selected laboratory parameters were investigated because of their potential relevance to early placental and embryonic development. Serum iron and ferritin were assessed as indicators of iron availability and body iron stores. Serum folate and vitamin B12 were evaluated because of their essential role in DNA synthesis, cell proliferation, and embryofetal development. Vitamin D, calcium, and magnesium were analyzed because of their potential involvement in implantation, placental development, and immune modulation required for the maintenance of normal pregnancy. Total serum proteins were included as a general indicator of maternal nutritional and metabolic status.

None of the patients had undergone hematological or biochemical assessment prior to conception, which represents an important limitation of the study.

Statistical Analysis

Continuous variables were assessed for normality using the Shapiro-Wilk test. Data are presented as mean (M) (standard deviation [SD]) and median [interquartile range] for continuous variables, and n (%) for categorical variables.

Between-group comparisons were performed using the Wilcoxon rank-sum test for continuous variables and Pearson chi-squared test (with Monte Carlo simulation when appropriate) for categorical variables. Statistical significance was set at $p < 0.05$.

Given the complete separation observed in standard logistic regression (due to strong group discrimination by nutritional biomarkers), multivariate analysis employed Firth penalized likelihood logistic regression, which provides stable and unbiased estimates in scenarios with small sample sizes or separation. Given the limited number of SAb cases, multivariable models were considered exploratory and were used only to assess whether the observed associations persisted after adjustment for selected covariates. These models were not intended to establish causality, temporality, or clinical prediction. No formal clinical prediction model was developed. Therefore, discrimination, calibration, and clinical utility metrics were not interpreted as primary outcomes, and the stability of the regression estimates remains limited by the small number of events, wide confidence intervals (CIs), and potential overfitting.

Variables with $p < 0.05$ in univariate analysis were included in model 1, whereas variables with $p < 0.10$ were included in model 2. For model 2, a liberal variable selection threshold of $p < 0.10$ was applied to minimize premature exclusion of clinically relevant variables prior to multivariable analysis. A third model, model 3, was additionally constructed based on clinically relevant confounders selected according to biological plausibility. Model fit was assessed using the Akaike information criterion (AIC).

All statistical analyses were performed using R version 4.x (R Foundation for Statistical Computing, Vienna, Austria), with the packages gtsummary, logistf, ggplot2, and patchwork.

RESULTS

Study Population

A total of 127 pregnant women were included in the analysis: 105 patients with ongoing pregnancy in the study arm and 22 patients with SAb in the control arm (**Table 1**).

Table 1. Baseline characteristics by study arm

Characteristic	Overall (n = 127[1])	Study (n = 105[1])	Control (n = 22[1])	p[2]
Age (years)	27.98 (5.40)/28.00 [24.00-32.00]	27.95 (5.54)/28.00 [24.00-32.00]	28.09 (4.81)/28.50 [25.00-32.00]	0.774
Gestational age (weeks)				0.504
6	15 (12%)	11 (10%)	4 (18%)	
7	24 (19%)	18 (17%)	6 (27%)	
8	23 (18%)	18 (17%)	5 (23%)	
9	24 (19%)	21 (20%)	3 (14%)	
10	24 (19%)	22 (21%)	2 (9.1%)	
11	17 (13%)	15 (14%)	2 (9.1%)	
Environment				0.975
Urban	61 (48%)	51 (49%)	10 (45%)	
Rural	66 (52%)	54 (51%)	12 (55%)	
Educational level				0.715
Primary school	0 (0%)	0 (0%)	0 (0%)	
Gymnasium	20 (16%)	16 (15%)	4 (18%)	
High school	69 (54%)	56 (53%)	13 (59%)	
University	38 (30%)	33 (31%)	5 (23%)	
Imminent abortion	105 (100%)	105 (100%)	0 (0%) NA	
Hyperemesis	22 (21%)	22 (21%)	0 (0%) NA	
Associated pathology				0.113
None	81 (64%)	63 (60%)	18 (82%)	
Urinary tract infection	24 (19%)	20 (19%)	4 (18%)	
Thrombophilia	7 (5.5%)	7 (6.7%)	0 (0%)	
Genital infection	15 (12%)	15 (14%)	0 (0%)	
Folic acid preconception	18 (14%)	18 (17%)	0 (0%)	0.078
Births (n)				< 0.001
1	52 (41%)	51 (49%)	1 (4.5%)	
2	41 (32%)	34 (32%)	7 (32%)	
3	22 (17%)	17 (16%)	5 (23%)	
4	9 (7.1%)	2 (1.9%)	7 (32%)	
5	2 (1.6%)	1 (1.0%)	1 (4.5%)	
6	1 (0.8%)	0 (0%)	1 (4.5%)	
Abortions (n)				0.209
0	52 (41%)	47 (45%)	5 (23%)	
1	43 (34%)	32 (30%)	11 (50%)	
2	24 (19%)	19 (18%)	5 (23%)	
3	8 (6.3%)	7 (6.7%)	1 (4.5%)	
Tobacco consumption	19 (15%)	16 (15%)	3 (14%)	> 0.999
Hemoglobin (g/dL)	11.01 (1.47)/10.80 [9.90-12.10]	11.43 (1.25)/11.20 [10.40-12.50]	9.04 (0.51)/9.05 [8.70-9.20]	< 0.001
Hematocrit (%)	33.44 (3.09)/32.80 [30.80-35.70]	34.08 (2.96)/33.80 [31.60-36.40]	30.38 (1.38)/30.10 [29.40-31.60]	< 0.001
Total proteins (g/dL)	6.13 (0.57)/6.12 [5.76-6.46]	6.17 (0.60)/6.14 [5.80-6.55]	5.93 (0.34)/6.00 [5.70-6.20]	0.074
Vitamin B12 (pg/mL)	340.86 (74.51)/327.00 [288.00-386.00]	336.41 (77.54)/318.00 [284.00-376.00]	362.09 (54.48)/373.50 [302.00-402.00]	0.036*

Table 1 (Continued).

Characteristic	Overall (n = 127[1])	Study (n = 105[1])	Control (n = 22[1])	p[2]
Serum iron (ug/dL)	68.97 (25.47)/68.83 [55.68-78.00]	73.06 (25.23)/72.48 [58.90-80.00]	49.48 (16.12)/48.18 [34.81-64.80]	< 0.001
Ferritin (ng/mL)	65.86 (25.93)/68.43 [48.27-84.88]	65.94 (25.66)/68.94 [49.00-84.70]	65.50 (27.85)/64.82 [45.76-88.40]	0.709
Serum folate (ng/mL)	14.21 (3.29)/14.18 [11.61-16.83]	14.84 (3.16)/14.98 [12.46-17.08]	11.18 (1.97)/11.27 [9.26-12.83]	< 0.001
Vitamin D (ng/mL)	26.03 (5.45)/26.52 [22.73-30.18]	27.75 (4.12)/27.36 [24.94-31.34]	17.85 (2.98)/18.05 [16.76-20.03]	< 0.001
Free calcium (mg/dL)	4.09 (0.26)/4.12 [3.96-4.23]	4.11 (0.25)/4.12 [3.98-4.26]	3.98 (0.27)/4.03 [3.78-4.18]	0.056
TSM (mg/dL)	1.83 (0.24)/1.85 [1.73-1.97]	1.83 (0.24)/1.85 [1.73-1.97]	1.81 (0.21)/1.82 [1.68-1.93]	0.562

Note. TSM: Total serum magnesium; [1]M (SD)/median [Q1-Q3]; n (%); [2]Wilcoxon rank-sum test for continuous variables; Pearson's Chi-squared test for categorical variables; NA: Not applicable; & *p < 0.05

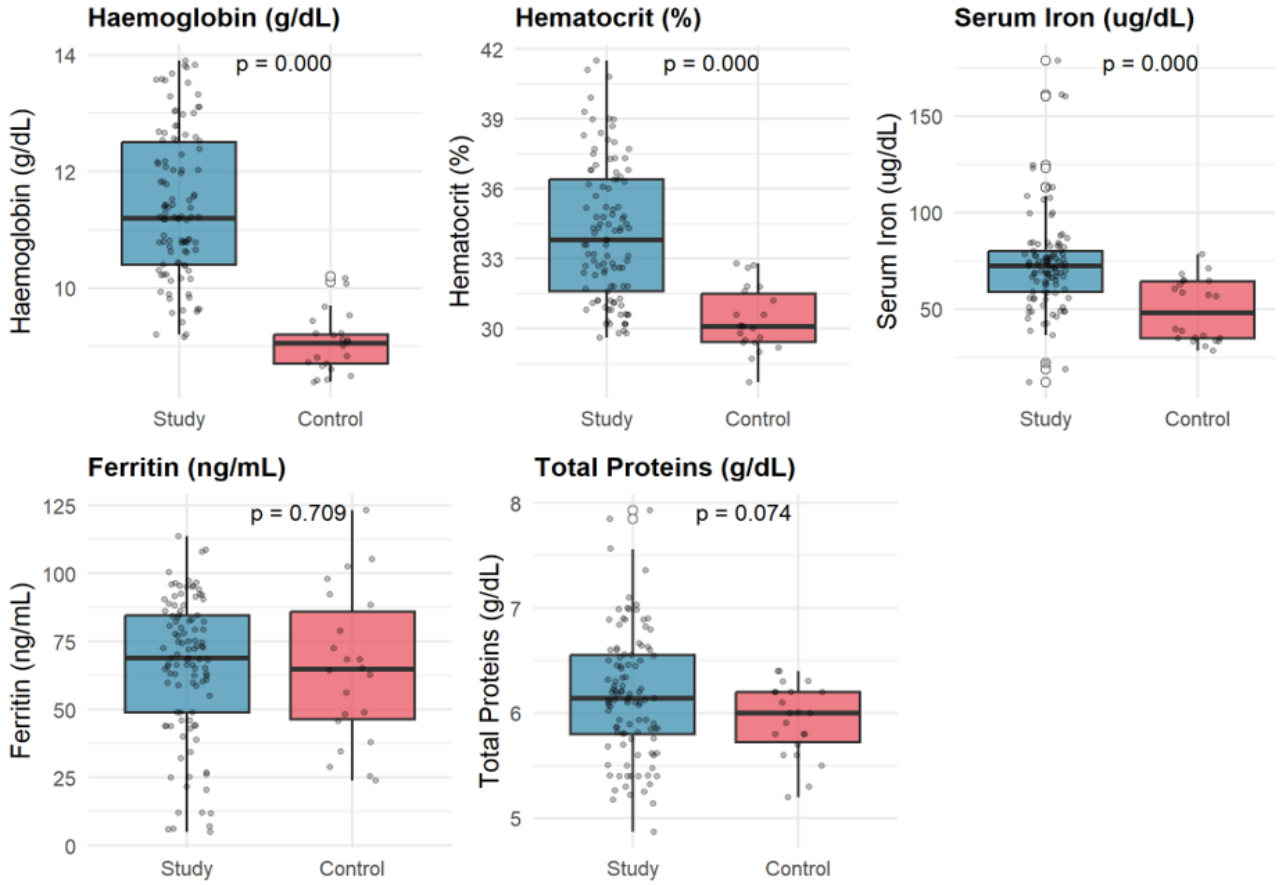


Figure 1. Comparison of hematological parameters between study and control arms (Box plots show median [horizontal line], interquartile range [box], and range [whiskers]; Individual data points are overlaid; p-values are from Wilcoxon rank-sum test) (the authors' own elaboration)

The median gestational age at admission was 8 weeks (range 6-11 weeks), with no significant difference between groups (p = 0.504).

The two groups were broadly comparable in terms of demographic characteristics, including age (p = 0.774), gestational age distribution (p = 0.504), area of residence, urban versus rural, (p = 0.975), and educational level (p = 0.715). Smoking rates were similar (15% in both groups, p > 0.999). Preconception folic acid supplementation was documented in 17% of the study arm and 0% of the control arm (p = 0.078), though this difference did not reach statistical significance at the 0.05 level.

Hematological Parameters

The study arm showed significantly higher hemoglobin levels (11.43 ± 1.25 g/dL) compared to control (9.04 ± 0.51 g/dL, p < 0.001). The control group M falls below the WHO threshold for anemia in pregnancy (< 11 g/dL). Hematocrit was higher in study arm (34.08 ± 2.96%) vs. control (30.38 ± 1.38%, p < 0.001), consistent with characteristics of the patients and sample structure (**Figure 1**). This finding may be largely explained by the amount of blood loss during SAb in the control group rather than a true nutritional deficiency.

Participants in the study arm had 48% higher serum iron (73.06 ± 25.23 µg/dL) compared to controls (49.48 ± 16.12 µg/dL, p < 0.001), indicating better iron status, while no significant difference between groups (p = 0.709) was observed for ferritin, suggesting iron stores were similar despite differences in circulating iron.

Micronutrient Status

A marked vitamin D deficiency was observed in the control arm (17.85 ± 2.98 ng/mL) compared to study arm (27.75 ± 4.12 ng/mL, p < 0.001). Controls averaged < 20 ng/mL, meeting criteria for vitamin D deficiency. In addition, study arm demonstrated 33% higher folate levels (14.84 ± 3.16 ng/mL) vs. controls (11.18 ± 1.97 ng/mL, p < 0.001) (**Figure 2**).

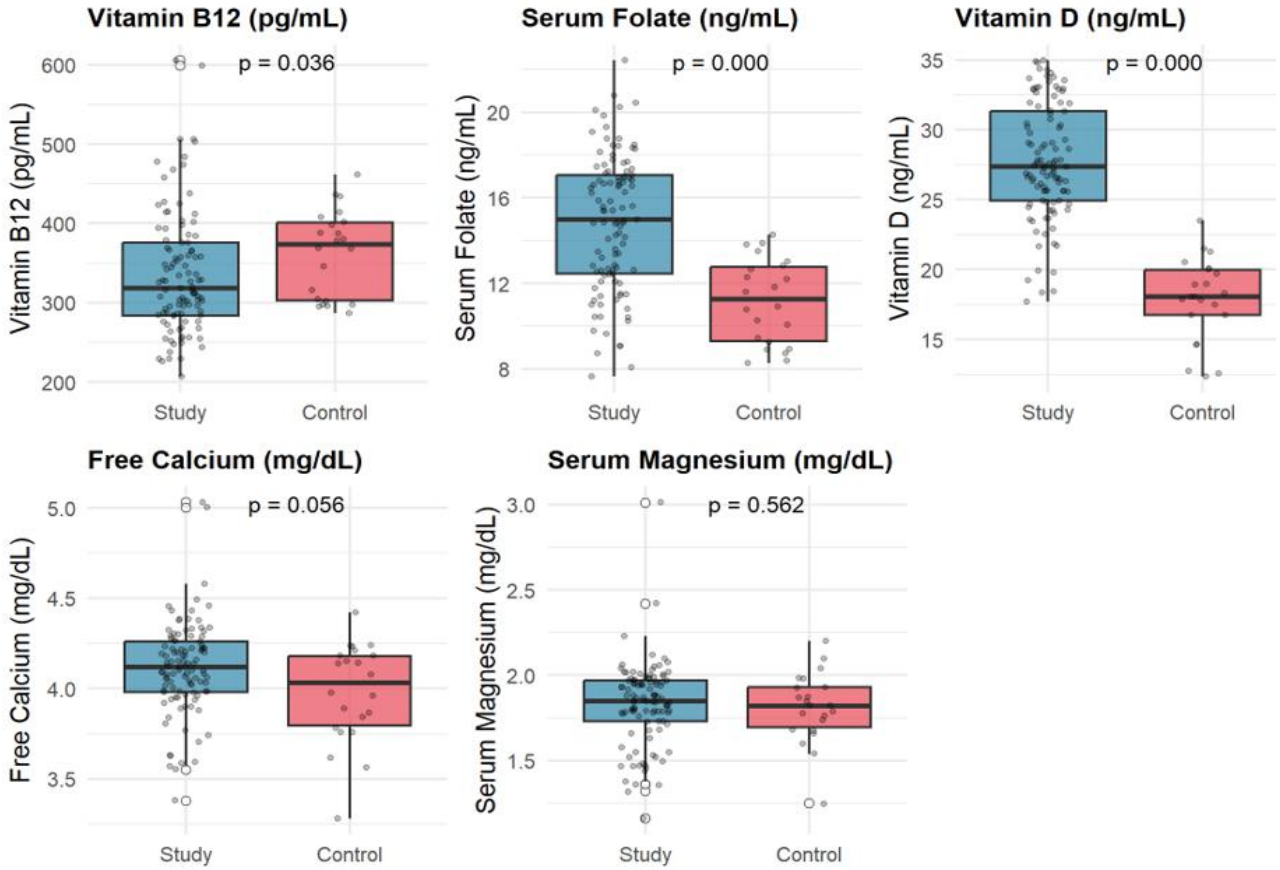


Figure 2. Vitamin and mineral status by study arm (Notable deficiencies were observed in the control arm for vitamin D and folate) (the authors' own elaboration)

Table 2. Multivariate Firth logistic regression – Model 1 (variables selected at $p < 0.05$)

Variable	OR (95% CI)	p
Hemoglobin (g/dL)	48.975 (0.617-1.86 × 10^{214})	0.072
Hematocrit (%)	0.608 (0-5.794)	0.515
Serum iron (ug/dL)	0.941 (0.113-1.085)	0.333
Serum folate (ng/mL)	1.202 (0-4.205)	0.657
Vitamin D (ng/mL)	1.31 (0.966-3.482)	0.084
Vitamin B12 (pg/mL)	0.975 (0.372-1.004)	0.097

Note. n = 127; AIC = 89.65; & CI: 95% profile likelihood CI

Table 3. Multivariate Firth logistic regression – Model 2 (variables selected at $p < 0.10$)

Characteristic	OR (95% CI)	p
Hemoglobin (g/dL)	76.549 (1.263-2.49 × 10^{45})	0.037*
Hematocrit (%)	0.453 (0.026-2.961)	0.421
Serum iron (ug/dL)	0.94 (0.192-1.056)	0.455
Serum folate (ng/mL)	1.15 (0.62-4.672)	0.642
Vitamin D (ng/mL)	1.232 (0.891-2.107)	0.180
Vitamin B12 (pg/mL)	0.976 (0.521-1.006)	0.107
Total proteins (g/dL)	1.685 (0.007-75767.675)	0.772
Free calcium (mg/dL)	7.176 (0.002-2.90 × 10^{30})	0.453
Folic acid preconception: Yes	0.076 (0-177.33)	0.231

Note. n = 127; AIC = 93.58; CI: 95% profile likelihood CI; &*p < 0.05

Modestly higher vitamin B12 levels were observed in control arm (362.09 ± 54.48 pg/mL) vs. study arm (336.41 ± 77.54 pg/mL, p = 0.036). Still, in both groups the vitamin B12 levels were within normal reference range (> 200 pg/mL). For calcium and magnesium, no significant differences were observed between the two groups (p = 0.056 and p = 0.562, respectively) (**Figure 2**).

Multivariate Analysis

Given the strong discrimination between groups (resulting in complete separation in standard logistic regression), we employed Firth penalized likelihood logistic regression for multivariate analysis (**Table 2**, **Table 3**, and **Figure 3**). This method provides unbiased estimates in scenarios with small sample sizes or perfect prediction.

Model 1 was a multivariable Firth logistic regression model including biomarkers that were significant in univariate comparisons. In this adjusted model, none of the included biomarkers retained a statistically significant association with SAb.

Model 2 included additional variables with $p < 0.10$ in univariate analysis (total proteins, free calcium, preconception folic acid). AIC was 93.58, compared to 89.65 for model 1, suggesting model 1 provides better parsimony without sacrificing fit. Considering model 2, hemoglobin was statistically significant (p = 0.037).

To address the potential influence of clinical confounders, a third multivariable Firth logistic regression model (model 3) was constructed incorporating a priori clinical variables alongside the key nutritional biomarkers. After adjustment for maternal age, obstetric history, tobacco use, preconception folate supplementation, and associated pathology, vitamin D (odds ratio [OR] = 0.440, 95% CI: 0.099-0.689, $p < 0.001$) and

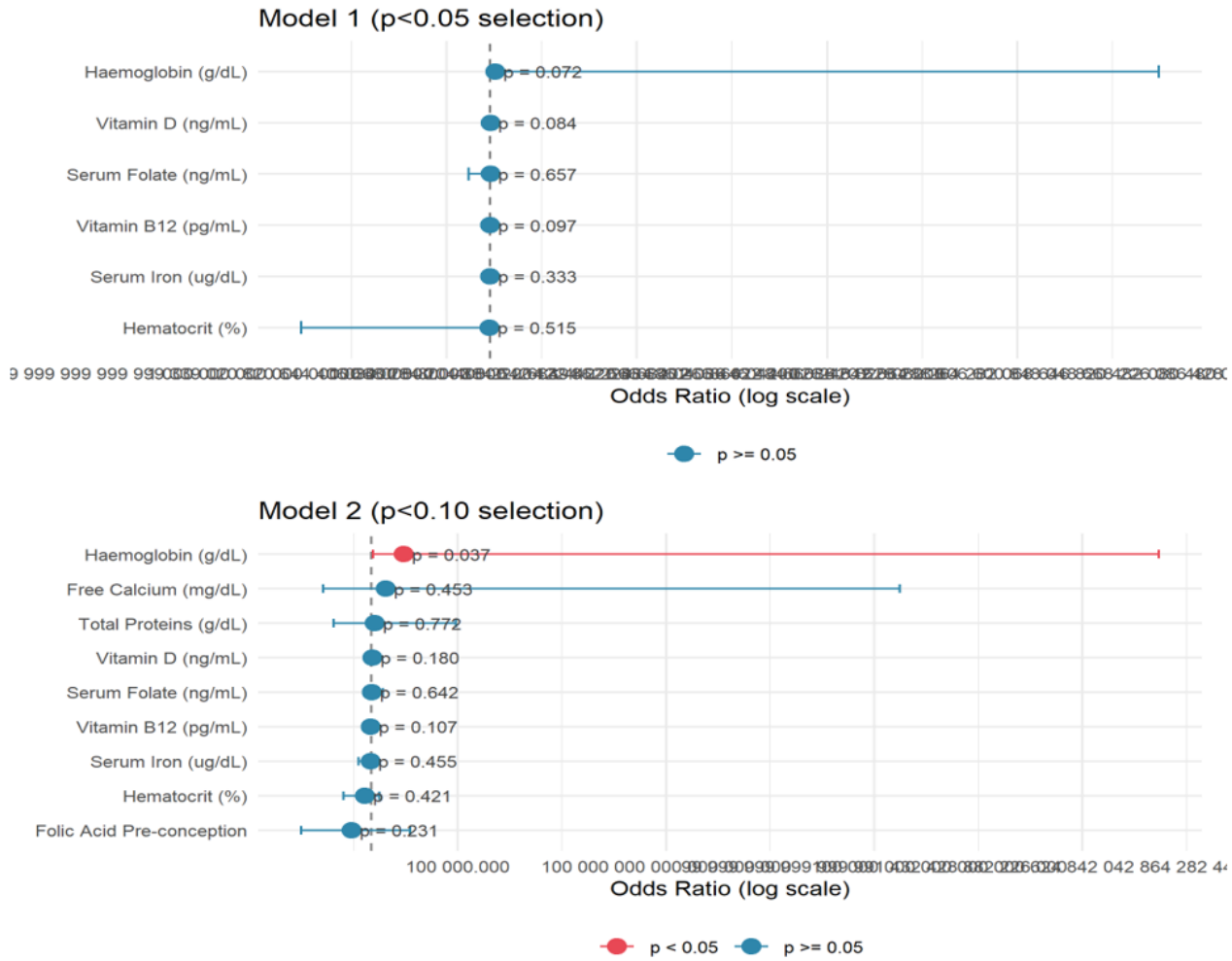


Figure 3. Forest plot showing odds ratios from multivariate Firth logistic regression (Model 1 [top] includes variables significant at p < 0.05; Model 2 [bottom] includes variables at p < 0.10; Error bars represent 95% profile likelihood Cis) (the authors' own elaboration)

Table 4. Multivariate Firth logistic regression – Model 3 (clinically relevant confounders)

Variable	OR (95% CI)	p
Associated pathology	0.303 (0.012-2.441)	0.274
Maternal age (years)	1.16 (0.87-1.868)	0.329
Preconception folate supplement	0.87 (0.006-23.882)	0.936
Prior abortions (n)	1.754 (0.554-11.042)	0.362
Serum folate (ng/mL)	0.609 (0.218-0.923)	0.015*
Serum iron (µg/dL)	0.989 (0.934-1.022)	0.525
Tobacco use	3.426 (0-1233.161)	0.657
Vitamin B12 (pg/mL)	1.011 (0.998-1.036)	0.104
Vitamin D (ng/mL)	0.44 (0.099-0.689)	< 0.001

Note. n = 127; AIC = 90.12; CI: 95% profile likelihood CI; & *p < 0.05

serum folate (OR = 0.609, 95% CI: 0.218-0.923, p = 0.015) remained associated with SAb after adjustment.

These findings should be interpreted as adjusted associations rather than causal or predictive relationships. Serum iron (OR = 0.989, p = 0.525) and vitamin B12 (OR = 1.011, p = 0.104) did not retain statistically significant associations after adjustment. None of the selected clinical covariates reached statistical significance in this exploratory model. However, given the limited number of SAb cases and the restricted statistical power, the absence of statistical significance should not be interpreted as evidence that these variables do not act as confounders (**Table 4** and **Figure 4**).

Post-Hoc Power Analysis

Post-hoc statistical power analysis was performed for the between-group comparisons of the assessed nutritional biomarkers (**Table 5**). The highest estimated post-hoc power was observed for hemoglobin, hematocrit, serum iron, serum folate, and vitamin D, consistent with the large standardized between-group differences for these parameters. In contrast, estimated power was low for vitamin B12, ferritin, magnesium, and total proteins, and moderate for free calcium. These results should be interpreted descriptively, as post-hoc power is derived from the observed effect sizes and does not replace prospective sample size estimation.

DISCUSSION

The present study supports the concept that maternal nutritional status is meaningfully associated with early pregnancy outcome, while also emphasizing that SAb should not be reduced to a single-deficiency model. In our cohort, women with SAb showed a pattern characterized by lower hemoglobin, hematocrit, serum iron, folate and vitamin D, together with a modest difference in vitamin B12 and no clear separation for calcium, magnesium or ferritin.

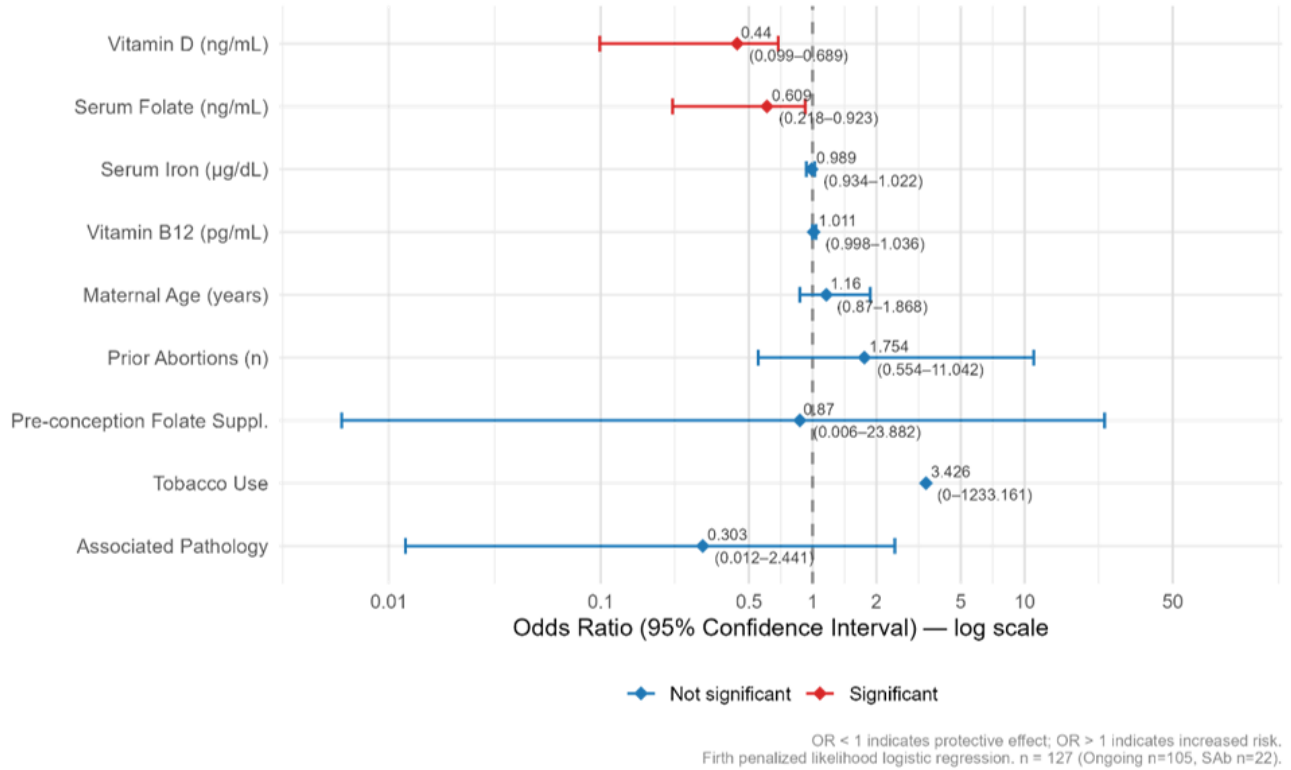


Figure 4. Forest plot showing odds ratios from multivariate Firth logistic regression model 3 (the authors' own elaboration)

Table 5. Post-hoc statistical power analysis for between-group comparisons of nutritional biomarkers

Biomarker	M ongoing pregnancy	SD ongoing pregnancy	Mean SAb	SD SAb	Cohen's d	Effect size category	Post-hoc power (%)	Wilcoxon p
Hemoglobin (g/dL)	11.43	1.25	9.04	0.51	2.062	Large	100	< 0.001
Hematocrit (%)	34.08	2.96	30.38	1.38	1.342	Large	100	< 0.001
Serum iron (µg/dL)	73.06	25.23	49.48	16.12	0.985	Large	98.6	< 0.001
Serum folate (ng/mL)	14.84	3.16	11.18	1.97	1.222	Large	99.9	< 0.001
Vitamin D (ng/mL)	27.75	4.12	17.85	2.98	2.504	Large	100	< 0.001
Vitamin B12 (pg/mL)	336.41	77.54	362.09	54.48	0.346	Small	31.1	0.036*
Ferritin (ng/mL)	65.94	25.66	65.5	27.85	0.017	Small	5.1	0.709
Free calcium (mg/dL)	4.11	0.25	3.98	0.27	0.531	Medium	61.3	0.056
Magnesium (mg/dL)	1.83	0.24	1.81	0.21	0.08	Small	6.3	0.562
Total poteins (g/dL)	6.17	0.6	5.93	0.34	0.423	Small	43.2	0.074

Note. Power estimates were calculated for comparisons between women with ongoing pregnancy (n = 105) and women with spontaneous abortion (n = 22), using a two-sided approximation corresponding to the Wilcoxon rank-sum test, with α = 0.05; Cohen's d represents the standardized mean difference between groups; Conventional thresholds were used to classify effect size as small, medium, or large; Post-hoc power (%) represents the estimated probability of detecting the observed between-group difference at α = 0.05; & *p < 0.05

These findings suggest that SAb was accompanied by a pattern of cumulative nutritional and metabolic alterations rather than by one isolated biochemical abnormality. This interpretation is consistent with broader literature describing SAb as a multifactorial event in which maternal micronutrient inadequacy may interact with oxidative stress, inflammatory activation, endocrine adaptation, placental development and fetal growth demands [3, 5].

Importantly, the same literature cautions that although micronutrient disturbances are biologically plausible contributors to adverse pregnancy outcomes, their clinical effect is often mediated through overlapping pathways rather than through a single direct causal chain.

All biomarkers were measured at clinical presentation and thus, the observed differences should be interpreted with caution in terms of event prediction or causality. This interpretive caution is particularly relevant for hematological parameters, since hemoglobin and hematocrit values measured after or during a miscarriage may reflect both the maternal nutritional status before conception and acute changes occurring at the time of pregnancy loss, especially blood loss and related haemodynamic adaptation.

This study was designed to investigate patients with ongoing pregnancy but, and it was found that, when compared the two study arms, the M values of hemoglobin and hematocrit were significantly lower in the women who had a miscarriage (control arm). The degree of anemia in the control arm may largely depend on the amount of blood loss during SAb and on the preconceptional hematological status of the patients. Anemia during pregnancy is a well-documented predisposing factor for stillbirth, preterm delivery, and low birth weight, which may be explained by reduced oxygen and nutrient delivery to the growing fetus and placenta [11, 18].

A large study reported that low preconceptional hemoglobin concentration increased the risk of miscarriage. In this case, the hemoglobin concentration prior to conception may be an important factor in the woman`s ability to sustain herself through the crucial early months of pregnancy [18]. Unfortunately, in our study, we could not assess the hematological status of the patients before pregnancy; therefore, low hemoglobin and hematocrit levels at admission in the control arm were interpreted primarily as secondary to blood loss during abortion.

The relationship between iron and SAb is an old theme with many different perspectives. Iron-related findings in SAb are not only due to the simple model of iron deficiency and low iron deposits. Serum iron was low in our SAb cases (control arm), whereas ferritin did not show any difference between the two groups of patients. Although ferritin is a storage iron protein it also plays a role in iron homeostasis. However, it is not always an accurate marker of iron bio-availability for maternal as well as fetal use in the active phase of a reproductive cycle [11]. Therefore, iron homeostasis is also a factor to be considered in the absence of iron deficiency in SAb [19]. In fact, serum ferritin has been reported to be lower in women with RSAb than in control women and is inversely correlated with the number of miscarriages [19, 20]. Moreover, serum iron is a routine test in antenatal care which possesses a low positive predictive value for SAb when tested alone [21]. Therefore, iron-related findings in SAb are far from being reduced to the simple model of iron deficiency or iron stores depletion.

Among the biochemical anomalies identified in this investigation, folate was recognized as one of the most significant. In our study, women experiencing SAb in the control arm exhibited markedly reduced serum folate concentrations, which is consistent with previous studies.

Folate is crucial for one-carbon metabolism, nucleotide synthesis, methylation reactions, DNA replication, and epigenetic regulation. Consequently, insufficient folate availability in the early stages of pregnancy may hinder the rapid cellular proliferation necessary for embryogenesis and trophoblast development [22, 23].

The literature consistently associates low folate levels with miscarriage, recurrent pregnancy loss, and other negative reproductive outcomes, suggesting that folate is significant not only for neural tube abnormalities but also for sustaining pregnancy [24-26]. The significance of folate in our group is further enhanced by the metabolic interdependence of folate and vitamin B12 within the same one-carbon cycle, indicating that folate deficiency may have clinically relevant consequences even in the absence of a significant reduction in serum vitamin B12 levels [12, 27]. When comparing our findings with previous studies, serum folate appears to be one of the nutritional biomarkers most consistently associated with early pregnancy loss, although its predictive or etiological significance cannot be established from the present design.

In our study, vitamin B12 levels exhibited moderate differences between groups; however, the degree of variation was far less pronounced compared to folate or vitamin D, and the values remained largely within the normal range. This is significant since substantial scientific evidence has linked decreased maternal vitamin B12 levels to miscarriage, early pregnancy loss, and reproductive risks associated with hyperhomocysteinemia [24, 25]. Nonetheless, our findings do not inherently contradict the existing literature; instead, they serve to enhance it. Vitamin B12 should be understood as an integral component of the one-carbon network rather than as an entirely autonomous signal, due to the fact that its biological effects are intricately linked with folate metabolism, methyl-group transfer, and homocysteine control [23, 27]. The evidence regarding folate-vitamin B12 imbalance indicates that detrimental pregnancy outcomes may result not solely from a complete lack of one nutrient, but also from an imbalance within the paired metabolic pathway [28]. In this context, the comparatively faint vitamin B12 signal in our group may suggest that folate disruption was the predominant one-carbon anomaly, while serum vitamin B12 alone lacked the sensitivity to differentiate women with ongoing pregnancy from patients with SAb. Another possible explanation is methodological: serum vitamin B12 may not accurately reflect intracellular or functional deficiency, especially in the absence of homocysteine or methylmalonic acid assessment. Consequently, our results do not identify vitamin B12 as a significant standalone factor in SAb, but they support the broader relevance of the folate-vitamin B12-homocysteine axis in early reproductive success.

Vitamin D was a clear discriminatory factor in this study, which is consistent with several previously published studies. Women with SAb had lower levels of vitamin D compared with the group of patients with ongoing pregnancy. The observation that vitamin D and serum folate remained associated with SAb after adjustment for selected clinical covariates (model 3) supports the robustness of these associations within the present dataset, but it does not establish causality or preevent prediction. Prior research has also reported associations between low folate or vitamin D levels and first-trimester miscarriage, although the interpretation of such findings depends strongly on study design, timing of biomarker assessment, and adjustment for clinical covariates. In the present study, the adjusted model should therefore be interpreted as exploratory. Vitamin D has a clear role in reproductive biology, as vitamin D receptors have been identified in the ovary, endometrium, decidua and placenta [29, 30]. Vitamin D may also play important roles in maternal-fetal tolerance, trophoblast invasion and in the process of placental vascular remodeling. Several studies from the literature state that vitamin D deficiency may lead to a pro-inflammatory state that predisposes women to pregnancy loss [31-33]. In addition, prospective cohort studies and systematic reviews have shown an association between lower vitamin D levels before conception or early in pregnancy and pregnancy loss [17, 34-36]. Our data are consistent with previous studies reporting lower vitamin D levels in women with pregnancy loss, including RSAb. Low levels of vitamin D may reflect an unfavorable biological context for the immunologic and placental functions required for pregnancy maintenance. Hypovitaminosis D is also a common finding in the general pregnant population, therefore, our data may also highlight the importance of adequate vitamin D supplementation for pregnancy in general.

Findings relating to magnesium and calcium should be treated with extreme caution. Serum magnesium levels did not differ between our groups and free calcium levels were at best borderline. At first glance, magnesium and calcium may appear less relevant to SAb. However, as previously discussed, the calcium/magnesium ratio, as well as magnesium and calcium levels, have been associated with abnormal pregnancy outcomes, including spontaneous miscarriage and other pregnancy-related complications [8, 9, 21]. Magnesium is an

important ion required for the activity of hundreds of enzymes, mitochondrial function, neuromuscular function, membrane integrity and also for the immune response and growth of tissues. Therefore, all of these functions are highly relevant to the growth and development of the placenta and fetus as well [37, 38]. In addition, serum magnesium only reflects a small portion of the total body pool and therefore normal serum levels do not exclude the presence of magnesium deficiency [39]. Furthermore, several mechanisms implicated in miscarriage, such as impaired calcium-dependent uterine excitability, hormonal changes, and decreased placental function, have been associated with hypomagnesemia [40, 41]. Therefore, a null finding for magnesium should not be considered conclusive and may instead suggest that the serum measurement is not a sensitive test for assessing magnesium status. Similarly, calcium should be interpreted with caution, as its role may be more subtle or related to the magnesium/calcium ratio, which may differ across subgroups of women.

Overall, the available evidence suggests that maternal nutritional biomarkers may be related to the biological profile of SAb, but their role should be interpreted within a multifactorial framework. Several of the nutrients for which differences between the study groups were demonstrated are supported by clinical and epidemiological studies. In contrast, iron-related parameters, including serum iron, ferritin, and haematologic variables, appear to contribute to the laboratory profile of SAb in a more complex manner, probably due to the effects of acute blood loss [42, 43]. Vitamin B12, magnesium, and calcium showed less clear-cut differences between the study groups. This may reflect their contribution to pregnancy only under specific conditions, possible interactions with other laboratory parameters, and variability in the clinical presentation of SAb among different women. Therefore, these findings support the need for further prospective studies evaluating whether individualized nutritional assessment before or very early in pregnancy may have clinical relevance, particularly for folate and vitamin D status.

Lastly, multivariable analysis serves as a significant conceptual foundation for the entire discourse. Although several biomarkers showed significant differences at the univariate level, the adjusted analyses should be interpreted cautiously because of the limited number of SAb cases, the imbalance between groups, and the potential instability of multivariable estimates. In the exploratory clinically adjusted model, lower serum folate and vitamin D levels remained associated with SAb, but these findings should be understood as adjusted associations rather than evidence of causality or preevent prediction. This should not be interpreted as a rejection of the previous findings, but rather as a realistic representation of the complex biology of SAb. The literature consistently depicts pregnancy loss as the culmination of interrelated nutritional, endocrine, immunologic, hematologic, and placental pathways, rather than a singular predominant biochemical abnormality.

The present study has several limitations that should be acknowledged. The sample size was determined by the availability of consecutive admissions over a four-month period. A central limitation of the present study is the timing of biomarker assessment. All hematological and biochemical parameters were measured at clinical presentation, when SAb had already occurred or was actively in progress. Consequently, the observed differences of hematological parameters cannot be interpreted as definitive pre-event predictive or etiological biomarkers. This limitation is particularly relevant for hematological and iron-related parameters, which may be influenced by acute bleeding, hemodynamic changes, inflammation, or systemic stress associated with the abortion process. For more stable nutritional biomarkers, such as vitamin D and serum folate, the measured concentrations may more plausibly reflect pre-existing maternal nutritional status; however, in the absence of preconceptional or pre-event measurements, reverse causality and acute-phase effects cannot be fully excluded. Although post-hoc power calculations were performed for the primary biomarker comparisons, these results should be interpreted with caution. Because post-hoc power is derived from the observed effect sizes, the high estimated values may partly reflect the large between-group differences observed in this sample rather than true robustness of the study design. Therefore, these analyses do not overcome the limitations imposed by the small and imbalanced sample, particularly the limited number of patients with SAb. Future prospective multicenter studies with larger samples and biomarker assessment before the onset of pregnancy loss are needed to clarify whether these parameters have predictive or etiological relevance.

The primary contribution of the current study is not the identification of a validated predictive biomarker, but the description of a concurrent maternal biomarker profile associated with a worse outcome in the first trimester of pregnancy, particularly involving folate, vitamin D, and iron-related disturbances.

CONCLUSIONS

In this single-center observational study, first-trimester SAb was associated with concurrent alterations in selected maternal hematological and biochemical nutritional biomarkers measured at clinical presentation. Women with SAb showed lower hemoglobin, hematocrit, serum iron, serum folate, and vitamin D levels compared with women with ongoing pregnancy, while ferritin, calcium, and magnesium did not differ significantly between groups. In exploratory adjusted analyses, lower serum folate and vitamin D levels remained associated with SAb; however, given the timing of biomarker assessment, these findings should not be interpreted as evidence of causality, antecedent nutritional deficiency, or preevent prediction. Larger prospective studies with preconceptional or early pregnancy biomarker assessment before the onset of pregnancy loss are required to clarify whether nutritional biomarkers have etiological or predictive relevance. Until such evidence is available, these biomarkers should be interpreted as part of the clinical and biological profile observed at presentation, rather than as validated tools for risk prediction.

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