

1 **Title:** Effects of vitamin B complex supplementation during pregnancy on neonatal vitamin B₁₂ status:
2 evidence from a cluster randomized controlled trial

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18 **Acknowledgements**

19 The authors thank all participants and researchers who participated in this study. The authors are also
20 grateful to the Xunyi County Hospital and the Xunyi County Maternal and Child Health Hospital for
21 their collaboration during the implementation of this trial. This study was sponsored by the National
22 Key Research and Development Program of China (grant number 2017YFC0907200, grant number
23 2017YFC0907201), the National Natural Science Foundation of China (grant number 81230016), the

24 Project of birth defect control and prevention in Shaanxi (grant number Sxwsjswzfcght2016-013),
25 the China Postdoctoral Science Foundation (grant number 2015M582678) and Xi'an Jiaotong
26 University (xjj2018146). The funders had no role in the design of the study; in the collection, analysis,
27 and interpretation of the data; in the writing of the manuscript, and in the decision to submit the
28 manuscript for publication.

29

30 **Abstract**

31 **Purpose:** Evidence about the effect of maternal vitamin B₁₂ supplementation during pregnancy on
32 offspring's vitamin B₁₂ status is limited. The present study aimed to evaluate the impact of antenatal
33 vitamin B complex supplementation on neonatal vitamin B₁₂ status.

34 **Methods:** In an ongoing cluster randomized controlled trial conducted in three rural counties in
35 northwest China, pregnant women <20 week of gestation were randomized to three treatment groups:
36 blank control, iron supplements, or vitamin B complex supplements. All women were administered
37 folic acid supplements during the periconceptional period. In a sub-study, we collected cord blood
38 samples of 331 participants from the control or vitamin B complex groups in the Xunyi county from
39 Jan.2017 to Dec.2017. Plasma concentrations of folate, vitamin B₁₂, and homocysteine were
40 measured. Two-level linear mixed models were used to compare differences between groups.

41 **Results:** Compared with newborns whose mothers were in the control group, newborns of the vitamin
42 B complex-supplemented women had significantly higher cord plasma vitamin B₁₂ concentrations (P
43 = 0.001) and lower homocysteine concentrations ($P = 0.033$). There was no significant difference in
44 cord plasma folate concentrations between groups ($P > 0.05$).

45 **Conclusions:** Maternal vitamin B complex supplementation during pregnancy is effective in
46 improving neonatal vitamin B₁₂ status in rural northwest China.

47 **Keywords:** Antenatal vitamin B complex supplementation; Newborns; Vitamin B₁₂ status; Cluster
48 randomized controlled trial; Rural northwest China

49

50

51 **Introduction**

52 Vitamin B₁₂, also known as cobalamin, is a water-soluble vitamin that is mainly acquired from
53 animal-source foods such as meat, poultry, eggs, seafood, and dairy products [1]. Together with folate,
54 vitamin B₁₂ is involved in the synthesis of purines and pyrimidines as well as the methylation of DNA,
55 RNA and protein [2]. To prevent the occurrence of neural tube defects, folic acid (FA) supplements
56 are widely recommended for women planning to become pregnant [3] and mandatory FA fortification
57 programs have been implemented in numerous countries [4]. Vitamin B₁₂ deficiency is common
58 among women of childbearing age in parts of the world, however, was less studies than folate [5]. In
59 a population-based cross-sectional study in Shaanxi province of northwest China, we reported that
60 the prevalence of vitamin B₁₂ deficiency among rural women aged 10-49 years was 52.0% [6], which
61 is comparable to that of South Asia [7-10]. Moreover, another survey in this region indicated that only
62 16.8% of pregnant women took vitamin B₁₂-containing supplements before conception or during
63 pregnancy [11].

64 Accumulating studies showed that suboptimal vitamin B₁₂ concentrations during pregnancy is
65 associated with neural tube defects [12], intrauterine growth retardation [13], as well as poor cognitive
66 function [14] and high insulin resistance [15] in children. We hypothesized that antenatal vitamin B₁₂
67 supplementation may be a cost-effective and feasible strategy to improve offspring's vitamin B₁₂
68 status and short-term and long-term health outcomes. To our knowledge, few clinical trials have
69 explored the effect of maternal vitamin B₁₂ supplementation on offspring's biochemical indicators
70 and most of them only focused on the South Asian populations [16-18].

71 A cluster randomized controlled trial in rural northwest China have been conducted to assess the
72 protective effect of micronutrient supplementation during pregnancy on congenital heart disease and
73 other adverse birth outcomes. Using data from the large trial, the present study aimed to examine

74 whether vitamin B complex supplementation during pregnancy could be effective to affect neonatal
75 folate, vitamin B₁₂, and homocysteine (Hcy) concentrations.

76

77 **Methods**

78 **Study design and participants**

79 This study was a biomarker sub-study of the ongoing cluster randomized controlled trial (registered
80 at clinicaltrials.gov as NCT02537392) that aimed to investigate the effect of micronutrient
81 supplementation during pregnancy on congenital heart disease and other adverse birth outcomes. This
82 trial was conducted in three rural counties (Changwu, Bin and Xunyi) in northwest China since July
83 2015. Women who were aged 15-49 years and have already been pregnant for less than 20 weeks
84 were invited to participate in this trial. Exclusion criteria at recruitment included 1) use of
85 supplements containing vitamin B complex or iron for more than two weeks, 2) having given birth to
86 children with congenital heart disease or other birth defects, and 3) having serious illnesses. As
87 pregnant women were administrated by township maternal and child health workers in northwest
88 China, a cluster randomized design, with a township as the unit of randomization, was used to
89 minimize the risk of contamination of the intervention. The township clusters were randomized to the
90 control, iron, or vitamin B complex groups with a 1:1:1 ratio before enrolment.

91 In accordance with the national policy for pregnant women in China, all women in this trial were
92 provided daily 400 µg FA supplements during pre-pregnancy and early pregnancy by County Health
93 Commission [19]. Women in the control group didn't receive any other micronutrient supplements.
94 Women in the iron group received a tablet (Ferrous sulfate/tablet; Jinan Yongning Pharmaceutical
95 Co., Ltd) containing 60 mg of iron, and those in the vitamin B complex group received supplements
96 (Weikangfu/tablet; Sino-American Shanghai Squibb Pharmaceuticals Ltd) containing 2 mg of

97 vitamin B₁, 2 mg of vitamin B₂, 2 mg of vitamin B₆, 2 µg of vitamin B₁₂, 5 mg of calcium pantothenate,
98 15 mg of nicotinamide (per tablet). Participants in both the iron and vitamin B complex groups were
99 instructed to take one tablet daily from enrolment to delivery. All micronutrient supplements were
100 commercially available during the period of this trial.

101 Pregnant women who were enrolled in the Xunyi county from Jan.2017 to Dec.2017 were further
102 invited to participate in the biomarker sub-study. Women were excluded from the sub-study if they
103 decided to deliver outside the Xunyi county or had twin births. Since this sub-study aimed to
104 investigate the effect of vitamin B complex supplementation on cord plasma concentrations, women
105 in the iron group were also excluded. Finally, cord blood samples were collected from 331 women.

106 The study was conducted according to the guidelines in the Declaration of Helsinki. Ethical
107 approval was obtained from the ethics review committee of the Xi'an Jiaotong University Health
108 Science Center (No.2012008). All participants provided written informed consent before their
109 participation in the study.

110

111 **Data collection**

112 At enrollment, sociodemographic characteristics, including age, education, occupation, household
113 wealth status and reproductive history, were collected by face-to-face interviews using a standardised
114 and structured questionnaire. After removal of heavy clothing and shoes, height was measured to the
115 nearest 0.1 cm with a stadiometer and weight was measured to the nearest 0.1 kg with an electronic
116 scale. Accordingly, BMI was calculated as weight in kilograms divided by height in meters squared

117 The number of dispensed vitamin B complex supplements and those returned at each bimonthly
118 antenatal check were record. Participants were also asked to report the use of FA supplements before
119 conception and during pregnancy within one week after delivery. All the information was recorded

120 by township maternal and child health workers through a web-based surveillance system.

121

122 **Cord blood collection and laboratory analysis**

123 Cord blood samples were collected from pregnant women at delivery using 5-mL EDTA-treated
124 vacutainer tubes and kept on 4 °C. Plasma and buffy coat were separated within 24 h and stored at -
125 80 °C until analysis. Plasma was analyzed within 12 months after collection by using the electro-
126 chemiluminescence assay for folate and vitamin B₁₂ and the enzymatic assay for Hcy. The intraassay
127 CVs for folate, vitamin B₁₂ and Hcy were 6.7%, 3.4% and 3.0%, and the interassay CVs were 8.1%,
128 7.0% and 7.3%, respectively.

129

130 **Statistical analysis**

131 The sample size in this sub-study was able to detect a 31.6% standard deviation unit difference
132 between groups with a 2-sided type I error rate of 5% and 80% power. Household wealth index was
133 constructed from an inventory of 15 household assets or facilities through principal component
134 analysis, and this index was classified as tertiles: low (poorest), medium (middle-income) and high
135 (richest) [20]. The compliance of vitamin B complex supplements was calculated by dividing the
136 number of supplements consumed by the number of days from enrollment to delivery. Mean ± SDs
137 or medians (25th, 75th percentiles) were used to describe normally or abnormally distributed
138 continuous variables, respectively. Numbers (%) were used to describe categorical variables.

139 All analyses were conducted based on the intention to treat principle. All biochemical indicators
140 were log-transformed before analyses. The cluster level baseline characteristics between groups were
141 compared by using Wilcoxon two-sample tests. To adjust for clustering effect, two-level mixed
142 models (township to level 2 and individual to level 1) were applied to assess the individual level

143 differences between groups, including baseline characteristics, number of FA supplements consumed,
144 and cord plasma concentrations of folate, vitamin B₁₂, and Hcy. Continuous variables and categorical
145 variables were analyzed by using linear mixed models and generalized linear mixed models,
146 respectively. Subgroup analyses according to baseline characteristics (gestational age at enrolment,
147 household wealth index, parity and maternal age) were further conducted. Potential interactions
148 between baseline characteristics and vitamin B complex supplementation were evaluated by
149 including an interaction term in models.

150 All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc.). Two
151 sided $P < 0.05$ were considered statistically significant.

152

153 **Results**

154 Figure 1 shows the flow chart of this study. A total of 760 pregnant women in the Xunyi county were
155 enrolled in the trial from January to December 2017. Of these participants, 392 were eligible for
156 inclusion in the biomarker sub-study. Of these eligible participants, seven were lost to follow-up, 19
157 had a spontaneous or induced abortion, one ended her pregnancy with stillbirth, and 34 refused cord
158 blood sampling. Ultimately, cord blood samples were available for 331 women, representing 84.4%
159 of the 392 eligible participants.

160 Baseline characteristics were similar between the participants with or without cord blood. Among
161 the participants with cord blood, the mean (SD) age was 26.4 (4.4) years, and 9.7% were educated
162 beyond junior school, and 92.2% were farmers. Primiparous women made up 52.3% of the study
163 sample. The mean gestational age at enrolment was 14.9 (4.6) and nearly one-third (29.6%) had a
164 gestational age <12 wk. The mean (SD) height, weight and BMI at enrolment was 159.5 (4.9) cm,
165 55.5 (7.3) kg and 21.8 (2.8) kg/m², respectively (Supplemental Table 1). The individual level baseline

166 characteristics were balanced between the participants in the control group and those in the vitamin
167 B complex group (Table 1). There were four clusters in the control group and five clusters in the
168 vitamin B complex group. The two groups were almost identical in terms of cluster level baseline
169 characteristics, including number of villages, area, population, number of pregnancies, per capita
170 gross domestic product and per capita net income (Supplemental Table 2).

171 The mean (SD) number of FA supplements consumed from three months before conception to
172 delivery was 77 (52) in the control group and 85 (63) in the vitamin B complex group, which was
173 similar between groups. The mean (SD) number of vitamin B complex supplements consumed from
174 enrollment to delivery was 129 (76) in the vitamin B complex group (Table 2). The mean (SD)
175 compliance with vitamin B complex supplementation was 69.5% (30.6%).

176 The intracluster correlation coefficients for cord plasma concentrations of folate, vitamin B₁₂, and
177 Hcy were 0, 0.03, and 0.02, respectively. The effect of vitamin B complex supplementation on cord
178 plasma concentrations is shown in Table 3. Newborns of mothers who received vitamin B complex
179 supplements had higher cord plasma vitamin B₁₂ concentrations (median: 218.1 pg/mL v. 183.7
180 pg/mL, $P = 0.001$) and lower Hcy concentrations (median: 15.7 $\mu\text{mol/L}$ v. 18.1 $\mu\text{mol/L}$, $P = 0.033$)
181 than did those in the control group. No significant difference was observed between groups in cord
182 plasma folate concentrations ($P > 0.05$). There was no evidence of a differential effect of the
183 intervention by baseline characteristics (gestational age at enrolment, household wealth index, parity
184 and maternal age) on cord plasma concentrations of vitamin B₁₂ and Hcy (Table 4).

185

186 **Discussion**

187 In this cluster randomized controlled trial of vitamin B complex supplementation during pregnancy,
188 we found that newborns born to supplemented mothers had higher cord plasma vitamin B₁₂

189 concentrations and lower Hcy concentrations, indicative of improved vitamin B₁₂ status.

190 Vitamin B₁₂ acts as a cofactor in the remethylation of Hcy and in the formation of succinyl-CoA
191 [21]. Therefore, vitamin B₁₂ deficiency can lead to accumulation of Hcy and methylmalonic acid [21].
192 Poor vitamin B₁₂ status, characterized as low vitamin B₁₂ concentrations and elevated concentrations
193 of Hcy and methylmalonic acid, has been thought to be highly prevalent among women of
194 childbearing age, especially in South Asia where the population is predominantly vegetarian [7-10].
195 There are limited data on vitamin B₁₂ status among Chinese women. A nutrition survey in Shaanxi
196 province of northwest China found that the vitamin B₁₂ concentrations of women aged 10-49 years
197 was low and nearly half of rural women were vitamin B₁₂ deficiency [6]. Given the high rate of
198 vitamin B₁₂ insufficiency and the pivotal role of vitamin B₁₂ in fetal growth and development, several
199 clinical trials of vitamin B₁₂ supplementation during pregnancy and early lactation have been
200 conducted in South Asia. The results from the Matlab Trial in Bangladesh showed that the prevalence
201 of vitamin B₁₂ deficiency among infants aged 6 months decreased by 11% after multivitamin
202 supplementation (containing 2.6 µg/day of vitamin B₁₂) from 14 week of gestation to 3-month
203 postpartum [16]. In the trial in India, when supplemented with 50 µg/day of vitamin B₁₂ from <14
204 week of gestation to 6-week postpartum, infant at 6 weeks of age had higher vitamin B₁₂
205 concentrations and lower Hcy and methylmalonic concentrations [17]. In another intervention study
206 in Bangladesh, supplementation with 250 µg/day of vitamin B₁₂ throughout pregnancy and 3-month
207 postpartum increased vitamin B₁₂ concentrations and lowered Hcy and methylmalonic acid
208 concentrations in both newborns and infants at 3 months of age^[18]. In our trial, pregnant women <20
209 week of gestation were allocated to receive or not to receive vitamin B complex supplements during
210 pregnancy. The supplements used in this trial contained 2 µg of vitamin B₁₂ per tablet. Even with a
211 low dose of vitamin B₁₂, newborns responded to vitamin B complex supplementation with

212 significantly higher cord plasma vitamin B₁₂ concentrations and lower Hcy concentrations. The
213 variation in the dose of vitamin B₁₂, the duration of supplementation and time point measured between
214 studies made it difficult to compare with each other. However, all these findings suggested that taking
215 vitamin B₁₂-containing supplements during pregnancy is an effective way to improve neonatal or
216 infant vitamin B₁₂ status in populations where vitamin B₁₂ deficiency is common.

217 It is noteworthy that even with vitamin B complex supplementation, cord plasma vitamin B₁₂ status
218 in study population was poorer in comparison with those previously measured in other populations.
219 The median cord plasma Hcy concentrations in the vitamin B complex group is much higher than the
220 values of 6.2-11.0 μmol/L reported in Norway [22], the Netherlands [23] and Bangladesh [18]. A
221 possible explanation for the poor vitamin B₁₂ status among newborns was that pregnant women
222 residing in this region are suffering from chronic malnutrition. Xunyi county is located in the southern
223 Loess Plateau of Shaanxi Province and is one of state-level poverty-stricken counties [24]. The
224 availability of animal products may be limited by poor natural environment and low economic level.
225 In addition, genetic factors may also affect the vitamin B₁₂ status of newborns. Methylene-
226 tetrahydrofolate reductase (MTHFR) is responsible for the conversion of 5,10-methylene-
227 tetrahydrofolate to 5-methyl- tetrahydrofolate, which is essential for the remethylation of Hcy [25].
228 The MTHFR 677C>T variant results in a thermolabile enzyme that has reduced activity in the
229 synthesis of 5-tetrahydrofolate [25]. Accordingly, individuals with the MTHFR 677T allele have
230 higher Hcy concentrations. The frequency of the MTHFR 677T allele in Shaanxi Province was
231 reported to be 43.3% [26], which is at a high level globally [27]. Our findings suggested that the dose
232 of 2 μg/day of vitamin B₁₂ during pregnancy may be inadequate to ensure the acceptable vitamin B₁₂
233 status of Chinese newborns. Additional studies are required to further explore the optimal dose of
234 vitamin B₁₂.

235 Hyperhomocysteinemia, which refers to abnormally high homocysteine concentrations in the
236 blood, has been considered as an independent risk factor for cardiovascular disease [28], cognitive
237 impairment [29] and adverse pregnancy outcomes [30]. Our finding of maternal vitamin B complex
238 supplementation during pregnancy lowering neonatal Hcy concentrations offered a potential strategy
239 for primary prevention. To further confirm the effects of antenatal vitamin B complex
240 supplementation on offspring's clinical outcomes, it would be necessary to conduct a long-term
241 follow-up of our trial.

242 The main strength of the present study is that the design of randomized controlled trial has the
243 ability to investigate causal association between maternal vitamin B complex supplementation and
244 neonatal vitamin B₁₂ status. However, our findings should be interpreted cautiously in light of some
245 limitations. First, neither participants nor researchers were blinded. On the basis of the fact that the
246 outcome indicators in this study were objective and the attrition rates between groups were similar
247 (vitamin B complex group v. control group: 17.0% v. 14.2%, $P = 0.444$), we believed that the non-
248 blinded design has a limited impact on estimated treatment effects [31]. Second, the unit of
249 randomization was a township rather than an individual, which might have introduced a larger random
250 error. To adjust for clustering effect, two-level mixed models were conducted in the present study.
251 Fortunately, both the cluster and individual level baseline characteristics were shown to be similar.
252 Third, except for vitamin B₁₂ and Hcy, other functional biomarkers of vitamin B₁₂ such as
253 methylmalonic acid and holo-transcobalamin have not been measured. Fourth, blood samples beyond
254 the newborn period have not been collected in this sub-study. Thus, the longer-term effect of vitamin
255 B complex supplementation on offspring's vitamin B₁₂ status remains unknown.

256 In conclusion, in the cluster randomized controlled trial in rural northwest China, we found that
257 maternal vitamin B complex supplementation during pregnancy could be effective in improving

258 neonatal vitamin B₁₂ status. Our study provided valuable evidence for the development of public
259 health policies on vitamin B₁₂ supplementation during pregnancy.

260

261 **Conflict of interest**

262 The authors have no conflicts of interest related to this study to disclose.

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Table 1 Baseline characteristics of pregnant women in control and vitamin B

complex groups.

	Vitamin B complex group (<i>n</i> =156)	Control group (<i>n</i> =175)	<i>P</i> ^a
Age (y)	26.8 ± 4.6	26.0 ± 4.1	0.140
<25 y	59 (37.8)	82 (46.9)	0.088
25-29 y	63 (40.4)	66 (37.7)	
≥30 y	34 (21.8)	27 (15.4)	
Education			0.975
Primary school or below	102 (65.4)	112 (64.0)	
Junior school	39 (25.0)	46 (26.3)	
Senior high school or above	15 (9.6)	17 (9.7)	
Occupation			0.930
Farmer	143 (91.7)	162 (92.6)	
Others	13 (8.3)	13 (7.4)	
Household wealth index	0.1 (1.4)	-0.1 (1.5)	0.477
Poor	46 (29.5)	60 (34.3)	0.585
Medium	50 (32.1)	64 (36.6)	
Rich	60 (38.5)	51 (29.1)	
Parity	0.5 ± 0.6	0.5 ± 0.5	0.691
Primiparous	82 (52.6)	91 (52.0)	0.979
Multiparous	74 (47.4)	84 (48.0)	

Gestational age at enrolment (wk)	14.7 ± 4.8	15.1 ± 4.4	0.777
<12 wk	46 (29.5)	52 (29.7)	0.554
≥12 wk	110 (70.5)	123 (70.3)	
Height (cm)	159.4 ± 5.0	159.7 ± 4.9	0.573
Weight at enrolment (kg)	55.1 ± 7.0	55.9 ± 7.7	0.352
BMI at enrolment (kg/m ²)	21.7 ± 2.5	21.9 ± 2.9	0.515

Values are means ± SDs or n (%).

^aDifferences between groups were assessed by using two-level linear mixed models for continuous variables or two-level generalized linear mixed models for categorical variables.

Table 2 Number of supplements consumed in control and vitamin B complex groups.

	Vitamin B complex group (<i>n</i> = 156)	Control group (<i>n</i> = 175)	<i>P</i> ^a
Folic acid supplements ^b	85 ± 63	77 ± 52	0.461
<60	57 (36.5)	59 (33.7)	0.799
60-119	62 (39.7)	82 (46.9)	
120-179	15 (9.6)	20 (11.4)	
≥180	22 (14.1)	14 (8.0)	
Vitamin B complex supplements ^c	129 ± 76	--	--
<60	19 (12.2)	--	--
60-119	48 (30.8)	--	
120-179	42 (26.9)	--	
≥180	47 (30.1)	--	

Values are means ± SDs or n (%).

^aDifferences between groups were assessed by using two-level linear mixed model for continuous variable or two-level generalized linear mixed model for categorical variable.

^bFrom three months before conception to delivery.

^cFrom enrollment to delivery.

Table 3 Effect of vitamin B complex supplementation during pregnancy on cord plasma concentrations of folate, vitamin B₁₂, and Hcy.

	Vitamin B complex group (<i>n</i> = 156)	Control group (<i>n</i> = 175)	<i>P</i> ^a
Plasma folate (ng/mL)	8.2 (5.9, 12.7)	7.6 (6.2, 11.3)	0.455
Plasma vitamin B ₁₂ (pg/mL)	218.1 (159.7, 298.1)	183.7 (141.7, 229.9)	0.001
Plasma Hcy (μmol/L)	15.7 (11.5, 22.4)	18.1 (12.9, 25.4)	0.033

Hcy, homocysteine.

Values are medians (25th, 75th percentiles).

^aDifferences between groups were assessed by using two-level linear mixed models.

Table 4 Stratified analysis of the effect of vitamin B complex supplementation on cord plasma concentrations of vitamin B₁₂ and Hcy.

	Vitamin B complex group (<i>n</i> = 156)	Control group (<i>n</i> = 175)	<i>P</i> ^a	<i>P</i> interaction
Plasma vitamin B ₁₂ (pg/mL)				
Gestational age at enrolment				0.293
<12 wk	224.5 (165.8, 287.0)	175.8 (144.0, 235.7)	0.001	
≥12 wk	213.1 (159.4, 301.9)	184.6 (139.3, 227.2)	0.002	
Household wealth index				0.842
Poor	216.7 (160.2, 325.5)	174.9 (133.3, 219.0)	0.006	
Medium	212.3 (157.1, 301.9)	187.3 (142.9, 230.1)	0.011	
Rich	224.5 (167.2, 288.8)	187.3 (148.4, 233.8)	0.044	
Parity				0.347
Primiparous	232.7 (180.1, 336.4)	201.2 (149.3, 256.8)	0.012	
Multiparous	204.7 (147.4, 273.5)	159.7 (133.7, 216.3)	<0.001	
Maternal age				0.742
<25 y	209.4 (160.2, 301.9)	175.2 (136.7, 233.8)	0.020	
25-29 y	204.9 (152.5, 273.5)	188.7 (145.3, 221.7)	0.010	
≥30 y	250.3 (179.6, 349.1)	178.4 (143.3, 239.7)	0.014	
Plasma Hcy (μmol/L)				
Gestational age at enrolment				0.150
<12 wk	14.6 (11.2, 19.9)	18.3 (13.3, 29.1)	0.014	

≥12 wk	16.3 (11.8, 22.4)	18.1 (12.9, 24.8)	0.236	
Household wealth index				0.146
Poor	15.2 (12.2, 22.3)	21.2 (16.4, 27.1)	0.008	
Medium	15.3 (11.4, 23.3)	16.9 (13.2, 25.0)	0.211	
Rich	16.3 (11.0, 21.5)	13.8 (10.6, 22.6)	0.935	
Parity				0.696
Primiparous	15.1 (11.3, 21.5)	15.8 (11.8, 24.3)	0.147	
Multiparous	16.3 (12.2, 22.4)	20.8 (15.2, 27.0)	0.055	
Maternal age				0.863
<25 y	15.2 (12.1, 22.0)	18.5 (12.8, 27.6)	0.050	
25-29 y	15.5 (10.6, 22.4)	16.5 (13.3, 24.8)	0.216	
≥30 y	16.9 (11.5, 22.4)	20.8 (13.4, 24.1)	0.425	

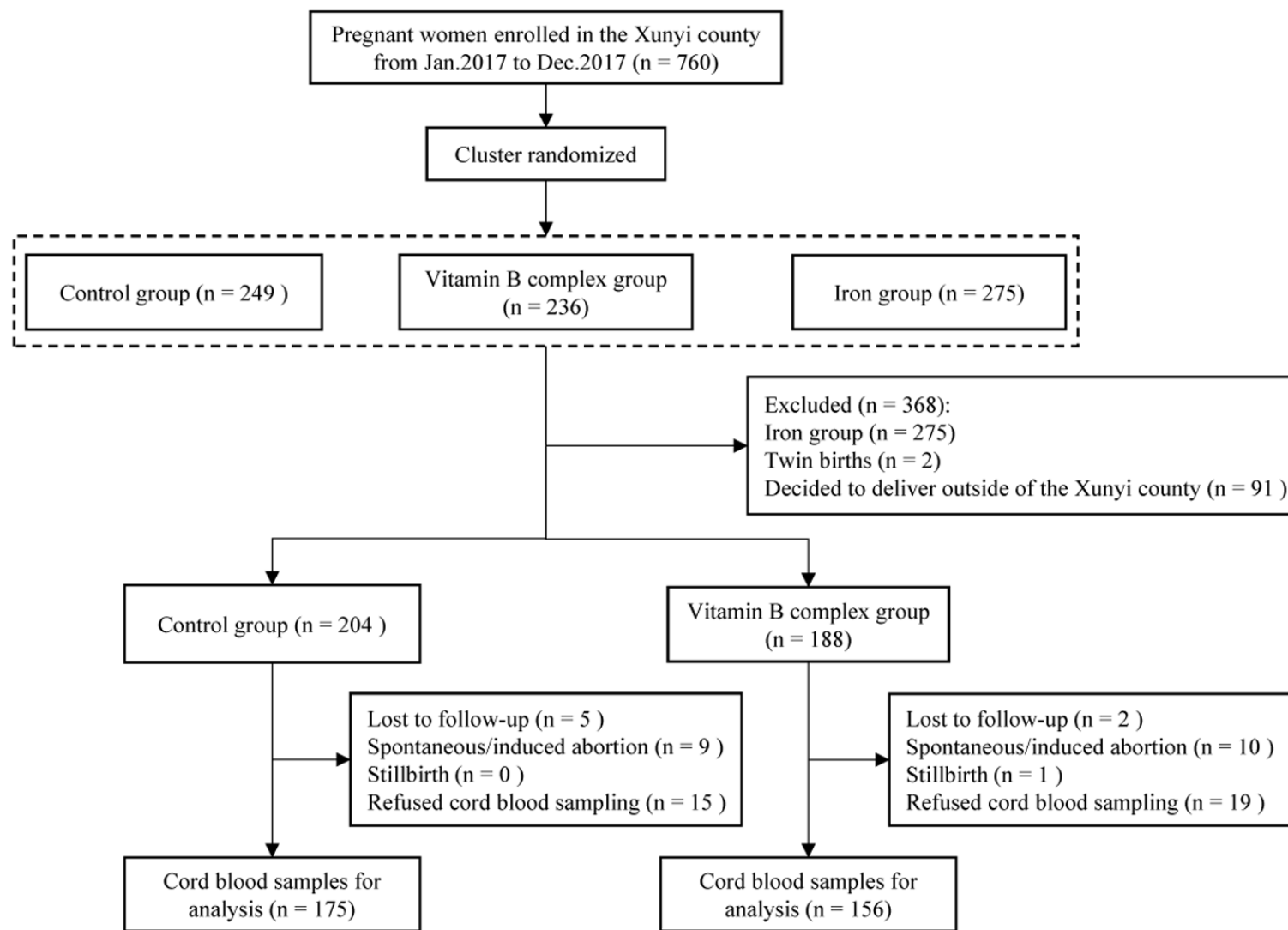
Hcy, homocysteine.

Values are medians (25th, 75th percentiles).

^aDifferences between groups were assessed by using two-level linear mixed models.

Figure legends

Fig.1 Study flow chart.



Supplemental Table 1 Baseline characteristics of pregnant women with cord blood and those without cord blood.

	Cord blood collected (<i>n</i> = 331)	Cord blood not collected (<i>n</i> = 61)	<i>P</i> ^a
Age (y)	26.4 ± 4.4	27.3 ± 4.3	0.169
<25 y	141 (42.6)	19 (31.2)	0.137
25-29 y	129 (39.0)	28 (45.9)	
≥30 y	61 (18.4)	14 (23.0)	
Education			0.053
Primary school or below	214 (64.7)	33 (54.1)	
Junior school	85 (25.7)	22 (36.1)	
Senior high school or above	32 (9.7)	6 (9.8)	
Occupation			0.270
Farmer	305 (92.2)	54 (88.5)	
Others	26 (7.9)	7 (11.5)	
Household wealth index	-0.001 (1.5)	0.004 (1.5)	0.562
Poor	113 (34.1)	18 (29.5)	0.722
Medium	107 (32.3)	24 (39.3)	
Rich	111 (33.5)	19 (31.2)	
Parity	0.5 ± 0.6	0.6 ± 0.6	0.506
Primiparous	173 (52.3)	30 (49.2)	0.516
Multiparous	158 (47.7)	31 (50.8)	

Gestational age at enrolment (wk)	14.9 ± 4.6	14.3 ± 5.5	0.355
<12 wk	98 (29.6)	25 (41.0)	0.070
≥12 wk	233 (70.4)	36 (59.0)	
Height (cm)	159.5 ± 4.9	158.5 ± 9.7	0.281
Weight at enrolment (kg)	55.5 ± 7.3	56.5 ± 7.8	0.367
BMI at enrolment (kg/m ²)	21.8 ± 2.8	22.9 ± 5.9	0.077

Values are means ± SDs or n (%).

^aDifferences between groups were assessed by using two-level linear mixed models for continuous variables or two-level generalized linear mixed models for categorical variables.

Supplementary Table 2 Baseline characteristics of clusters in control and vitamin B complex groups.

	Vitamin B complex group	Control group	<i>P</i> ^a
No. of clusters	5	4	--
No. of villages	12 (11, 12)	13 (9, 19)	1.000
Area (km ²)	42 (40, 74)	81 (55, 133)	0.623
Population	17125 (16858, 19321)	19180 (11072, 31405)	1.000
No. of pregnancies	156 (135, 187)	209 (134, 348)	0.806
Per capita gross domestic product (RMB yuan)	54400 (50180, 102750)	63076 (32444, 73371)	0.713
Per capita net income (RMB yuan)	10339 (9810, 11156)	9688 (9428, 11405)	0.540

Values are medians (25th, 75th percentiles).

^aDifferences between groups were assessed by Wilcoxon two-sample test.