Preplanned Studies

Molybdenum Concentration and the Risk of Spontaneous Preterm Birth: A Nested Case-Control Study — Beijing Municipality, China, 2018–2020

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Summary

What is already known about this topic?

The level of molybdenum (Mo) in a mother's urine has been linked to the growth rate of the fetus and the blood pressure levels in children.

What is added by this report?

We evaluated the variations in maternal plasma Mo concentrations throughout pregnancy and their potential association with the risk of spontaneous preterm birth (SPB).

What are the implications for public health practice?

Future research must determine the Mo levels in pregnant women across various regions in China. Moreover, particular attention needs to be given to the potential increase in Mo concentration throughout pregnancy and its possible adverse impacts on the health of both the mother and the fetus.

Spontaneous preterm births (SPB), with no identifiable causes, account for a significant 60%–70% of all preterm births (*1*). These are responsible for about 35% of neonatal fatalities linked to premature births (*2*). Given its high incidence and consequences, the prevention of preterm births is increasingly gaining traction as a critical public health concern. An essential ultratrace element, molybdenum (Mo), plays a role in several human metalloflavoproteins. Studies have revealed that the maternal urinary Mo concentration during pregnancy bears an inverse association with fetal abdominal circumference and estimated fetal weight while also positively correlating with childhood blood pressure (*3*–*4*). This study aimed to monitor Mo levels in pregnant women's plasma and analyze any potential link to SPB risk. We found the maternal plasma Mo concentration in the SPB group was significantly greater than that in the control group during the first trimester [2.12 (1.56–2.69) *vs.* 2.02 (1.46–2.50) ng/mL, *P*=0.027]. Higher Mo levels (≥ 2.584 ng/mL) recorded in the first trimester were linked with an elevated SPB risk after adjusting for maternal age, ethnicity, pre-pregnancy body mass index (PPBMI), parity, mode of delivery, education, and income levels [odds ratio (*OR*)=2.074 (1.212, 3.550), *P*=0.008]. However, when the Mo concentration stood at ≥3.554 ng/mL during the third trimester, the *OR* for SPB reached 1.732 (1.016, 2.953), *P*=0.044, again adjusted for the aforementioned factors. Furthermore, it is crucial to note that the current reference range for blood Mo concentrations in pregnant women across various Chinese regions remains undefined. Hence, clinicians must be vigilant against illnesses potentially arising from high Mo concentrations.

We conducted a nested case-control study with subjects drawn from the Beijing birth cohort. In estimating our sample size, we predicated on a high-level Mo exposure rate of approximately 50% in the term delivery group, a common odds ratio of 2 (*5*), a two-sided importance level of α =0.05 and β=0.10, and a case-control proportion of 1∶1. Calculations performed using the PASS (version 11.0; NCSS statistical software, Kaysville, USA), indicated a minimum sample size of 179 patients required for the SPB group. Eligibility criteria included women aged between 18 and 44 years willing to undergo routine prenatal examinations and deliveries at the Beijing Obstetrics and Gynecology Hospital, and who provided signed consent. Following an overnight fast (≥8 hours), we obtained 2.0 mL venous blood samples from each participant during the first (7 to 9 weeks of gestation) and third trimesters (33 to 34 weeks). SPB was defined as preterm birth with intact fetal membranes and preterm premature rupture of membranes before the 37th week of gestation, excluding iatrogenic preterm birth caused by medical interventions such as maternal fetal induction or

cesarean section. After excluding women with twin pregnancies, cervical inadequacies, a history of preterm birth, placental abruption, iatrogenic preterm birth due to hypertension, diabetes, central placental previa, abnormal liver function, acute fatty liver, cholestasis, appendicitis, and significant cardiac insufficiency, liver, kidney, and brain diseases, as well as those on bed-rest; we selected a cohort of 29,303 live singletons, 617 of whom were SPB cases. We further narrowed it down to 236 SPB patients who had blood samples collected during both the first and third trimesters. A control group of 236 subjects composed of healthy pregnant women with gestational weeks of <39 or >41 was also selected (Supplementary Figure S1, available athttps://weekly.chinacdc.cn/). The study was granted approval by the Ethics Committee of the Beijing Obstetrics and Gynecology Hospital, Capital Medical University.

The Mo concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, PerkinElmer, USA). Standardized plasma (ClinChek® - Plasma Control, Level II, Germany) samples served as controls for quality assurance. The detection limit (LOD) for Mo in the plasma was set at 0.04 ng/mL and our analysis found that the concentrations of Mo in all samples exceeded this threshold. In the standard plasma samples, the median reference value for Mo was 5.96 ng/mL, ranging from a minimum of 4.77 ng/mL to a maximum of 7.15 ng/mL. To investigate the correlation between Mo levels and the risk of SPB anomalies, we executed an unconditional logistic regression analysis utilizing the IBM SPSS Statistics software (version 26.0; IBM Corporation, Armonk, NY, USA). We deemed a two-tail *P*-value of less than 0.05 as statistically significant in all statistical analyses.

The study groups, consisting of 236 patients each in the SPB cohort and the control group, demonstrated comparable demographic characteristics with respect to maternal education, personal monthly income, maternal ethnicity, and fetal gender. Nevertheless, significant differences were observed concerning maternal delivery time (35.53±0.69 weeks for SPB *vs.* 39.70±0.74 weeks for controls, *P*<0.001), maternal age [under 35 years: 177 (75%) SPB *vs.* 196 (83.05%) controls; 35 years and older: 59 (25%) SPB *vs.* 40 (16.95%) controls, *P*=0.032], PPBMI [under 18.5 kg/m^2: 29 (12.29%) SPB *vs.* 29 (12.29%) controls; 18.5–25 kg/m^2: 149 (63.14%) SPB *vs.* 176 (74.58%) controls; 25 kg/m^2 and above: 58 (24.57%) SPB *vs.* 31 (13.13%) controls, *P*=0.021], parity [nulliparous: 154 (65.25%) SPB *vs.* 184 (77.97%) controls; multiparous: 82 (34.75%) SPB *vs.* 52 (22.03%) controls, *P*=0.002], mode of delivery [vaginal: 145 (61.44%) SPB *vs.* 176 (74.58%) controls; cesarean section: 91 (38.56%) SPB *vs.* 60 (25.42%) controls, *P*=0.002], fetal birth weight (2696.46±338.75 g SPB *vs.* 3429.94±299.46 g controls, *P*<0.001), and birth length (47.70±1.93 cm SPB *vs.* 50.25±0.90 cm controls, *P*<0.001) (Table 1).

During the first trimester, median maternal plasma Mo concentrations were measured at 2.07 (1.53–2.58) ng/mL for the entire cohort. Within this cohort, women in the SPB group had a slightly higher median Mo concentration of 2.12 (1.56–2.69) ng/mL compared to 2.02 (1.46–2.50) ng/mL in the control group, and this difference was statistically significant (*P*=0.027). By the third trimester, the overall median Mo concentration had increased to 2.59 (1.66–3.55) ng/mL. The SPB group's median concentration rose to 2.66 (1.69–3.69) ng/mL, while the control group's median was 2.54 (1.58–3.41) ng/mL, though this inter-group difference was not statistically significant (*P*=0.147). Notably, third trimester Mo concentrations were significantly elevated when compared to first trimester levels (*P*<0.001). Between the first and third trimesters, the SPB group experienced an increase in Mo concentration of 0.37 (range: −0.86 to 1.73) ng/mL and the control group an increase of 0.35 (range: −0.84 to 1.73) ng/mL, with no significant difference between the groups (*P*=0.775) (Table 2).

During the first trimester, when maternal plasma Mo concentration was at or above 2.584 ng/mL, the odds of SPB as determined by logistic regression analyses with various adjustments, were significantly increased with *OR*s of 1.878 [95% confidence interval (*CI*): 1.129, 3.124, *P*=0.015], 1.892 (95% *CI*: 1.118, 3.201, *P*=0.018), and 2.074 (95% *CI*: 1.212, 3.550, *P*=0.008). In the third trimester, the occurrence of SPB at a Mo concentration of 3.554 ng/mL or higher had an adjusted *OR* of 1.732 (95% *CI*: 1.016, 2.953, *P*=0.044), after factoring in variables such as maternal age, ethnicity, PPBMI, number of births (parity), mode of delivery, educational background, and income levels. In comparison to the lowest quartile of plasma Mo levels, the odds of experiencing SPB were 1.217 (95% *CI*: 0.715, 2.072), 1.084 (95% *CI*: 0.635, 1.848), and 2.074 (95% *CI*: 1.212, 3.550) for the second, third, and fourth quartiles, respectively, showing a significant trend (P_{trend}=0.017) in the third logistic regression model. In the first trimester, the P_{trend} values for the remaining two models were 0.036

TABLE 1. Characteristics of women in the SPB and control groups.

Characteristics	SPB (*n*=236)	Control (*n*=236)	*P*
Delivery time (weeks) (mean±SD)	35.53±0.69	39.70±0.74	<0.001*
Age (year), *n* (%)			0.032*
<35	177 (75)	196 (83.05)	
≥35	59 (25)	40 (16.95)	
PPBMI (kg/m^2), *n* (%)			0.021*
<18.5	29 (12.29)	29 (12.29)	
18.5–25	149 (63.14)	176 (74.58)	
≥25	58 (24.57)	31 (13.13)	
Parity, *n* (%)			0.002*
0	154 (65.25)	184 (77.97)	
≥1	82 (34.75)	52 (22.03)	
Education, *n* (%)			0.130
<Bachelor's degree	63 (26.69)	49 (20.76)	
≥Bachelor's degree	173 (73.31)	187 (79.24)	
Personal monthly income (CNY), *n* (%)			0.271
<10,000	58 (24.58)	48 (20.34)	
≥10,000	178 (75.42)	188 (79.66)	
Ethnicity, *n* (%)			0.616
Han	215 (91.10)	218 (92.37)	
Other ethnicities	21 (8.90)	18 (7.63)	
Delivery way, *n* (%)			0.002*
Vaginal delivery	145 (61.44)	176 (74.58)	
Cesarean section	91 (38.56)	60 (25.42)	
Birth weight (g) (mean±SD)	2696.46±338.75	3429.94±299.46	<0.001*
Birth length (cm) (mean±SD)	47.70±1.93	50.25±0.90	<0.001*
Fetal gender, *n* (%)			0.310
Boy	132 (55.93)	121 (51.27)	
Girl	104 (44.07)	115 (48.73)	

Abrreviation: SPB=spontaneous preterm birth; Mo=molybdenum; PPBMI=pre-pregnancy body mass index; CNY=Chinese Yuan.
* P<0.05.

and 0.039, respectively. For the third trimester, P_{trend} was found to be significant at 0.038 in logistic regression model 3 (Table 3).

The subgroup analysis results suggested a correlation between plasma Mo concentration and the risk of SPB in overweight, obese, and multiparous mothers during the first trimester (Supplementary Tables S1 and S2, available at https://weekly.chinacdc.cn/). Additionally, our sensitivity analysis results corroborate with our preliminary findings (Supplementary Table S3, available at https://weekly.chinacdc.cn/).

DISCUSSION

Our results indicate a positive correlation between elevated levels of Mo in maternal plasma and an amplified risk of SPB during the first trimester, specifically in multiparous or overweight/obese women. Although these findings are suggestive, additional studies are necessary to clarify the exact mechanisms by which Mo affects SPB. Considering these results, it is essential to enhance awareness around Mo consumption and environmental exposure during early pregnancy due to their potential contribution to SPB risk.

Our study showed an elevated risk of SPB with observed Mo concentrations of ≥2.584 ng/mL in the first trimester and ≥3.554 ng/mL in the third trimester. Comparisons with other studies provides additional context. For example, the National Institute of Child Health and Human Development (NICHD)

TABLE 2. Median concentrations of molybdenum in maternal plasma.

Groups	Total M (IQR)	SPB M (IQR)	Control M (IQR)	$P^{\dagger}$
Plasma				
1st	2.07 (1.53–2.58)	2.12 (1.56–2.69)	2.02 (1.46–2.50)	0.027*
3rd	2.59 (1.66–3.55)	2.66 (1.69–3.69)	2.54 (1.58–3.41)	0.147
Increment	0.36 (−0.85 to 1.73)	0.37 (−0.86 to 1.73)	0.35 (−0.84 to 1.73)	0.775
$P^{\S}$	<0.001*	<0.001*	<0.001*	

Note: The designated plasma unit is ng/mL.
* P<0.05.
† The SPB group was contrasted with the control group.
§ Comparison between the first and third trimesters of pregnancy. Increment refers to the amount of Mo that increased from the first trimester to the third trimester.
Abbreviation: SPB=spontaneous preterm birth; Mo=molybdenum; IQR=interquartile range.

TABLE 3. Relationship between maternal molybdenum concentration and the risk of spontaneous premature birth.

Mo concentration	SPB	Control	Odds ratio (95% *CI*)					
			Model 1†	*P*	Model 2§	*P*	Model 3¶	*P*
Plasma 1st, *n* (%)								
<1.531	53 (22.46)	64 (27.12)	1		1		1	
1.531–2.074	59 (25.00)	60 (25.42)	1.179 (0.713, 1.950)	0.521	1.206 (0.714, 2.037)	0.485	1.217 (0.715, 2.072)	0.469
2.074–2.584	54 (22.88)	64 (27.12)	1.000 (0.604, 1.656)	1	1.031 (0.611, 1.739)	0.908	1.084 (0.635, 1.848)	0.768
≥2.584	70 (29.66)	48 (20.34)	1.878 (1.129, 3.124)	0.015*	1.892 (1.118, 3.201)	0.018*	2.074 (1.212, 3.550)	0.008*
P_{trend}			0.036*		0.039*		0.017*	
Plasma 3rd, *n* (%)								
<1.664	57 (24.15)	61 (25.96)	1		1		1	
1.664–2.593	55 (23.31)	62 (26.38)	0.968 (0.585, 1.600)	0.898	0.995 (0.590, 1.678)	0.984	0.977 (0.574, 1.665)	0.933
2.593–3.554	59 (25.00)	60 (25.53)	1.085 (0.656, 1.795)	0.750	1.129 (0.669,1.903)	0.649	1.132 (0.667, 1.923)	0.645
≥3.554	65 (27.54)	52 (22.13)	1.437 (0.868, 2.379)	0.159	1.570 (0.931, 2.649)	0.091	1.732 (1.016, 2.953)	0.044*
P_{trend}			0.140		0.079		0.038*	

Note: The designated plasma unit is ng/mL. In the Plasma 1st: SPB: *n*=236; Control: *n*=236; In the Plasma 3rd: SPB: *n*=236; Control: *n*=235.
Abbreviation: SPB=spontaneous preterm birth; Mo=molybdenum; *CI*=confidence interval; PPBMI=pre-pregnancy body mass index.
* P<0.05.
† Unconditional logistic regression with no adjustments.
§ Unconditional logistic regression with adjustments for maternal age, PPBMI, parity, and delivery way.
¶ Unconditional logistic regression with adjustments for maternal age, ethnicity, PPBMI, parity, delivery way, education, and income levels.

Fetal Growth Studies reported maternal plasma Mo concentration of 1.9 [interquartile range (IQR): 1.3] μg/L at 10–13 weeks gestation among 1,720 subjects (*6*). Yin et al. (*7*) reported Mo concentrations in the serum of pregnant women recorded as 2.378 (1.757–2.938) ng/mL, 2.413 (1.835–2.970) ng/mL, 2.327 (1.727–2.930) ng/mL, and 2.816 (2.392–3.496) ng/mL in cases of total orofacial clefts, cleft lip with cleft palate, cleft lip only (*n*=130), and in controls (*n*=260) respectively. These samples were collected at <28, 28–37, or ≥37 gestational weeks. In another study, maternal serum Mo levels were 2.51 (1.43–3.07) ng/mL and 2.66 (2.03–3.27) ng/mL in neural tube defects (NTDs) cases (*n*=273) and controls (*n*=477), respectively. These blood samples were collected at <28, 28–37, or ≥37 gestational weeks (*8*). Since Mo binds to α 2-macroglobulins in the form of molybdate, and spectrin on erythrocytes (*9*), it is expected that the Mo concentrations in plasma and serum would be nearly identical (*10*).

As a metal element, Mo is widely used in metallurgy, electronics, missiles and many other important areas (*11–13*). It is also found in everyday items like toys, clothing, as well as household and plant care products (*10*), suggesting an increased risk of exposure to Mo (*14*). The primary sources of Mo in humans are water and dietary intake. Foods rich in Mo include beans, wheat, oats, asparagus, milk, and cheese, with its

concentration in food varying based on the levels found in the local soil and water where the food was cultivated (*15*). Once absorbed, Mo is primarily localized in the kidneys, liver, and bones, with negligible levels crossing the placental barrier (*16–18*). Although Mo deficiencies are uncommon given their ultratrace occurrence, excessive consumption can result in health complications such as joint pain, hyperuricuria, hallucinations, and seizures (*19*). Moreover, Mo metabolism is closely linked with copper and sulfur metabolism. Excessive intake of molybdate can heighten the formation of copper sulfide, causing copper deficiency (*20–21*).

Certain limitations in our study merit attention. First, our participant pool came exclusively from Beijing, which should be considered when generalizing our findings to other regions. Furthermore, data collection did not include nutrient supplements or medication intake during pregnancy.

Our study reveals a preliminary correlation between maternal Mo levels and SPB risk, providing new insights into Mo's potential toxic effects on SPB. Currently, many regions in China lack reference ranges for trace element concentrations, and the risk of disease due to excessive trace elements is often overlooked by clinicians. Our findings emphasize the need for vigilant monitoring of Mo levels during pregnancy, particularly in Mo mining areas. Timely monitoring of trace element concentrations, such as Mo, in early pregnancy stages enables clinicians to intervene swiftly, thereby diminishing potential health risks to the mother and fetus. Going forward, comprehensive, nationwide multi-center studies that include dietary and nutritional surveys are needed to further investigate these issues.

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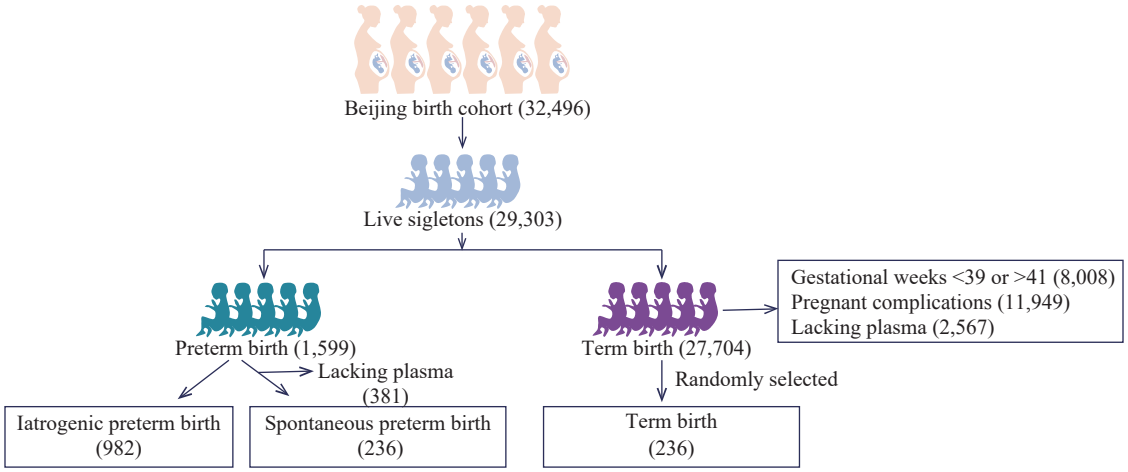
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S1. Flow chart illustrating the selection of cases and controls.

SUPPLEMENTARY TABLE S1. Association between maternal plasma molybdenum concentration and the risk of spontaneous preterm birth at varying pre-pregnancy body mass indexes.

PPBMI levels	Odds ratio (95% *CI*)					
	Model 1[†]	*P*	Model 2[§]	*P*	Model 3[¶]	*P*
1st						
<18.5	1.995 (1.007, 3.952)	0.048*	1.855 (0.926, 3.716)	0.081	2.076 (0.853, 5.053)	0.107
18.5–25	1.200 (0.971, 1.483)	0.092	1.203 (0.972, 1.489)	0.090	1.237 (0.994, 1.541)	0.057
≥25	1.529 (0.854, 2.736)	0.153	1.633 (0.899, 2.967)	0.107	2.698 (1.209, 6.020)	0.015*
3rd						
<18.5	1.182 (0.775, 1.804)	0.437	1.174 (0.753, 1.831)	0.479	1.348 (0.790, 2.299)	0.274
18.5–25	1.161 (0.980, 1.374)	0.084	1.190 (1.002, 1.414)	0.048*	1.200 (1.007, 1.431)	0.042*
≥25	0.909 (0.561, 1.473)	0.698	0.900 (0.553, 1.465)	0.672	0.944 (0.514, 1.734)	0.853

Abbreviation: SPB=spontaneous preterm birth; Mo=molybdenum; *CI*=confidence interval; PPBMI=pre-pregnancy body mass index.
* *P*<0.05.
[†] Unconditional logistic regression with no adjustments.
[§] Unconditional logistic regression with adjustments for maternal age, parity, and delivery way.
[¶] Unconditional logistic regression with adjustments for maternal age, ethnicity, parity, delivery way, education, and income levels.

SUPPLEMENTARY TABLE S2. Association between maternal plasma molybdenum concentration and the risk of spontaneous preterm birth at varying parity levels.

Parity	Odds ratio (95% *CI*)					
	Model 1[†]	*P*	Model 2[§]	*P*	Model 3[¶]	*P*
1st						
0	1.185 (0.957, 1.467)	0.119	1.173 (0.946, 1.454)	0.146	1.219 (0.976, 1.523)	0.080
≥1	1.762 (1.144, 2.713)	0.010*	1.755 (1.124, 2.740)	0.013*	2.271 (1.315, 3.921)	0.003*
3rd						
0	1.189 (0.996, 1.419)	0.055	1.194 (0.997, 1.430)	0.054	1.204 (1.002, 1.445)	0.047*
≥1	1.104 (0.837, 1.456)	0.485	1.108 (0.832, 1.476)	0.482	1.117 (0.813, 1.533)	0.496

Abbreviation: SPB=spontaneous preterm birth; Mo=molybdenum; *CI*=confidence interval; PPBMI=pre-pregnancy body mass index.
* *P*<0.05.
[†] Unconditional logistic regression without any adjustments.
[§] Implementation of unconditional logistic regression adjustments considering variables such as maternal age, PPBMI, and mode of delivery.
[¶] Unconditional logistic regression was utilized, accounting for variables including maternal age, ethnicity, PPBMI, mode of delivery, educational attainment, and income brackets.

SUPPLEMENTARY TABLE S3. Association between maternal Mo concentration and SPB in the sensitivity analysis.

Mo concentration	SPB	Control	Odds ratio (95% *CI*)					
			Model 1[†]	*P*	Model 2[§]	*P*	Model 3[¶]	*P*
Plasma 1st								
<1.529	48 (21.92%)	61 (27.98%)	1		1		1	
1.529–2.070	56 (25.57%)	54 (24.77%)	1.280 (0.760, 2.156)	0.353	1.329 (0.781, 2.261)	0.295	1.379 (0.802, 2.370)	0.246
2.070–2.571	49 (22.37%)	60 (27.52%)	1.074 (0.637, 1.810)	0.790	1.127 (0.660, 1.925)	0.661	1.204 (0.698, 2.076)	0.505
≥2.571	66 (30.14%)	43 (19.73%)	2.024 (1.189, 3.445)	0.009*	2.105 (1.225, 3.620)	0.007*	2.235 (1.286, 3.885)	0.004*
P_{trend}			0.025*		0.018*		0.011*	
Plasma 3rd								
<1.692	56 (25.57%)	53 (24.42%)	1		1		1	
1.692–2.612	49 (22.37%)	60 (27.65%)	0.868 (0.514, 1.467)	0.597	0.880 (0.512, 1.511)	0.642	0.885 (0.511, 1.532)	0.662
2.612–3.558	55 (25.11%)	54 (24.88%)	1.018 (0.604, 1.717)	0.946	1.038 (0.606, 1.777)	0.892	1.131 (0.597, 1.778)	0.914
≥3.558	59 (26.95%)	50 (23.05%)	1.258 (0.745, 2.124)	0.391	1.421 (0.772, 2260)	0.310	1.686 (1.003, 2.794)	0.048*
P_{trend}			0.313		0.245		0.041*	

Note: The unit used is ng/mL. Excluded: Vaginal infection cases involving group B streptococcus, fungal, and mycoplasma infections were not included in both the SPB group (*n*=17) and the TB group (*n*=18). In the first plasma group: SPB: *n*=219; Control: *n*=218. In the third plasma group: SPB: *n*=219; Control: *n*=217.

Abbreviation: SPB=spontaneous preterm birth; Mo=molybdenum; *CI*=confidence interval; PPBMI=pre-pregnancy body mass index.

* *P*<0.05.

[†] Unconditional logistic regression without any adjustments.

[§] Unconditional logistic regression was performed, adjusting for factors such as maternal age, PPBMI, parity, and mode of delivery.

[¶] Unconditional logistic regression was utilized, while controlling for factors such as maternal age, ethnicity, pre-pregnancy body mass index, parity, method of delivery, educational attainment, and income levels.