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Preplanned Studies

Trends in the Incidence of High-Risk Newborns Based on a New Conceptual Framework — Beijing Municipality, China, 2013–2022

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Summary

What is already known on this topic?

A new conceptual term, small and vulnerable newborns (SVN), bringing preterm birth, small for gestational age (SGA), or low birth weight (LBW) together is being advocated for assessing whether a child is at high risk.

What is added by this report?

According to the new conceptual term, the increasing incidence of high-risk newborns (from 9.82% to 10.96%) has been observed among 2,005,408 newborns over the period from 2013 to 2022, which is higher than using any of the three definitions of SVN. Maternal age ≥35, primiparity, and multiple births are high risks for SVN.

What are the implications for public health practice?

The new conceptual framework should be used to better assess the number of high-risk newborns. Attention should be paid to multiple births to prevent preterm-related SVN. To reduce term newborns who are SGA, we need to be concerned not only with multiple pregnancies but also with first-time mothers.

The new conceptual framework, small and vulnerable newborns (SVN), encompasses preterm birth, small for gestational age (SGA), and low birth weight (LBW). This framework identifies these high-risk infants, who share a markedly increased risk of short-term and long-term adverse consequences, as a single group (*1*). There are two primary reasons for adopting this framework: first, using any of the three individual definitions would underestimate the number of high-risk newborns by 50% (*2*); and second, because the three definitions are not mutually exclusive, assessing the total burden on a region or country becomes difficult. This study aimed to assess the burden trends of high-risk newborns in Beijing from 2013 to 2022 under this new SVN conception, using data collected from the Maternal and Children Information System in Beijing Municipality, China. The study among 2,005,408 newborns found an increasing incidence of SVN in Beijing, with rates rising from 9.82% of births in 2013 to 10.96% in 2022. Additionally, the average incidence over the ten years for SVN, preterm birth, SGA, and LBW were 9.94%, 6.35%, 4.51%, and 4.80%, respectively. Notably, while the incidence of preterm-SGA and preterm-nonSGA showed a significant rising trend, the incidence of term-SGA decreased significantly. It is essential for public health practitioners to adopt this new conceptual framework to better estimate the total burden of high-risk newborns. Considering the increasing trends over the past decade, more interventions should be implemented to reduce the burden on high-risk newborns, especially for preterm infants, regardless of SGA status.

SVN include preterm, SGA, and LBW infants. Since these three definitions are not mutually exclusive, SVN births can be classified into three mutually exclusive types: 1) preterm-SGA: newborns who are both preterm and SGA, 2) preterm-nonSGA: newborns who are preterm but not SGA, and 3) term-SGA: newborns who are born at term but are SGA (*1*).

This study utilized data from the Maternal and Children Information System in Beijing, a registry for births. All midwifery agencies in Beijing are required to register birth information, including birth weight and gestational age. SVN were defined as newborns who were preterm (<37 weeks of gestational age), had LBW (<2,500 g at any gestational age), or had a birth weight less than the 10th percentile for gestational age according to the "Reference Standards for Fetal Weight Estimation at different gestational age in Chinese Population" in the *Expert Consensus on Fetal Growth Restriction* (2019 edition). This classification can be divided into three mutually exclusive types: term-SGA, preterm-SGA, and preterm-nonSGA. Non-live births and births with a gestational age of less than 28 weeks were excluded.

A linear regression model, implemented in R software (version 4.4.0; The R Foundation for Statistical Computing, Vienna, Austria), was used to analyze the trend between year and incidence of SVN. Logistic regression, also conducted in R software, was used to estimate the association between biological characteristics and SVN and its subtypes. Results were expressed as adjusted odds ratios (a*OR*s) with 95% confidence intervals (*CI*s). *P* values equal to or less than 0.05 (two-tailed) were considered statistically significant.

Between 2013 and 2022, a total of 2,005,408 births were recorded. Of these, 96,236 (4.80%) were LBW babies, 127,422 (6.35%) were preterm births, and 90,508 (4.51%) were SGA babies. Using the new definition, 199,254 (9.94%) were SVN babies. The basic characteristics of the births are displayed in Table 1.

Figure 1 illustrates the trends in incidence for each subtype between 2013 and 2022. Over this period, the incidence of SVN demonstrated an increasing trend, rising from 9.82% in 2013 to 10.96% in 2022 (Linear Regression, t=4.597, P=0.002). The incidence of both preterm-SGA and preterm-nonSGA also manifested upward trends. Preterm-SGA incidence climbed from 0.80% in 2013 to 1.11% in 2022 (Linear Regression, t=8.905, P=0.000), while preterm-nonSGA incidence rose from 4.43% to 6.34% (Linear Regression, t=5.674, P=0.000). In contrast, the incidence of term-SGA decreased from 4.60% in 2013 to 3.51% in 2022 (Linear Regression, t=−2.582, P=0.033).

Table 2 presents the associations between biological characteristics and SVN subtypes, as estimated by logistic regression. Maternal age, parity, and number of births were adjusted for in the model. Maternal age was not associated with term-SGA newborns. However, multiple pregnancies (a*OR*=6.98, 95% *CI*: 6.79, 7.17) and primiparity (a*OR*=2.11, 95% *CI*: 2.07, 2.15) were associated with an increased risk of term-SGA newborns. Multiple pregnancies were also a significant predictor for both preterm-nonSGA (a*OR*=17.15, 95% *CI*: 16.83, 17.48) and preterm-SGA newborns (a*OR*=32.75, 95% *CI*: 31.70, 33.84).

DISCUSSION

This study is the first to apply a new conceptual framework to evaluate the incidence of high-risk newborns in Beijing. Data from the Beijing Maternal and Children Information System indicate an overall SVN incidence of 9.94% over the past decade. The system also reveals incidences of 4.51% for SGA, 4.80% for LBW, and 6.35% for preterm births over the same period. Using any subtype of SVN to assess the number of high-risk newborns would lead to an underestimation of 40%–50%, a finding similar to a Brazilian study (*2*). The new definition encompasses SGA, LBW, and preterm birth under the umbrella term SVN. In this study, routine individual-level data collection in midwifery hospitals allows for accurate assessment of the burden associated with being born small or preterm. Because these three definitions are not mutually exclusive, a new SVN classification method is used: term-SGA, preterm-SGA, and preterm-non-SGA. Although this new classification

TABLE 1. Basic characteristics of births in Beijing Municipality, China, from 2013 to 2022 (*N*=2,005,408).

Variable	Total, *N* (%)	Preterm, *N* (%)	LBW, *N* (%)	SGA, *N* (%)	SVN, *N* (%)
Sexual					
Boy	1,042,072 (51.96)	70,629 (6.78)	46,368 (4.45)	34,715 (3.33)	97,001 (9.31)
Girl	963,038 (48.02)	56,750 (5.89)	49,813 (5.17)	55,743 (5.79)	102,179 (10.61)
Gender ambiguity	298 (0.01)	43 (14.43)	54 (18.12)	49 (16.44)	73 (24.5)
Number of birth					
Single birth	1,940,193 (96.75)	97,382 (5.02)	66,841 (3.45)	76,613 (3.95)	161,920 (8.35)
Multiple births	65,215 (3.25)	30,040 (46.06)	29,395 (45.07)	13,895 (21.31)	37,334 (57.25)
Mode of delivery					
Vaginal delivery	1,162,877 (57.99)	48,965 (6.75)	33,665 (2.89)	46,251 (3.98)	91,077 (7.83)
Cesarean section	842,435 (42.01)	78,447 (5.81)	62,563 (7.43)	44,250 (5.25)	108,164 (12.84)
Total	2,005,408 (100.00)	127,422 (6.35)	96,236 (4.80)	90,508 (4.51)	199,254 (9.94)

Abbreviation: LBW=low birth weight; SGA=small for gestational age; SVN=small and vulnerable newborns.

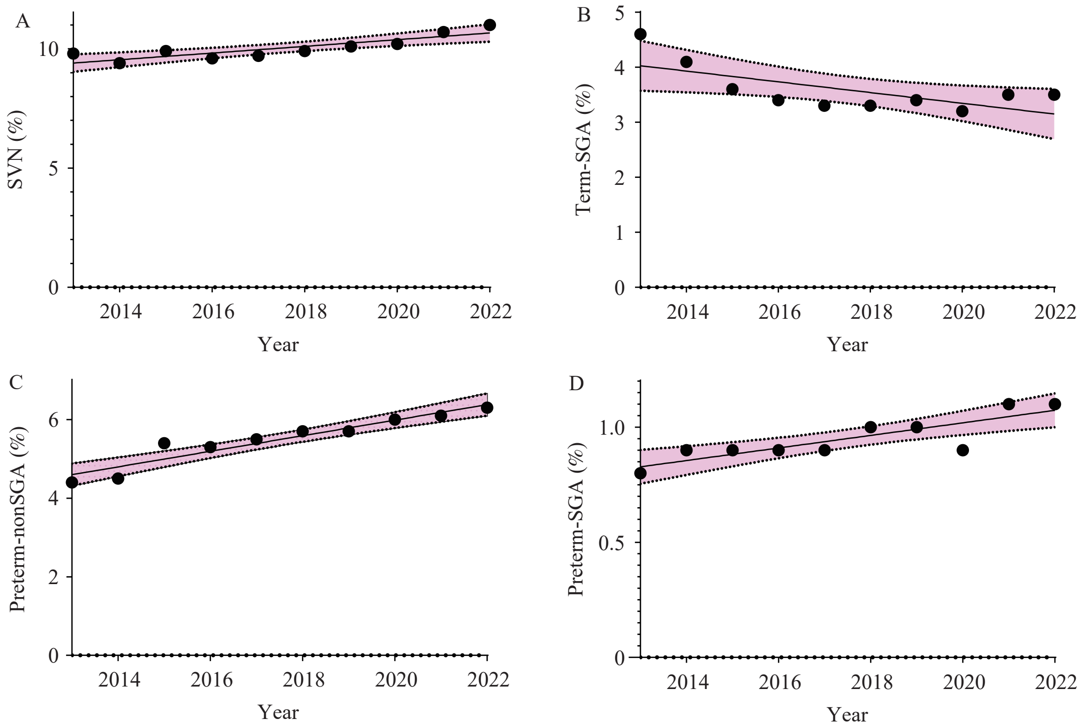

FIGURE 1. Trends between years and incidence of high-risk newborns by linear regression, in Beijing, China, from 2013 to 2022 (%). (A) The trend between years and incidence of SVN (t=4.597, P=0.002). (B) The trend between years and incidence of term-SGA (t=−2.582, P=0.033). (C) The trend between years and incidence of preterm-nonSGA (t=5.674, P=0.000). (D) The trend between years and incidence of preterm-SGA (t=8.905, P=0.000).
Abbreviation: SVN=small and vulnerable newborns; Preterm-SGA=preterm newborns also small for gestational age; Preterm-nonSGA=preterm newborns but not small for gestational age; Term-SGA=term newborns but small for gestational age.

TABLE 2. Risk of SVN and its subtypes in pregnant women with different biological characteristics in Beijing, China.

Variable		Term-SGA* (*N*=71,832)	Preterm-SGA * (*N*=18,676)	Preterm-nonSGA* (*N*=108,746)	SVN* (*N*=199,254)
		a*OR* (95% *CI*)	a*OR* (95% *CI*)	a*OR* (95% *CI*)	a*OR* (95% *CI*)
Parity	Multipara	Reference	Reference	Reference	Reference
	Primipara	2.11 (2.07, 2.15)	1.43 (1.44, 1.54)	0.97 (0.96, 0.98）	1.31 (1.29, 1.32)
Age, years	<35	Reference	Reference	Reference	Reference
	35–40	0.92 (0.90, 0.94)	1.41 (1.36, 1.47)	1.40 (1.38, 1.42）	1.23 (1.22, 1.25)
	≥40	1.01 (0.95, 1.06)	2.17 (2.01, 2.33)	1.88 (1.82, 1.95）	1.60 (1.56, 1.65)
Number of births	Single birth	Reference	Reference	Reference	Reference
	Multiple birth	6.98 (6.79, 7.17）	32.75 (31.70, 33.84)	17.15 (16.83, 17.48）	14.10 (13.91, 14.38)

Abbreviation: SVN=small and vulnerable newborns; Preterm-SGA=preterm newborns also small for gestational age; Preterm-nonSGA=preterm newborns but not small for gestational age; Term-SGA=term newborns but small for gestational age; a*OR*=adjusted odds ratio; *CI*=confidence interval.
* Comparison of the study group with the normal newborn group by logistic regression, adjusting for parity, maternal age, and number of births.

covered 95.43% (88,355/92,586) of LBW babies in our study, slightly lower than in previous studies (*1*), public health practitioners are advised to use SVN when assessing the number of high-risk newborns. This recommendation is based on the new conceptual framework's ability to better estimate the total burden of high-risk newborns.

The incidence of SVNs in this study was markedly lower than the 26.2% reported for 195 countries and areas in 2020. The global study indicated that of all SVNs, 1.1% were preterm-SGA, 8.8% were preterm-nonSGA, and 16.3% were term-SGA (*3*). Over the past ten years in Beijing, the incidences of SVNs (9.94%), preterm-nonSGA (5.42%), and term-SGA (3.58%) were significantly lower than global data, while the incidence of preterm-SGA (0.93%) was slightly lower. While few studies have used this new conceptual framework, previous research has shown that the incidences of preterm birth, LBW, and SGA vary significantly between countries (*4–6*). Similarly, preterm birth incidence varies by region within China (*7*). Regional differences in economic development, maternal demographic characteristics, and antenatal care capabilities should be considered when comparing SVN incidence.

The incidence of term-SGA decreased from 4.60% in 2013 to 3.51% in 2022 (linear regression, $t=-2.582$, $P=0.033$). This decrease may be partially attributed to Beijing's status as one of the most economically developed areas in China, with a higher level of antenatal care compared to most other areas. Increasing the quantity and quality of antenatal contact with healthcare providers can reduce or prevent the birth of SVN (*8*). The Chinese government's implementation of the Basic Public Health Service project in 2009, which included establishing annual maternal health records and prenatal examinations, can further improve maternal health services and reduce maternal mortality (*9*). Furthermore, an analysis of maternal and child healthcare quality in 31 Chinese provincial-level administrative divisions (PLADs) from 2010 to 2020, using 6 indicators (maternal mortality rate, maternal health record establishment rate, pregnancy systematic management rate, prenatal examination rate, postpartum visit rate, and hospital delivery rate), ranked Beijing among the top areas for maternal and child health quality (*10*). Despite the decrease in term-SGA incidence, this condition remains a concern as it accounted for 26.4% of SVN-associated neonatal deaths (*1*).

Despite some progress, a significant health concern remains. This study found no decrease in the incidence of SVN, with preterm-SGA and preterm-nonSGA births increasing over the past 10 years. This suggests a need for improved interventions, particularly those targeting preterm infants. Our study found a strong association between multiple pregnancies and preterm birth (preterm-SGA: a*OR*=32.75, 95% *CI*: 31.70, 33.84; preterm-nonSGA: a*OR*=17.15, 95% *CI*: 16.83, 17.48). Interventions aimed at reducing SVN should prioritize women of advanced maternal age, primiparous women, and those with multiple pregnancies.

This study is among the first to group preterm birth, SGA, and LBW together under the umbrella term SVN. This study has several additional strengths. First, the Maternal and Children Information System in Beijing collects birth data from all midwifery hospitals in the city and uses all data for birth registration, allowing it to accurately represent the occurrence of SVN in Beijing without sampling bias. Second, the diagnostic criteria for SVN are relatively objective. These criteria are based on the newborn's birth weight and gestational age, eliminating potential diagnostic access bias due to inconsistent criteria among different midwifery hospitals.

This study was subject to some limitations. The occurrence of SVN is influenced by economic and cultural conditions. While this study offers a comprehensive representation of SVN occurrence in Beijing, it is important to note the significant variations in economic levels, basic demographic characteristics, and antenatal care quality across different regions of China. Consequently, these findings may not accurately reflect the overall SVN situation in China. In addition, the study did not take into account pregnancy-related diseases. As far as we are aware, pregnancy complications and comorbidities are among the crucial factors that significantly impact pregnancy outcomes. This study investigates the applicability of the new concept for assessing high-risk newborns in Beijing and offers insights for interventions. However, this study did not consider maternal health status; therefore, further research is needed to develop more precise interventions.

Conflicts of interest: No conflicts of interest.

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Preplanned Studies

Changes in Maternal Socio-Demographic Characteristics and Pregnancy Outcomes Across Monitoring Regions — Six Provinces, China, 2016–2022

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Summary

What is already known about this topic?

In recent years, there has been a significant increase in the proportion of women of advanced maternal age (AMA), accompanied by a rise in adverse pregnancy outcomes in certain regions of China.

What is added by this report?

From 2016 to 2022, there was an observed increase in the proportion of AMA, educational levels, and incidences of preterm birth and low birth weight (LBW) in both primiparous and multiparous women. Concurrently, there was a declining trend in the rate of cesarean deliveries and the incidence of macrosomia among multiparous women.

What are the implications for public health practice?

In addition to focusing on health management for AMA individuals, proactive steps should be undertaken to enhance the quality of medical services and promote childbirth at optimal ages, thereby reducing the incidence of adverse pregnancy outcomes.

In recent years, China has faced significant challenges due to a declining fertility rate, an aging population, and an unbalanced demographic structure. Concurrently, there have been shifts in maternal characteristics and pregnancy outcomes. While existing research predominantly focuses on maternal age and pregnancy results and often only within specific provinces (*1–4*), there remains a lack of comprehensive understanding of socio-demographic traits across various regions. This retrospective study examines maternal data from monitoring sites across China from 2016 to 2022, providing a systematic evaluation of the changes in maternal socio-demographic characteristics and perinatal outcomes. The findings reveal that from 2016 to 2022, there was an increase in the number of advanced maternal age (AMA) mothers, educational attainment, and instances of preterm birth and low birth weight (LBW) among both primiparous and multiparous mothers. Conversely, the rates of cesarean delivery and macrosomia among multiparous mothers showed a declining trend. These trends underscore the need for targeted healthcare improvements and policies encouraging childbirth at optimal ages to mitigate adverse pregnancy outcomes.

Data for this study were obtained from the Maternal and Newborn Health Monitoring System (MNHMS), established by the National Center for Women and Children's Health (NCWCH) in 2013. The cohort comprised all pregnant women who were either resident or had resided for more than six months in the designated monitoring areas and were enrolled during their initial antenatal care (ANC) visit. Data, including ANC visits throughout pregnancy and delivery, were captured and maintained in the MNHMS. To ensure data continuity and integrity, this study analyzed maternal case data from 6 provinces monitored from January 1, 2016, to December 31, 2022. The regions covered included Fujian Province (Haicang and Jimei districts) and Guangdong Province (Zijin and Longchuan counties) in the eastern region, Hebei Province (Zhengding County) and Hubei Province (Luotian County) in the central region, and Sichuan Province (Gongjing District and Rong County) and Yunnan Province (Tonghai and Huaning counties) in the western region. Inclusion criteria were: 1) delivery occurred between January 1, 2016 and December 31, 2022; 2) singleton pregnancies; and 3) live birth. Exclusion criteria included: 1) missing last menstrual period (LMP) or delivery dates; 2) availability of only prenatal examination records or absence of delivery records. Ultimately, 253,389 pregnant women and their infants were included in the analysis. The Maternal and Newborn Health Monitoring Program (MNHMP) data collection was approved by the Ethics Committee of the National Center for Women and Children's Health, China CDC (No. FY2015–007), and underwent annual ethical reviews (Nos. FY2018-

14, FY2019-12, FY2021-13, FY2022-11). Additionally, the use of data in this study was also approved by this committee (No. FY2024-12).

In this study, women residing in the district were categorized as urban residents, while those living in the county were classified as rural residents. Preterm delivery was defined as childbirth occurring between 28 and less than 37 weeks of gestation, and LBW was defined as a birth weight below 2,500 g. The dataset was imported from Excel into SAS software (version 9.4, SAS Institute, Cary, NC, USA) for data cleaning. The χ^2 test was employed to evaluate differences between primiparas (first-time mothers) and multiparas (mothers with one or more previous births). The trend χ^2 test was utilized for univariate analysis to assess trends in maternal age, literacy, preterm birth, LBW, and macrosomia incidence annually.

In the monitored regions, the majority of mothers were aged between 18 and 34 years (88.9%). The proportion of women aged 35 years or older was significantly higher among multiparas (16.0%) compared to primiparas (3.9%). Educational attainment differed between the groups; 43.6% of primiparas had at least a college degree, and 30.9% had completed high school or vocational training. In contrast, the largest segment of multiparas had reached junior high school education (39.5%). Vaginal delivery was the predominant mode of delivery for both primiparas (63.8%) and multiparas (61.4%). The rates of preterm birth and macmultiparas (mothers with one or more previous births). The trend thers, whereas primiparous mothers experienced a significantly higher incidence of LBW compared to multipar test was utilized for univariate analysis to assess trends in maternal age, literacy, preterm birth, LBW, and macrosomia incidence annually (Table 1).

From 2016 to 2022, there was a significant increase in the proportion of AMA among both primiparas and multiparas, with all P<0.01. Specifically, the rise in the proportion of advanced age among multiparas (6.8%) was significantly greater than that among primiparas (2.4%). By 2022, the share of advanced-age multiparas peaked at 19.7%. Over the same period, the proportions of both primiparas and multiparas in eastern areas showed a consistent annual increase (χ^2_{trend}=4,625.873, P<0.01 and χ^2_{trend}=4,912.240, P<0.01, respectively). Additionally, from 2016 to 2022, there was a rising trend in the proportion of individuals with a college degree or higher among both primiparas and multiparas (χ^2_{trend}=2,757.522, P< 0.01 and χ^2_{trend}=1,896.743, P<0.01, respectively). From 2018 to 2022, the predominant educational level among primiparas was college or above. Among multiparas, although the predominant educational level was junior high school from 2016 to 2020, the proportion with college degrees or higher grew to become the majority by 2021 (38.1%) and further increased in 2022 (41.4%).

From 2016 to 2022, the distribution of delivery methods among primiparas remained largely unchanged. However, there was a notable decrease in the proportion of cesarean sections among multiparas (χ^2_{trend}=16.358, P<0.01). During the same period, the incidence of adverse pregnancy outcomes such as preterm birth and LBW increased among both primiparas and multiparas (all P<0.01). Conversely, the incidence of macrosomia in multiparas showed a declining trend (χ^2_{trend}=8.231, P<0.01) (Table 2).

DISCUSSION

This study is a retrospective cohort investigation spanning several years, encompassing various provinces and regions across China. It specifically focused on all pregnant women present at the study sites throughout the duration of the study. The findings indicate that between 2016 and 2022, the proportion of AMA among both multiparas and primiparas has been increasing, with a notably higher proportion observed in multiparas compared to primiparas. These findings are largely in agreement with prior research conducted in Zhejiang (*1*) and Hebei (*2*). The rise in AMA among primiparas can likely be attributed to delayed childbirth, driven by socio-economic advancements and subsequent postponements in marriage and first-time childbearing (*5*). Additionally, during this period, revisions in fertility policy potentially facilitated the realization of deferred reproductive intentions among older women, contributing to the observed increase in AMA among multiparas. From 2016 to 2022, the proportion of both primiparas and multiparas in the eastern region of China showed a yearly increase. This trend could be influenced by the region's economic growth, which fosters fertility; however, this effect varies across regions and is influenced by differing levels of economic development (*6*). As the eastern region offers higher living standards, better social security, and medical facilities, coupled with more favorable fertility policies, it attracts more women to conceive and have additional children.

The results indicated that the educational level

TABLE 1. Maternal socio-demographic characteristics and pregnancy outcomes in 6 provinces, China, 2016–2022.

Variable	Total *n* (%; 95% *CI*)	Primiparas *n* (%; 95% *CI*)	Multiparas *n* (%; 95% *CI*)	*P*
Age (years)				
<18	1,168 (0.5; 0.4, 0.5)	1,087 (1.0; 0.9, 1.0)	81 (0.1; 0.1, 0.1)	< 0.001
18–34	225,272 (88.9; 88.8, 89.0)	107,582 (95.1; 95.0, 95.2)	117,690 (83.9; 83.7, 84.1)	< 0.001
≥35	26,949 (10.6; 10.5, 10.8)	4,456 (3.9; 3.8, 4.1)	22,493 (16.0; 15.9, 16.2)	<0.001
Residence				
Urban	92,236 (36.4; 36.2, 36.6)	37,345 (33.0; 32.7, 33.3)	54,891 (39.1; 38.9, 39.4)	<0.001
Rural	161,153 (63.6; 63.4, 63.8)	75,780 (67.0; 66.7, 67.3)	85,373 (60.9; 60.6, 61.1)	
Region				
Eastern	142,992 (56.4; 56.2, 56.6)	55,352 (48.9; 48.6, 49.2)	87,640 (62.5; 62.2, 62.7)	< 0.001
Central	58,362 (23.0; 22.9, 23.2)	34,427 (30.4; 30.1, 30.7)	23,935 (17.1; 16.9, 17.3)	< 0.001
Western	52,035 (20.5; 20.4, 20.7)	23,346 (20.6; 20.4, 20.9)	28,689 (20.5; 20.2, 20.7)	0.255
Educational level				
Primary school or lower	8,635 (3.4; 3.3, 3.5)	2,383 (2.1; 2.0, 2.2)	6,252 (4.5; 4.4, 4.6)	< 0.001
Junior high school	81,837 (32.3; 32.1, 32.5)	26,481 (23.4; 23.2, 23.7)	55,356 (39.5; 39.2, 39.7)	<0.001
Senior high school/vocational training	68,935 (27.2; 27.0, 27.4)	34,942 (30.9; 30.6, 31.2)	33,993 (24.2; 24.0, 24.5)	<0.001
College or above	93,982 (37.1; 37.0, 37.3)	49,319 (43.6; 43.3, 43.9)	44,663 (31.8; 31.6, 32.1)	<0.001
Delivery mode				
Vaginal delivery	158,340 (62.5; 62.3, 62.7)	72,173 (63.8; 63.5, 64.1)	86,167 (61.4; 61.2, 61.7)	<0.001
Cesarean section	95,049 (37.5; 37.3, 37.7)	40,952 (36.2; 35.9, 36.5)	54,097 (38.6; 38.3, 38.8)	
Preterm delivery				
Yes	11,609 (4.6; 4.5, 4.7)	4,763 (4.2; 4.1, 4.3)	6,846 (4.9; 4.8, 5.0)	<0.001
No	241,780 (95.4; 95.3, 95.5)	108,362 (95.8; 95.7, 95.9)	133,418 (95.1; 95.0, 95.2)	
Low birth weight				
Yes	9,703 (3.8; 3.8, 3.9)	4,776 (4.2; 4.1, 4.3)	4,927 (3.5; 3.4, 3.6)	<0.001
No	243,686 (96.2; 96.1, 96.2)	108,349 (95.8; 95.7, 96.0)	135,337 (96.5; 96.4, 96.6)	
Macrosomia				
Yes	8,442 (3.3; 3.3, 3.4)	3,368 (3.0; 2.9, 3.1)	5,074 (3.6; 3.5, 3.7)	<0.001
No	244,947 (96.7; 96.6, 96.7)	109,757 (97.0; 96.9, 97.1)	135,190 (96.4; 96.3, 96.5)	
Total	253,389 (100.0)	113,125 (100.0)	140,264 (100.0)	

among both primiparas and multiparas has progressively improved, aligning with findings from other studies (*7*). Furthermore, this study revealed a significant shift in the predominant educational attainment of multiparas from junior high school to college or above in recent years. This trend may be attributed to the overall enhancement of societal educational standards. According to the statistical monitoring report from the "Development Outline for Chinese Women," the number of female students in higher education rose by 1.225 million from 2021, reaching 29.033 million in 2022 (*8*). Additionally, the link between higher fertility rates and advanced education underscores that individuals with higher educational levels often possess higher incomes, more stable employment, and better salaries, which collectively facilitate the financial means necessary for child-rearing. Chen (*9*) investigated the causal impact of women's educational level on fertility and found that a higher educational level increases women's desire for children.

In this study, we observed a decrease in the rate of cesarean sections among multiparous women. This may be attributed to a growing preference among women in recent years to have two or more children, leading them to opt for vaginal delivery during their first childbirth. This choice appears to reduce the prevalence of scarred uteri among multiparas,

TABLE 2. Univariate analysis of primiparous and multiparous individuals across 6 PLADs in China, 2016–2022 [*n* (%, 95% *CI*)].

Variable	Primiparas								Multiparas							
	2016	2017	2018	2019	2020	2021	2022	*P*	2016	2017	2018	2019	2020	2021	2022	*P*
Age (years old)																
<18	209 (1.1; 0.9, 1.2)	182 (1.1; 1.0, 1.3)	192 (1.1; 1.0, 1.3)	175 (1.0; 0.8, 1.1)	138 (0.9; 0.7, 1.1)	117 (0.8; 0.7, 1.0)	74 (0.6; 0.5, 0.8)	0.001	9 (0.0; 0.0, 0.1)	15 (0.1; 0.0, 0.1)	20 (0.1; 0.1, 0.1)	10 (0.0; 0.0, 0.1)	11 (0.1; 0.0, 0.1)	7 (0.0; 0.0, 0.1)	9 (0.1; 0.0, 0.1)	0.701
18–34	18819 (96.1; 95.8, 96.3)	15,802 (95.3 95.0, 95.6)	16,554 (95.0; 94.7, 95.3)	17,438 (95.2; 94.8, 95.5)	14,406 (94.8; 94.4, 95.1)	13,184 (94.5 94.2, 95.0)	11,379 (94.2; 93.8, 94.6)	<0.001	17,239 (87.1; 86.6, 87.5)	17,264 (83.7; 83.2, 84.2)	18,871 (83.7; 83.2, 84.2)	20,387 (85.0; 84.5, 85.4)	17,171 (83.5; 83.0, 84.1)	14,805 (82.9; 82.3, 83.4)	11,953 (80.2; 79.6, 80.9)	<0.001
≥35	549 (2.8; 2.6, 3.0)	592 (3.6; 3.3, 3.9)	675 (3.9; 3.6, 4.2)	711 (3.9; 3.6, 4.2)	658 (4.3; 4.0, 4.7)	644 (4.6; 4.3, 5.0)	627 (5.2; 4.8, 5.6)	<0.001	2,548 (12.9; 12.4, 13.3)	3,354 (16.3; 15.8, 16.8)	3,646 (16.2; 15.7, 16.7)	3,590 (15.0; 14.5, 15.4)	3,370 (16.4; 15.9, 16.9)	3,052 (17.1; 16.5, 17.6)	2,933 (19.7; 19.1, 20.3)	<0.001
Residence																
Urban	5,585 (28.5; 27.9, 29.2)	2,710 (16.3; 15.8, 16.9)	5,803 (33.3; 32.6, 34.0)	6,656 (36.3; 35.6, 37.0)	6,018 (39.6; 38.8, 40.4)	5,558 (39.9; 39.0, 40.7)	5,015 (41.5; 40.6, 42.4)	<0.001	7,183 (36.3; 35.6, 37.0)	4,877 (23.6; 23.1, 24.2)	9,706 (43.1; 42.4, 43.7)	10,027 (41.8; 41.2, 42.4)	9,196 (44.7; 44.1, 45.2)	7,661 (42.9; 42.2, 43.6)	6,241 (41.9; 41.1, 42.7)	<0.001
Rural	13,992 (71.5; 70.8, 72.1)	13,866 (83.7; 83.1, 84.2)	11,618 (66.7; 66.0, 67.4)	11,668 (63.7; 63.0, 64.4)	9,184 (60.4; 59.6, 61.2)	8,387 (60.1; 59.3, 61.0)	7,065 (58.5; 57.6, 59.4)		12,613 (63.7; 63.0, 64.4)	15,756 (76.4; 75.8, 76.9)	12,831 (56.9; 56.3, 57.6)	13,960 (58.2; 57.6, 58.8)	11,356 (55.3; 54.6, 55.9)	10,203 (57.1; 56.4, 57.8)	8,654 (58.1; 57.3, 58.9)	<0.001
Region																
Eastern	6,688 (34.2; 33.5, 34.8)	5,695 (34.4; 33.6, 35.1)	8,361 (48.0; 47.2, 48.7)	10,076 (55.0; 54.3, 55.7)	8,865 (58.3; 57.5, 59.1)	8,378 (60.1; 59.3, 61.0)	7,289 (60.3; 59.5, 61.2)	<0.001	9,082 (45.9; 45.2, 46.6)	10,325 (50.0; 49.4, 50.7)	14,016 (62.2; 61.6, 62.8)	16,308 (68.0; 67.4, 68.6)	14,443 (70.3; 69.6, 70.9)	12,683 (71.0; 70.3, 71.7)	10,783 (72.4; 71.7, 73.1)	<0.001
Central	7,174 (36.6; 36.0, 37.3)	6,677 (40.3; 39.5, 41.0)	5,683 (32.6; 31.9, 33.3)	5,201 (28.4; 27.7, 29.0)	3,819 (25.1; 24.4, 25.8)	3,169 (22.7; 22.0, 23.4)	2,704 (22.4; 21.6, 23.1)	<0.001	5,323 (26.9; 26.3, 27.5)	4,674 (22.7; 22.1, 23.2)	3,764 (16.7; 16.2, 17.2)	3,540 (14.8; 14.3, 15.2)	2,603 (12.7; 12.2, 13.1)	2,291 (12.8; 12.3, 13.3)	1,740 (11.7; 11.2, 12.2)	<0.001
Western	5,715 (29.2; 28.6, 29.8)	4,204 (25.4; 24.7, 26.0)	3,377 (19.4; 18.8, 20.0)	3,047 (16.6; 16.1, 17.2)	2,518 (16.6; 16.0, 17.2)	2,398 (17.2; 16.6, 17.8)	2,087 (17.3; 16.6, 18.0)	<0.001	5,391 (27.2; 26.6, 27.9)	5,634 (27.3; 26.7, 27.9)	4,757 (21.1; 20.6, 21.6)	4,139 (17.3; 16.8, 17.7)	3,506 (17.1; 16.5, 17.6)	2,890 (16.2; 15.6, 16.7)	2,372 (15.9; 15.3, 16.5)	<0.001
Educational level																
Primary school or lower	467 (2.4; 2.2, 2.6)	340 (2.1; 1.8, 2.3)	332 (1.9; 1.7, 2.1)	255 (1.4; 1.2, 1.6)	236 (1.6; 1.4, 1.8)	565 (4.1; 3.7, 4.4)	188 (1.6; 1.3, 1.8)	0.116	1,315 (6.6; 6.3, 7.0)	1,089 (5.3; 5.0, 5.6)	1,074 (4.8; 4.5, 5.1)	869 (3.6; 3.4, 3.9)	793 (3.9; 3.6, 4.1)	639 (3.6; 3.3, 3.9)	473 (3.2; 2.9, 3.5)	<0.001
Junior high school	4,856 (24.8; 24.2, 25.4)	4,621 (27.9; 27.2, 28.6)	4,407 (25.3; 24.7, 26.0)	4,414 (24.1; 23.5, 24.7)	3,397 (22.3; 21.7, 23.0)	2,690 (19.3; 18.6, 20.0)	2,096 (17.4; 16.7, 18.0)	<0.001	8,773 (44.3; 43.6, 45.0)	8,858 (42.9; 42.3, 43.6)	8,881 (39.4; 38.8, 40.0)	9,611 (40.1; 39.4, 40.7)	7,867 (38.3; 37.6, 38.9)	6,316 (35.4; 34.7, 36.1)	5,050 (33.9; 33.1, 34.7)	<0.001
Senior high school/vocational training	7,052 (36.0; 35.3, 36.7)	6,325 (38.2; 37.4, 38.9)	5,642 (32.4; 31.7, 33.1)	5,585 (30.5; 29.8, 31.2)	4,291 (28.2; 27.5, 28.9)	3,363 (24.1; 23.4, 24.8)	2,684 (22.2; 21.5, 23.0)	<0.001	5,085 (25.7; 25.1, 26.3)	5,212 (25.3; 24.7, 25.9)	5,632 (25.0; 24.4, 25.6)	5,874 (24.5; 23.9, 25.0)	4,879 (23.7; 23.2, 24.3)	4,105 (23.0; 22.4, 23.6)	3,206 (21.5; 20.9, 22.2)	<0.001
College or above	7,202 (36.8; 36.1, 37.5)	5,290 (31.9; 31.2, 32.6)	7,040 (40.4; 39.7, 41.1)	8,070 (44.0; 43.3, 44.8)	7,278 (47.9; 47.1, 48.7)	7,327 (52.5; 51.7, 53.4)	7,112 (58.9; 58.0, 59.8)	<0.001	4,623 (23.4; 22.8, 23.9)	5,474 (26.5; 25.9, 27.1)	6,950 (30.8; 30.2, 31.4)	7,633 (31.8; 31.2, 32.4)	7,013 (34.1; 33.5, 34.8)	6,804 (38.1; 37.4, 38.8)	6,166 (41.4; 40.6, 42.2)	<0.001

Continued

Variable	Primiparas 2016	Primiparas 2017	Primiparas 2018	Primiparas 2019	Primiparas 2020	Primiparas 2021	Primiparas 2022	Primiparas *P*	Multiparas 2016	Multiparas 2017	Multiparas 2018	Multiparas 2019	Multiparas 2020	Multiparas 2021	Multiparas 2022	Multiparas *P*
Delivery mode																
Vaginal delivery	12,737 (65.1; 64.4, 65.7)	10,383 (62.6; 61.9, 63.4)	11,124 (63.9; 63.1, 64.6)	12,061 (65.8; 65.1, 66.5)	9,529 (62.7; 61.9, 63.5)	8,844 (63.4; 62.6, 64.2)	7,495 (62.0; 61.2, 62.9)	<0.001	11,127 (56.2; 55.5, 56.9)	11,993 (58.1; 57.4, 58.8)	13,772 (61.1; 60.5, 61.7)	15,247 (63.6; 63.0, 64.2)	13,000 (63.3; 62.6, 63.9)	11,481 (64.3; 63.6, 65.0)	9,547 (64.1; 63.3, 64.9)	<0.001
Cesarean section	6,840 (34.9; 34.3, 35.6)	6,193 (37.4; 36.6, 38.1)	6,297 (36.1; 35.4, 36.9)	6,263 (34.2; 33.5, 34.9)	5,673 (37.3; 36.5, 38.1)	5,101 (36.6; 35.8, 37.4)	4,585 (38.0; 37.1, 38.8)	<0.001	8,669 (43.8; 43.1, 44.5)	8,640 (41.9; 41.2, 42.6)	8,765 (38.9; 38.3, 39.5)	8,740 (36.4; 35.8, 37.0)	7,552 (36.7; 36.1, 37.4)	6,383 (35.7; 35.0, 36.4)	5,348 (35.9; 35.1, 36.7)	<0.001
Preterm delivery																
Yes	732 (3.7; 3.5, 4.0)	612 (3.7; 3.4, 4.0)	726 (4.2; 3.9, 4.5)	753 (4.1; 3.8, 4.4)	714 (4.7; 4.4, 5.0)	662 (4.7; 4.4, 5.1)	564 (4.7; 4.3, 5.1)	<0.001	861 (4.3; 4.1, 4.6)	890 (4.3; 4.0, 4.6)	1,080 (4.8; 4.5, 5.1)	1,179 (4.9; 4.6, 5.2)	1,036 (5.0; 4.7, 5.3)	959 (5.4; 5.0, 5.8)	841 (5.6; 5.3, 6.0)	<0.001
No	18,845 (96.3; 96.0, 96.5)	15,964 (96.3; 96.0, 96.6)	16,695 (95.8; 95.5, 96.1)	17,571 (95.9; 95.6, 96.2)	14,488 (95.3; 95.0, 95.6)	13,283 (95.3; 94.9, 95.6)	11,516 (95.3; 94.9, 95.7)	<0.001	18,935 (95.7; 95.4, 95.9)	19,743 (95.7; 95.4, 96.0)	21,457 (95.2; 94.9, 95.5)	22,808 (95.1; 94.8, 95.4)	19,516 (95.0; 94.7, 95.3)	16,905 (94.6; 94.3, 95.0)	14,054 (94.4; 94.0, 94.7)	<0.001
Low birth weight																
Yes	730 (3.7; 3.5, 4.0)	641 (3.9; 3.6, 4.2)	718 (4.1; 3.8, 4.4)	762 (4.2; 3.9, 4.5)	683 (4.5; 4.2, 4.8)	662 (4.7; 4.4, 5.1)	580 (4.8; 4.4, 5.2)	<0.001	616 (3.1; 2.9, 3.4)	632 (3.1; 2.8, 3.3)	778 (3.5; 3.2, 3.7)	912 (3.8; 3.6, 4.1)	735 (3.6; 3.3, 3.8)	673 (3.8; 3.5, 4.1)	581 (3.9; 3.6, 4.2)	<0.001
No	18,847 (96.3; 96.0, 96.5)	15,935 (96.1; 95.8 , 96.4)	16,703 (95.9; 95.6, 96.2)	17,562 (95.8; 95.5, 96.1)	14,519 (95.5; 95.2, 95.8)	13,283 (95.3; 94.9, 95.6)	11,500 (95.2; 94.8, 95.6)	<0.001	19,180 (96.9; 96.6, 97.1)	20,001 (96.9; 96.7, 97.2)	21,759 (96.5; 96.3, 96.8)	23,075 (96.2; 96.0, 96.4)	19,817 (96.4; 96.2, 96.7)	17,191 (96.2; 95.9, 96.5)	14,314 (96.1; 95.8, 96.4)	<0.001
Macrosomia																
Yes	634 (3.2; 3.0, 3.5)	466 (2.8; 2.6, 3.1)	515 (3; 2.7, 3.2)	538 (2.9; 2.7, 3.2)	504 (3.3; 3.0, 3.6)	393 (2.8; 2.5, 3.1)	318 (2.6; 2.4, 2.9)	0.057	775 (3.9; 3.6, 4.2)	742 (3.6; 3.3, 3.9)	816 (3.6; 3.4, 3.9)	856 (3.6; 3.3, 3.8)	803 (3.9; 3.6, 4.2)	609 (3.4; 3.1, 3.7)	473 (3.2; 2.9, 3.5)	0.004
No	18,943 (96.8; 96.5, 97.0)	16,110 (97.2; 96.9, 97.4)	16,906 (97.0; 96.8, 97.3)	17,786 (97.1; 96.8, 97.3)	14,698 (96.7; 96.4, 97.0)	13,552 (97.2; 96.9, 97.5)	11,762 (97.4; 97.1, 97.6)	0.057	19,021 (96.1; 95.8, 96.4)	19,891 (96.4; 96.1, 96.7)	21,721 (96.4; 96.1, 96.6)	23,131 (96.4; 96.2, 96.7)	19,749 (96.1; 95.8, 96.4)	17,255 (96.6; 96.3, 96.9)	14,422 (96.8; 96.5, 97.1)	0.004
Total	19,577 (100.0)	16,576 (100.0)	17,421 (100.0)	18,324 (100.0)	15,202 (100.0)	13,945 (100.0)	12,080 (100.0)		19,796 (100.0)	20,633 (100.0)	22,537 (100.0)	23,987 (100.0)	20,552 (100.0)	17,864 (100.0)	14,895 (100.0)	

subsequently lowering their cesarean rates (*3*). Additionally, the findings revealed that the incidence of macrosomia was higher in multiparas compared to primiparas, potentially linked to a greater prevalence of gestational diabetes in multiparas, a condition known to elevate the risk of macrosomia. Between 2016 and 2022, there was a rising trend in the incidences of preterm birth and LBW among both primiparas and multiparas. This increase is likely related to higher maternal ages, which have been strongly associated with both preterm birth and LBW (*10*).

This study's strengths lie in its reliance on continuous, systematic data monitoring, a substantial sample size, and broad temporal scope, which collectively provide a detailed insight into evolving maternity patterns within the monitored regions.

However, the study is limited by its focus on just six provinces, restricting the applicability of its findings to the national level and suggesting a need for more geographically inclusive data. Furthermore, while this study has concentrated on preterm births, LBW, and macrosomia, future research should include a wider range of adverse outcomes to expand upon these findings.

In conclusion, between 2016 and 2022, there was a notable increase in the proportion of older mothers, along with a rise in the incidences of preterm births and LBWs, particularly among multiparas in the monitored regions. Consequently, it is vital for perinatal healthcare workers and obstetricians to enhance the management of pregnant women, particularly among AMA groups, and to improve the quality of medical services to mitigate the risk of adverse pregnancy outcomes. Additionally, measures should be implemented to encourage women to conceive at an optimal age to foster maternal and child health.

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Preplanned Studies

Fertility Intentions Among Reproductive-Age Women — Three Provinces, China, 2023

Yuting Qiao[1]; Jiangli Di[1,#]; Sidi Chen[1]; Huanqing Hu[1]

Summary

What is already known about this topic?

Low fertility rates have become the most important risk affecting the balanced development of the population in China.

What is added by this report?

About 80.0% of childless women had fertility intentions, 31.9% of women with one child and 11.3% of women with two children intended to have a second and third child, respectively. Women with one child who had an agricultural *Hukou**, were younger than 30 years old, were remarried, and had received a deduction or reimbursement for childbirth expenses during their first delivery were more willing to have a second child. Women with two children who had an agricultural *Hukou* and a upper-middle personal income, were self-employed, and had two daughters were more willing to have a third child.

What are the implications for public health practice?

In China, women's willingness to have more children is not optimistic. To increase their desire for more children, creating a more favorable fertility environment for reproductive-age women and providing more preferential fertility policies for pregnant women will be necessary.

The global decline in fertility rates is a pressing concern. The United Nations reports that the global fertility rate fell from 3.2 to 2.5 live births per woman between 1990 and 2019, with projections reaching 1.9 by 2100. Similarly, China's 2020 census revealed a total fertility rate of 1.3, signifying a very low fertility society. Despite policy adjustments since 2013, China's fertility rate continues to dwindle, with 1.38 million fewer newborns in 2021 compared to 2020 (*1*). In response, 17 government departments, including the National Health Commission and the National Development and Reform Commission, introduced 20 fertility support measures in July 2022, encompassing taxation, insurance, education, housing, and employment (*2*). While fertility desire is a strong predictor of actual fertility behavior, research on fertility intentions across Chinese provinces since the implementation of these 20 measures remains limited. Therefore, this study investigates whether these supportive measures have effectively stimulated fertility intentions among childbearing-age women. A cross-sectional survey of 8,002 women aged 20–49 was conducted in Shandong, Hunan, and Yunnan provinces between August and September 2023. Preliminary findings indicate higher childbearing intentions among childless women. However, the intention for a second or third child remains notably low among one-child and two-child women, respectively. The decision to have children correlates with factors such as age, education, occupation, *Hukou*, marital status, income, children's gender, and childbirth expense reimbursements. Creating a more supportive fertility environment and providing preferential policies for pregnant women are crucial steps towards addressing this issue.

This cross-sectional survey was conducted in August and September 2023 in Shandong, Hunan, and Yunnan provinces. Within each province, the provincial capital (urban area) and one county under its jurisdiction (rural area) were selected as investigation sites. A multi-stage stratified random cluster sampling method was used to select women aged 20–49 years at each site. First, one street (urban) or town (rural) was randomly selected. Second, two neighborhoods (urban) or villages (rural) were randomly selected, stratified by distance from the administrative office to ensure representation of areas closer and farther away. Finally, within each selected

**Hukou* is a record in the system of household registration required by law in China. It officially identifies a person as a resident of an area and includes identifying information such as name, parents, spouse, and date of birth. There are two kinds of *Hukou* in China, namely agricultural *Hukou* and non-agricultural *Hukou*.

neighborhood or village, all eligible women aged 20–49 years were recruited until the target sample size was reached. Data were collected through face-to-face interviews. Ethical approval was obtained from the Ethical Review Committee of the National Center for Women and Children's Health, China CDC (No. FY2023-8), and all participants provided informed consent. Chi-square tests were used to compare differences between groups. A multiple logistic regression model examined factors associated with fertility intentions, with unwillingness (marked as 0) and willingness (marked as 1) as the dependent variables, and factors identified as statistically significant in the Chi-square analysis as independent variables.

This study included 8,002 women: 2,595 (32.4%) from Shandong Province, 2,767 (34.6%) from Hunan Province, and 2,819 (35.2%) from Yunnan Province. Of these, 4,410 (55.1%) resided in urban areas and 3,771 (47.1%) in rural areas. The women's average age was 34.11±7.30 years. A total of 5,769 (72.1%) women had children, including 2,539 (31.7%) with one child and 3,051 (38.1%) with two children.

Among the 2,412 childless women, the majority were aged 25–29 (35.0%), resided in urban areas (61.0%), held non-agricultural *Hukou* (63.5%), had attained a college degree or higher (72.9%), and worked as professionals (e.g., doctors, teachers, engineers) (31.3%). Of the 2,539 one-child women, the largest group (25.8%) were aged 30–34, 67.3% had a college degree or higher, and 24.4% were professionals. Among the 3,051 two-child women, the largest group (32.2%) were aged 35–39. Most two-child women (69.8%) held agricultural *Hukou*, 74.7% had attained a senior high school diploma, and 31.5% worked as farmers. Detailed sociodemographic characteristics are presented in Table 1.

Among the 2,412 childless women, 1,933 (80.1%) intended to have at least one child, with 1,015 (42.1%) desiring one child and 918 (38.0%) desiring more than one. The remaining 479 (19.9%) reported no intention to have children. Fertility intentions for a first child varied by region: 39.2% (273/697) in the east, 45.6% (450/986) in the central region, and 40.1% (292/729) in the west. Univariate analysis revealed that childless women who were more likely to intend to have children (P<0.05) shared the following characteristics: residence in the eastern region (84.4%) or rural areas (85.6%), agricultural *Hukou* (82.4%), age between 35–39 years (92.4%), junior high school education or lower (92.3%), first marriage (90.3%), employment as farmers (89.4%), average monthly individual income (87.9%) or household income (85.5%) between 5,000–9,999 Yuan, sexually active (88.7%), history of pregnancy (91.9%), and presence of reproductive system diseases (84.9%) (Table 1).

Among one-child women who intended to have a second child (31.9%, n=810), univariate analysis revealed significant associations (P<0.05) with several factors. These included residing in a rural area (36.3%), having an agricultural Hukou (36.4%), being younger than 25 years old (50.0%), being remarried (42.7%), being unemployed (36.3%), and having a spouse younger than 25 years old (45.0%). Additionally, a history of abortion (36.7%), complications during first childbirth (40.3%), abnormal delivery history (39.2%), receiving free antenatal care (33.1%), and receiving childbirth expense deductions or reimbursements (33.7%) were also significantly associated with the intention to have a second child (Table 1).

Among two-child women, only 98 (11.3%) intended to have a third child. Univariate analysis revealed that the following characteristics were significantly associated with a higher likelihood of intending to have a third child (P<0.05): agricultural *Hukou* (12.5%), self-employment (15.1%), average monthly income of 10,000–30,000 Chinese Yuan (CNY) (25.0%), having daughters (14.4%), and a history of abortion (13.3%) (Table 1).

Multiple logistic regression analysis revealed distinct factors associated with the willingness to have more children. Childless women residing in rural areas, possessing an agricultural *Hukou*, aged 35–39 years, with a junior high school education or lower, married, and having an average monthly household income of 3,000–9,999 CNY demonstrated a higher willingness to have children. Among one-child women, factors associated with a greater willingness to have a second child included having an agricultural *Hukou*, being younger than 30 years old, being remarried, and having received deductions or reimbursements for childbirth expenses during their first delivery. Two-child women who were more willing to have a third child were more likely to have an agricultural *Hukou*, an average monthly income of 5,000–3,0000 CNY, be self-employed, and have daughters (Table 2).

DISCUSSION

This study, the first known cross-sectional survey in China to employ a multi-stage stratified random

TABLE 1. Univariate analysis of the association of characteristics with fertility intentions in Shandong, Hunan, and Yunnan provinces, China, 2023.

Characteristics	Fertility intentions of childless women			Fertility intentions to have a second child of one-child women			Fertility intentions to have a third child of two-child women		
	N	***n* (%)**	***P***	***N***	***n* (%)**	***P***	***N***	***n* (%)**	***P***
Region			0.001			0.407			0.696
Eastern	697	588 (84.4)		870	267 (30.7)		965	116 (12.0)	
Central	986	785 (79.6)		796	268 (33.7)		941	104 (11.1)	
Western	729	560 (76.8)		873	275 (31.5)		1,145	125 (10.9)	
Place of residence			<0.001			<0.001			0.083
Urban	1,472	1,128 (76.6)		1,381	390 (28.2)		1,449	179 (12.4)	
Rural	940	805 (85.6)		1,158	420 (36.3)		1,602	166 (10.4)	
Age, years			<0.001			<0.001			0.081
<25	570	379 (66.5)		1,220	21 (50.0)		921	4 (28.6)	
25–29	833	654 (78.5)		1,319	168 (43.4)		2,130	26 (14.4)	
30–34	386	332 (86.0)			240 (36.7)			73 (9.4)	
35–39	249	230 (92.4)		2,406	143 (28.5)		2,867	108 (11)	
40–44	182	159 (87.4)		133	79 (19.2)		184	78 (11.7)	
45–49	192	164 (85.4)			159 (29.3)			56 (12.9)	
Hukou			<0.001	42		<0.001	14		0.017
Non-agricultural	1,532	671 (76.3)		387	330 (27)		181	77 (8.3)	
Agricultural	880	1,262 (82.4)		654	480 (36.4)		773	268 (12.5)	
Ethnicity			0.199	502		0.382	983		0.5
Han	2,266	1,822 (80.4)		411	763 (31.7)		665	327 (11.4)	
Minority	146	111 (76.0)		543	47 (35.3)		435	18 (9.8)	
Educational level			<0.001			0.498			0.625
Junior high school or below	336	310 (92.3)		323	112 (34.7)		773	90 (11.3)	
Senior high school	317	283 (89.3)		507	157 (31)		2,278	75 (10.4)	
College or higher	1,759	1,340 (76.2)		1,709	541 (31.7)		1,559	180 (11.8)	
Marital status			<0.001			<0.001			0.118
First marriage	1,327	1,243 (93.7)		2,348	756 (32.2)		2,849	314 (11.0)	
Remarriage	72	65 (90.3)		89	38 (42.7)		149	26 (17.4)	
Single	1,013	625 (61.7)		102	16 (15.7)		53	5 (9.4)	
Occupation			<0.001			0.001			0.046
Farmer	359	321 (89.4)		376	133 (35.4)		960	105 (10.9)	
Factory worker	103	87 (84.5)		148	30 (20.3)		115	10 (8.7)	
Civil servant	243	175 (72)		278	82 (29.5)		203	25 (12.3)	
Doctor/teacher/engineer	754	586 (77.7)		619	225 (36.3)		580	63 (10.9)	
Clerk	228	190 (83.3)		226	65 (28.8)		163	19 (11.7)	
Service staff	214	169 (79.0)		304	80 (26.3)		326	30 (9.2)	
Self-employment person	235	195 (83.0)		286	90 (31.5)		383	58 (15.1)	
Unemployed	155	122 (78.7)		175	67 (38.3)		209	22 (10.5)	
Other	121	88 (72.7)		127	38 (29.9)		112	13 (11.6)	
Average monthly income, CNY			0.001			0.216			0.002
<3,000	882	688 (78)		833	246 (29.5)		1,402	146 (10.4)	
3,000–4,999	976	767 (78.6)		1,055	332 (31.5)		980	101 (10.3)	

Continued

Characteristics	Fertility intentions of childless women			Fertility intentions to have a second child of one-child women			Fertility intentions to have a third child of two-child women		
	N	***n* (%)**	***P***	***N***	***n* (%)**	***P***	***N***	***n* (%)**	***P***
5,000–9,999	322	283 (87.9)		448	156 (34.8)		333	48 (14.4)	
10,000–30,000	55	42 (76.4)		32	11 (34.4)		32	8 (25.0)	
>30,000	18	14 (77.8)		14	5 (35.7)		22	1 (4.5)	
No income	159	139 (87.4)		157	60 (38.2)		282	41 (14.5)	
Have insurance			0.261			0.084			0.176
Yes	1,776	1433 (80.7)		2,044	636 (31.1)		2,175	235 (10.8)	
No	636	500 (78.6)		495	174 (35.2)		876	110 (12.6)	
Have maternity insurance			0.398			0.296			0.086
Yes	1,410	1132 (80.3)		1,588	485 (30.5)		1,372	155 (11.3)	
No	366	301 (82.2)		456	151 (33.1)		803	72 (9.0)	
Family average monthly income, CNY			<0.001			0.265			0.45
<3,000	547	406 (74.2)		414	124 (30)		798	103 (12.9)	
3,000–4,999	818	646 (79.0)		732	229 (31.3)		936	105 (11.2)	
5,000–9,999	691	591 (85.5)		960	300 (31.3)		928	93 (10.0)	
10,000–30,000	312	255 (81.7)		401	147 (36.7)		342	38 (11.1)	
>30,000	44	35 (79.5)		32	10 (31.3)		47	6 (12.8)	
Gender of children			−			0.708			<0.001
Boys	−	−		1,443	456 (31.6)		743	80 (10.7)	
Girls	−	−		1,096	354 (32.3)		749	108 (14.4)	
Boy and girl	−	−		−	−		1,559	235 (10.8)	
History of abortion			0.099			0.006			0.021
Yes	201	185 (92.0)		788	289 (36.7)		1,080	144 (13.3)	
No	1,060	933 (88.0)		1,259	388 (30.8)		1,292	126 (9.8)	
Complications of pregnancy			−			<0.001			0.115
Yes	−	−		365	147 (40.3)		426	55 (12.9)	
No	−	−		2,174	663 (30.5)		2,625	290 (11.0)	
History of abnormal delivery			−			0.045			0.441
Yes	−	−		153	60 (39.2)		217	28 (12.9)	
No	−	−		2,386	750 (31.4)		2,834	317 (11.2)	
Mode of delivery			−			0.915			0.687
Vaginal delivery	−	−		1,521	484 (31.8)		1,720	191 (11.1)	
Caesarean section	−	−		1,018	326 (32.0)		1,331	154 (11.6)	
Free antenatal examination			−			0.035			0.693
Yes	−	−		962	318 (33.1)		1,388	140 (10.8)	
No	−	−		1,055	303 (28.7)		1,192	137 (11.5)	
Deduction or reimbursement for childbirth expenses			−			0.012			0.357
Yes	−	−		1,575	531 (33.7)		2,151	222 (11.1)	
No	−	−		964	279 (28.9)		900	103 (11.9)	
Painless delivery						0.332			0.258
Yes	−	−		1,076	332 (30.9)		1,137	119 (10.5)	
No	−	−		1,462	478 (32.7)		1,914	226 (11.8)	

Note: "−" means no data.
Abbreviation: CNY=Chinese Yuan.

TABLE 2. Multiple logistic regression model for fertility intentions — Shandong, Hunan, and Yunnan provinces, China, 2023

Variables	estimated *β*	Wald χ^2	*P*	*OR*	95% *CI*
Fertility intentions of childless women					
Place of residence					
Urban				Ref	
Rural	0.421	11.034	0.001	1.52	1.19, 1.95
Hukou					
Non-agricultural				Ref	
Agricultural	0.422	11.426	0.001	1.53	1.19, 1.95
Age, years					
<25				Ref	
25–29	0.209	2.15	0.143	1.23	0.93, 1.63
30–34	0.331	2.668	0.102	1.39	0.94, 2.1
35–39	0.655	5.118	0.024	1.92	1.09, 3.39
40–44	0.092	0.102	0.750	1.10	0.62, 1.93
45–49	0.635	3.32	0.068	1.89	0.95, 3.74
Educational level					
College or higher				Ref	
Senior high school	0.389	2.187	0.139	1.48	0.88, 2.5
Junior high school or below	0.472	4.683	0.030	1.60	1.05, 2.46
Marital status					
Single				Ref	
First marriage	1.943	158.727	<0.001	6.98	5.16, 9.44
Remarriage	1.241	7.916	0.005	3.46	1.46, 8.21
Household average monthly income, CNY					
<3,000				Ref	
3,000–4,999	0.595	12.406	<0.001	1.81	1.30, 2.53
5,000–9,999	0.768	15.911	<0.001	2.16	1.48, 3.14
10,000–30,000	0.433	3.119	0.077	1.54	0.95, 2.49
>30,000	0.715	2.35	0.125	2.04	0.80, 5.10
Fertility intentions to have a second child of one-child women					
Hukou					
Non-agricultural				Ref	
Aricultural	0.317	9.685	0.002	1.37	1.13, 1.68
Marital status					
First marriage				Ref	
Remarriage	0.535	4.517	0.034	1.71	1.04, 2.80
Single	−0.849	6.510	0.011	0.43	0.22, 0.82
Age, years					
45–49				Ref	
<25	0.747	4.167	0.041	2.11	1.00, 4.33
25–29	0.549	10.005	0.002	1.73	1.23, 2.44
30–34	0.184	1.390	0.238	1.20	0.89, 1.63
35–39	−0.213	1.605	0.205	0.81	0.58, 1.12
40–44	−0.547	8.573	0.003	0.58	0.40, 0.84

Continued

Variables	estimated β	Wald χ^2	P	OR	95% CI
Deduction or reimbursement for childbirth expenses					
No				Ref	
Yes	0.266	6.292	0.012	1.31	1.10, 1.61
Fertility intentions to have a third child of two-child women					
Hukou					
Non-agricultural				Ref	
Agricultural	0.691	6.606	0.010	2.00	1.18, 3.40
Average monthly income, CNY					
<3,000				Ref	
3,000–4,999	0.020	0.005	0.942	1.02	0.59, 1.76
5,000–9,999	0.787	5.530	0.019	2.20	1.14, 4.23
10,000–30,000	1.590	7.418	0.006	4.91	1.56, 15.40
>30,000	−17.451	0.000	0.998	0.00	
No income	0.442	1.461	0.227	1.56	0.76, 3.19
Gender of children					
Boy				Ref	
Girl	0.953	10.404	0.001	2.59	1.45, 4.63
Boy and girl	0.113	0.146	0.703	1.12	0.63, 2.00
Occupation					
Farmer				Ref	
Factory worker	−0.105	0.019	0.890	0.90	0.20, 3.97
Civil servant	−1.342	1.670	0.196	0.26	0.03, 2.00
Doctor/teacher/engineer	0.540	2.421	0.120	1.72	0.87, 3.39
Clerk	0.339	0.406	0.524	1.40	0.50, 3.98
Service staff	0.243	0.312	0.577	1.28	0.54, 2.99
Self-employed	0.901	7.554	0.006	2.46	1.30, 4.68
Unemployed	0.560	1.722	0.189	1.75	0.76, 4.04
Other	0.891	2.920	0.088	2.44	0.88, 6.77

Abbreviation: CNY=Chinese Yuan; *CI*=confidence interval; *OR*=odds ratio.

cluster sampling method to examine fertility intentions and associated factors among women of childbearing age across different regions, revealed discrepancies in fertility intentions compared to previous research (*3*–*6*). The current study found a lower intention rate for a second child among one-child women (31.9%) than Jing's 2022 survey (55.6%) (*3*) and a lower intention rate for a third child among two-child women (11.3%) than previous surveys (12.2%) (*5*). These differences may be attributed to the online methodology of the previous surveys, potentially attracting a higher proportion of participants who were already interested in fertility policies and having more children. Notably, this study observed higher fertility intentions for a first child among childless women (45.6%) and for a second child among one-child women (33.7%) in Hunan compared to Chen Qian's 2022 Hunan-based study (*6*), which also employed a multi-stage sampling method but found that 35.9% of childless women and 29.7% of one-child women intended to have a/nother child. This suggests that the continuous adjustment of fertility policy and implementation of supportive measures may lead to a gradual realization of childbirth potential among childless and one-child women.

This study found that residing in rural areas or having an agricultural *Hukou* was associated with higher fertility intentions, consistent with previous research (*7*–*9*). Notably, *Hukou* was the only factor influencing childbearing desires across all three groups:

childless, one-child, and two-child women. This aligns with previous findings (*8*) suggesting that traditional cultural influences contribute to a stronger desire for larger families among rural women. Furthermore, the differential impact of the one-child policy — imposing looser restrictions on women with agricultural *Hukou* and stricter ones on those with non-agricultural *Hukou* — might explain the slower increase in childbearing desires among urban women following the policy's relaxation.

This study revealed that childless women aged 35–39 exhibited the strongest desire for children. This finding may be attributed to younger women prioritizing educational attainment and career advancement, leading to delayed marriage and childbearing. By ages 35–39, with increased career stability, the desire for children may become more pronounced. Additionally, advancements in Chinese medical technology and reproductive policies likely contribute to this trend. These improvements offer greater support for high-risk pregnancies, potentially boosting the confidence of older women considering childbearing.

Consistent with previous studies, women with middle or higher household incomes were more likely to intend to have children than those with lower incomes (*10*). This suggests that financial stability, which allows families to meet both childcare and daily expenses, may increase the desire for children. However, while higher individual income was positively correlated with birth intentions, a large majority (77.7%) of self-employed women also reported intending to have a child. Furthermore, among women with two children, professional women constituted the largest group intending to have a third child. These findings suggest that flexible work schedules, often available to self-employed and professional women, may mitigate the negative career impacts associated with larger families.

Contrary to previous studies, this study found no association between education level and the intention to have more children. Instead, maternity subsidies, such as free antenatal examinations and hospital delivery subsidies, emerged as major influencing factors. This suggests that effectively implemented fertility support measures could further increase desired family size. Additionally, univariate analysis revealed that women with a history of miscarriage or previous pregnancy complications were more likely to have a second child, highlighting the need for targeted support for this demographic.

This study reveals a low intention to have second or third children among Chinese women. To effectively increase birth rates, China must cultivate a more supportive fertility environment. This includes implementing measures to eliminate employment barriers for mothers and alleviate work-family conflict. Additionally, expanding access to preferential fertility policies, particularly those supporting pregnant women, is crucial to enhancing reproductive desires.

This study benefits from its multi-stage stratified random cluster sampling method across diverse regions. Furthermore, it uniquely examines the influence of socio-demographic characteristics, pregnancy history, and service utilization during pregnancy on fertility intentions.

There were some limitations in this study. First, the geographic limitation to six counties/districts within three provinces may restrict the generalizability of findings to regional and national levels. Second, factors like childcare and children's education were not considered in this study.

Conflicts of interest: No conflicts of interest.

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Preplanned Studies

Reproductive Characteristics and Trends of Major Pregnancy Outcomes of Women at Different Parities — Beijing Municipality, China, 2013–2022

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Summary

What is already known about this topic?

With socioeconomic development, the increase of older pregnancies and multiparas has brought risks to mothers and infants.

What is added by this report?

As parities increased, the proportion of women of advanced maternal age (AMA) and non-local domicile increased, while the proportion of women with higher education levels decreased. Women with ≥3 parities are more likely to have preterm birth (PTB) and macrosomia.

What are the implications for public health practice?

A comprehensive analysis of pregnancy traits among women at different parities offers a robust foundation for tailored strategies against adverse pregnancy outcomes.

Studies have shown significant changes in the composition of the pregnant population and pregnancy outcomes over the past decade (*1*–*2*). With the rising number of older mothers and high-risk pregnancies, the incidences of birth defects, preterm birth (PTB), and low birth weight (LBW) have also increased. Higher parity is associated with a higher incidence of these adverse pregnancy outcomes. However, the overall understanding of the reproductive characteristics of women with different parities in Beijing Municipality, China, remains limited. Therefore, a thorough analysis of the characteristics and pregnancy outcomes of women with different parities in Beijing is particularly valuable to inform better healthcare services and decision-making. This study analyzed data from 1,963,445 women with a gestational week of delivery ≥28 weeks and permanent residence in Beijing who gave birth between January 1, 2013 and December 31, 2022. The results showed that as parity increased, the proportions of advanced maternal age (AMA) and non-local domicile increased, while those with higher education levels decreased. Women with ≥3 parities had a higher risk of PTB and macrosomia. Therefore, strengthening maternal healthcare for multiparous women, especially those who are AMA or non-local, is essential.

This study utilized data from birth medical certificates issued through the Beijing Maternal and Child Health Network Information System. This system is used by all midwifery institutions in Beijing to issue birth certificates, with the average annual number issued accounting for 99.5% of newborns from these institutions. Given that the hospital delivery rate in Beijing is 99.99%, the data accurately reflects the fertility status of women in Beijing (*3*). This study analyzed data from 1,963,445 women residing in Beijing who delivered at ≥28 weeks between January 1, 2013 and December 31, 2022. Women were grouped by parity: P1 (primipara, parity=1), P2 (multipara, parity=2), and P≥3 (multipara, parity≥3). Chi-square tests and analysis of variance (ANOVA) were used for data analysis, with the Bonferroni method applied for multiple comparisons using R software (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria). Joinpoint regression program (version 5.0.2, Statistical Research and Applications Branch, National Cancer Institute) analyzed the annual percentage change (APC) in the proportion of cesarean section (C-section), PTB, LBW, and macrosomia across parity groups. All statistical analyses used a two-sided test with a significance level of $P<0.05$. The study was approved by the Medical Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Ethics Approval Numbers: 2020-KY-032-01; 2021-KY-054-01).

This study included 1,331,201 (67.8%) primiparous women and 632,244 (32.2%) multiparous women. Among multiparous women, 95.1% were P2, and

4.9% were P≥3. The average maternal age was 30.4 years, and 17.5% of women were of AMA (≥35 years).

Table 1 demonstrates that the proportion of AMA increases with parity: 9.7%, 33.3%, and 43.6% for P1, P2, and P≥3 groups, respectively. To further analyze reproductive characteristics and major pregnancy outcomes, the results were stratified by maternal age (<35 or ≥35 years). Among women younger than 35 years, the proportion with an undergraduate education was highest in the P1 and P2 groups (66.9% and 54.5%, respectively). Conversely, the P≥3 group was predominantly composed of women with a junior secondary or primary school education (44.8%). The proportion of women with non-local domicile increased with parity: 33.6%, 41.2%, and 74.5% for P1, P2, and P≥3 groups, respectively. Regarding occupation, the proportion of office workers/professional and technical personnel was highest in the P1 and P2 groups (53.8% and 43.7%, respectively), while the P≥3 group had the largest proportion of unemployed/freelance individuals (31.0%). Within the AMA stratum, the P1 and P2 groups also exhibited the highest proportion of women with undergraduate education. In the P≥3 group, the most common education level was undergraduate (36.9%), followed by junior secondary or primary school (34.2%). Similar to the younger age stratum, the proportion of women with non-local domicile increased with parity. The largest proportion of women in the AMA stratum were office workers/professional and technical personnel (54.3%, 55.7%, and 27.9% for P1, P2, and P≥3 groups, respectively). However, the proportion of unemployed/freelance individuals in the P≥3 group was also higher in this stratum (23.1%).

Among women younger than 35 years, the proportion of C-section births was lowest in the primiparous group (36.9%), followed by those with three or more prior deliveries (38.7%) and the second-time delivering group (42.0%) (Table 2). Newborn outcomes also varied by parity in this age group. The incidence of PTB was highest (8.0%) among women with three or more prior deliveries and lowest (5.4%) in the second-time delivering group. The incidence of LBW was highest in the primiparous group (4.7%) and lowest (3.1%) in the second-time delivering group. Macrosomia increased with parity, with rates of 6.7%, 7.8%, and 9.3% in the primiparous, second-time delivering, and three or more prior deliveries groups, respectively. Among women of AMA, the proportion of C-section decreased as parity increased. The highest proportions of PTB and LBW were observed in the primiparous group (10.5% and 8.6%, respectively), followed by the three or more prior deliveries group (9.8% and 5.3%) and the second-time delivering group (7.5% and 4.5%). The incidence of macrosomia was highest (9.6%) in the group with three or more prior deliveries.

Figure 1 displays the trends of major pregnancy outcomes over the past decade. Among women younger than 35 years, the C-section rate in the P1 group decreased from 43.2% in 2013 to 35.2% in 2015 ($APC_{2013–2015}$=−11.8%, P<0.05), then increased to 37.3% by 2022 ($APC_{2015–2022}$=2.0%, P<0.05). The P2 group showed a decline from 2013 to 2022 ($APC_{2013–2022}$=−1.9%, P<0.05), while the P≥3 group experienced an increase until 2020 ($APC_{2013–2020}$= 4.6%, P<0.05), followed by a decrease ($APC_{2020–2022}$= −4.5%, P>0.05). Similar trends were observed in the AMA group. For women younger than 35 years, the incidence of PTB rose across all parity groups from 2013 to 2022 (P1: $APC_{2013–2015}$=9.4%, $APC_{2015–2022}$=2.1%; P2: $APC_{2013–2022}$=3.0%; P3: $APC_{2013–2022}$=7.0%; all P<0.05). In the AMA group, the incidence of PTB in the P1 group fluctuated between 9.2% and 11.1% ($APC_{2013–2016}$=7.1% and $APC_{2016–2022}$=−1.0%, both P>0.05), while in the P2 group, it increased from 6.4% in 2013 to 8.2% in 2022 ($APC_{2013–2022}$=2.8%, P<0.05). The incidence of PTB in the P≥3 group fluctuated between 7.9% and 10.0% ($APC_{2013–2022}$=2.0%, P>0.05). The incidence of LBW increased in the P1, P2, and P≥3 groups among women younger than 35 years over the past decade (P1: $APC_{2013–2022}$=4.3%; P2: $APC_{2013–2022}$= 3.6%; P3: $APC_{2013–2022}$=7.0%; all P<0.05). In the AMA group, the incidence of LBW fluctuated across all parity groups (P1: $APC_{2013–2016}$=9.0%, P<0.05; $APC_{2016–2022}$=−0.1%, P>0.05; P2: $APC_{2013–2017}$= −0.4%, P>0.05; $APC_{2017–2022}$=4.2%, P<0.05; P≥3: $APC_{2013–2022}$=2.9%, P<0.05). The incidence of macrosomia in the P1 group decreased from 8.1% in 2013 to 4.4% in 2022 ($APC_{2013–2018}$=−5.0%, $APC_{2018–2022}$=−8.9%, both P<0.05). It also declined in the P2 group from 8.8% to 6.4% ($APC_{2013–2022}$=−3.9%, P<0.05). The P≥3 group initially saw an increase ($APC_{2013–2015}$=13.0%, P>0.05) followed by a decrease ($APC_{2015–2022}$=−6.6%, P<0.05). In the AMA group, the incidences of macrosomia decreased in all parity groups (P1: $APC_{2013–2022}$=−7.0%; P2: $APC_{2013–2022}$=−5.6%; P3: $APC_{2013–2022}$=−5.8%, all P<0.05).

TABLE 1. Comparison of basic characteristics of women of different parities in Beijing Municipality, 2013–2022.

Variables	Total	Primipara	Multipara		*P*
		Parity=1	Parity=2	Parity≥3	
Maternal age (M±SD)	30.4±4.3	29.3±4.0	32.7±4.2*	33.7±4.7*,†	<0.001
AMA (*n*, %)	342,670 (17.5)	128,790 (9.7)	200,473 (33.3)*	13,407 (43.6)*,†	<0.001
Maternal age<35 years	1,620,775	1,202,411	401,077	17,287	
Education level (*n*, %)					<0.001
Postgraduate	221,268 (14.9)	182,382 (16.8)	38,380 (9.8)*	506 (3.2)*,†	
Undergraduate	941,347 (63.2)	725,239 (66.9)	211,824 (54.5)*	4,284 (27.1)*,†	
Senior secondary school/Secondary vocational school	198,136 (13.3)	118,504 (10.9)	75,769 (19.5)*	3,863 (24.5)*,†	
Junior secondary school/Primary school	127,188 (8.5)	57,410 (5.3)	62,699 (16.1)*	7,079 (44.8)*,†	
Illiterate/semi-literate	525 (0.1)	233 (0.1)	231 (0.1)*	61 (0.4)*,†	
Domicile (*n*, %)					<0.001
Local	1,030,612 (64.1)	791,323 (66.4)	234,933 (58.8)*	4,356 (25.5)*,†	
Non-local	577,822 (35.9)	400,267 (33.6)	164,854 (41.2)*	12,701 (74.5)*,†	
Residence (*n*, %)					<0.001
Urban	647,874 (50.2)	498,890 (52.8)	143,489 (43.0)*	5,495 (43.5)*	
Suburban	538,899 (41.8)	377,111 (39.9)	155,598 (46.7)*	6,190 (49.1)*,†	
Rural	103,699 (8.0)	68,575 (7.3)	34,193 (10.3)*	931 (7.4)†	
Ethnicity (*n*, %)					<0.001
Han	1,523,386 (94.2)	1,128,320 (94.1)	378,691 (94.5)*	16,375 (95.7)*,†	
Others	93,643 (5.8)	71,052 (5.9)	21,848 (5.5)*	743 (4.3)*,†	
Occupation (*n*, %)					<0.001
Office worker/professional and technical personnel	824,732 (50.9)	646,506 (53.8)	175,242 (43.7)*	2,984 (17.3)*,†	
Commercial/service worker	239,170 (14.8)	165,452 (13.8)	70,598 (17.6)*	3,120 (18.1)*	
Agricultural workers	13,982 (0.9)	6,659 (0.5)	6,778 (1.7)*	545 (3.1)*,†	
Others	188,684 (11.6)	129,284 (10.7)	56,164 (14.0)*	3,236 (18.7)*,†	
Unemployed/freelance	183,248 (11.3)	107,163 (8.9)	70,723 (17.6)*	5,362 (31.0)*,†	
Student	6,567 (0.4)	5,949 (0.5)	602 (0.2)*	16 (0.1)*	
Unknown	164,392 (10.1)	141,398 (11.8)	20,970 (5.2)*	2,024 (11.7)†	
Maternal age ≥35 years (AMA)	342,670	128,790	200,473	13,407	
Education level (*n*, %)					<0.001
Postgraduate	65,383 (20.4)	25,035 (22.2)	39,205 (20.0)*	1,143 (9.2)*,†	
Undergraduate	185,565 (57.8)	72,268 (64.2)	108,699 (55.4)*	4,598 (36.9)*,†	
Senior secondary school/secondary vocational school	38,379 (11.9)	10,005 (8.9)	26,065 (13.3)*	2,309 (18.5)*,†	
Junior secondary school/primary school	31,487 (9.8)	5,245 (4.7)	21,974 (11.2)*	4,268 (34.2)*,†	
Illiterate/semi-literate	390 (0.1)	38 (0.0)	201 (0.1)*	151 (1.2)*,†	
Domicile (*n*, %)					<0.001
Local	240,603 (70.5)	92,794 (72.7)	142,277 (71.0)*	5,532 (41.6)*,†	
Non-local	100,534 (29.5)	34,816 (27.3)	57,962 (29.0)*	7,756 (58.4)*,†	
Residence (*n*, %)					<0.001
Urban	154,978 (60.3)	60,550 (68.2)	90,191 (56.5)*	4,237 (49.1)*,†	
Suburban	86,293 (33.5)	25,769 (29.0)	56,895 (35.6)*	3,629 (42.1)*,†	
Rural	15,897 (6.2)	2,492 (2.8)	12,645 (7.9)*	760 (8.8)*,†	

Continued

Variables	Total	Primipara	Multipara		*P*
		Parity=1	Parity=2	Parity≥3	
Ethnicity (*n*, %)					<0.001
Han	319,198 (93.6)	118,958 (93.1)	187,720 (93.8)*	12,520 (94.7)*,†	
Others	21,899 (6.4)	8,778 (6.9)	12,417 (6.2)*	704 (5.3)*,†	
Occupation (*n*, %)					<0.001
Office workers/professional and technical personnel	185,276 (54.1)	69,934 (54.3)	111,602 (55.7)*	3,740 (27.9)*,†	
Commercial/service workers	47,832 (14.0)	15,742 (12.2)	29,725 (14.8)*	2,365 (17.6)*,†	
Agricultural workers	5,344 (1.6)	560 (0.4)	4,301 (2.2)*	483 (3.6)*,†	
Others	41,957 (12.2)	14,600 (11.4)	24,901 (12.4)*	2,456 (18.3)*,†	
Unemployed/freelance	35,455 (10.3)	9,617 (7.5)	22,743 (11.3)*	3,095 (23.1)*,†	
Student	337 (0.1)	186 (0.1)	141 (0.1)*	10 (0.1)	
Unknown	26,469 (7.7)	18,151 (14.1)	7,060 (3.5)*	1,258 (9.4)*,†	

Abbreviation: AMA=advanced maternal age.
* Compared with Parity=1, *P*<0.05.
† Compared with Parity=2, *P*<0.05.

TABLE 2. Major pregnancy outcomes of different parities in Beijing Municipality, 2013–2022.

Variables	Total	Parity=1	Parity=2	Parity≥3	*P*
Maternal age <35 years					
Maternal	1,620,775	1,202,411	401,077	17,287	
Mode of delivery (*n*, %)					<0.001
Vaginal delivery	1,002,496 (61.9)	759,207 (63.1)	232,690 (58.0)*	10,599 (61.3)*,†	
Cesarean section	618,279 (38.1)	443,204 (36.9)	168,387 (42.0)*	6,688 (38.7)*,†	
Newbirth	1,644,231	1,221,934	404,748	17,549	
Preterm birth (*n*, %)	93,492 (5.7)	70,408 (5.8)	21,688 (5.4)*	1,396 (8.0)*,†	<0.001
Low birth weight (*n*, %)	71,031 (4.3)	57,558 (4.7)	12,721 (3.1)*	752 (4.3)*,†	<0.001
Macrosomia (*n*, %)	114,848 (7.0)	81,472 (6.7)	31,751 (7.8)*	1,625 (9.3)*,†	<0.001
Maternal age ≥35 years (AMA)					
Maternal	342,670	128,790	200,473	13,407	
Mode of delivery (*n*, %)					<0.001
Vaginal delivery	150,310 (43.9)	51,069 (39.7)	92,446 (46.1)*	6,795 (50.7)*,†	
Cesarean section	192,360 (56.1)	77,721 (60.3)	108,027 (53.9)*	6,612 (49.3)*,†	
Newbirth	350,485	134,145	202,728	13,612	
Preterm birth (*n*, %)	30,671 (8.8)	14,135 (10.5)	15,196 (7.5)*	1,340 (9.8)*,†	<0.001
Low birth weight (*n*, %)	21,373 (6.1)	11,485 (8.6)	9,164 (4.5)*	724 (5.3)*,†	<0.001
Macrosomia (*n*, %)	25,134 (7.2)	8,286 (6.2)	15,548 (7.7)*	1,300 (9.6)*,†	<0.001

Abbreviation: AMA=advanced maternal age.
* Compared with Parity=1, *P*<0.05.
† Compared with Parity=2, *P*<0.05.

DISCUSSION

This study analyzed delivery information from Beijing over a decade, revealing that the proportion of AMA mothers increased with parity. Concurrently, the proportion of women with undergraduate education levels decreased, while the proportion of women of non-local domicile increased. Among those with ≥3 parities, the proportions of non-local domicile, lower education levels, and unemployed/freelance status were relatively higher. Xu et al. found similar trends in a cross-sectional study in five provinces in China, noting

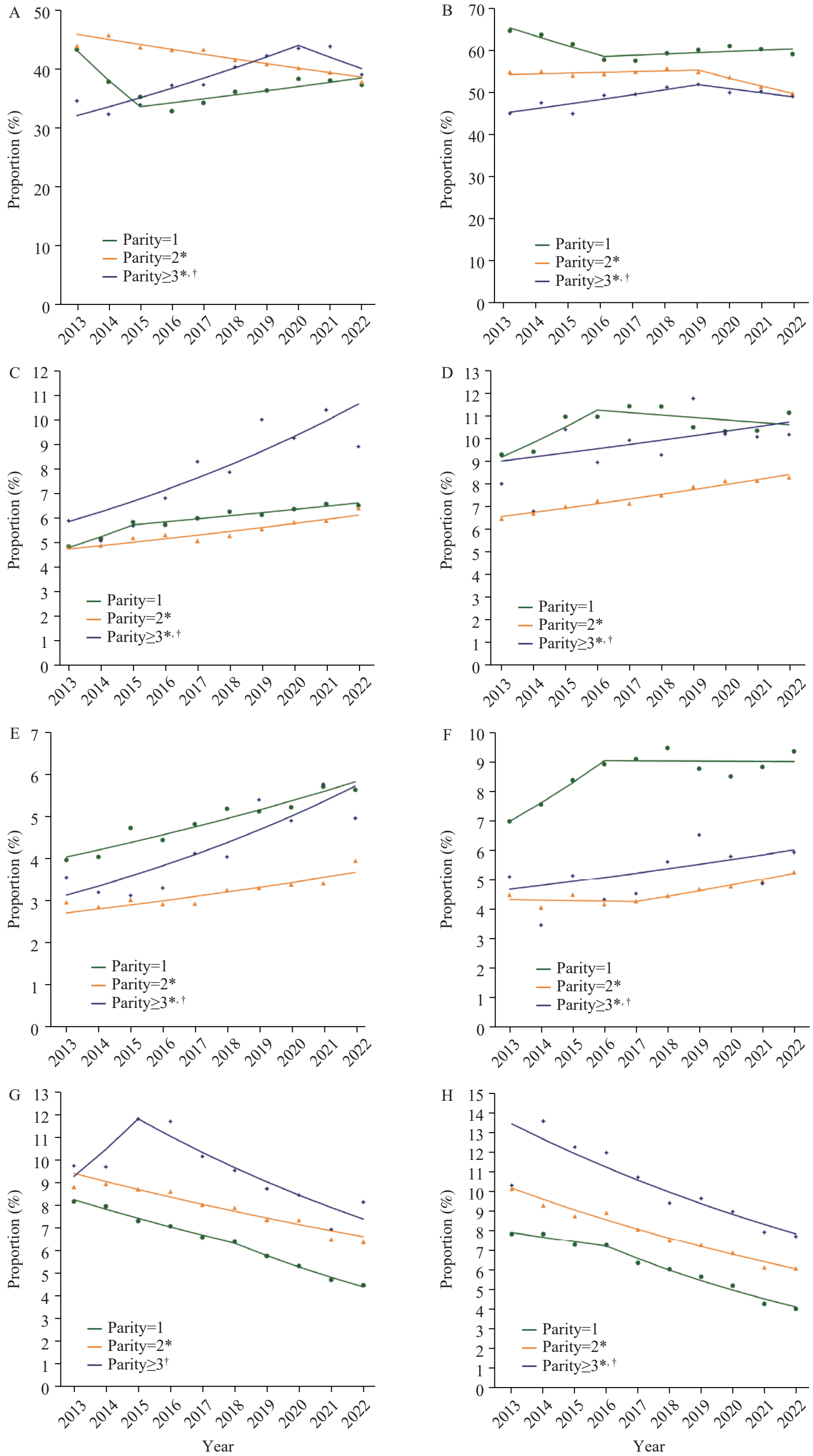

FIGURE 1. Trends of the proportion of C-section, PTB, LBW, and macrosomia among women of different parities in Beijing Municipality, 2013–2022. (A) The proportion of C-sections among maternal age <35 years; (B) The proportion of C-sections among maternal age ≥35 years; (C) The proportion of PTB among maternal age <35 years; (D) The proportion of PTB among maternal age ≥35 years; (E) The proportion of LBW among maternal age <35 years; (F) The proportion of LBW among maternal age ≥35 years; (G) The proportion of macrosomia among maternal age <35 years; (H) The proportion of macrosomia among maternal age ≥35 years.

Abbreviation: C-sections=cesarean section; PTB=preterm birth; LBW=low birth weight.

* Compared with Parity=1, *P*<0.05;

† Compared with Parity=2, *P*<0.05.

that women with low education and low income were more likely to have a second child (*3*). Studies in developed countries, such as in Europe, have also shown that highly educated women tend to have fewer children than those with lower education levels (*4*). Furthermore, women with high parities, believing themselves to be experienced in pregnancy and childbirth (*5*), tend to prioritize their own health less than primiparous women, potentially leading to pregnancy-related complications. This speculation is supported by neonatal outcomes, as the incidences of PTB and macrosomia were higher in the P≥3 group than in the P1 and P2 groups among younger women (maternal age <35 years). The mobility and economic conditions of non-local women further complicate access to perinatal care and elevate the risk of adverse pregnancy outcomes. An analysis of maternal mortality trends in Tianjin from 2011 to 2020 showed that, among maternal deaths, the proportion of women without stable occupations, those who were unemployed, and those with education levels below undergraduate were much higher than those with local domicile or higher education (*6*). These findings highlight challenges for maternal healthcare services in Beijing, emphasizing the crucial need for increased attention and targeted healthcare services for this demographic to improve birth outcomes.

Analysis of C-section births in Beijing over the past decade showed a decreasing trend followed by an increasing trend among primiparous women, regardless of maternal age. Unnecessary C-sections have adverse effects on both mothers and newborns; therefore, their use has been strictly controlled globally and nationally. According to data from 438 hospitals nationwide, the C-section rate among primiparous women decreased from 46.7% in 2013 to 37.9% in 2016 (*7*). In comparison, C-section use among primiparous women in Beijing has been lower than the national average. However, it rebounded after 2017, potentially due to delayed childbearing age. As maternal health declines and the incidence of gynecological diseases increases with age, the risk of C-section also increases. However, controlling C-section use remains a global challenge. C-section use in most countries and regions is currently rising, influenced by various factors such as family preferences, medical personnel skills, and healthcare payment models, making it difficult to control consistently with a single method (*8*).

Another concern is the rising trend of PTB incidence in Beijing, potentially attributable to rising maternal age or improved medical standards that allow preterm infants, especially early preterm infants, to survive (*9*). Notably, the incidence of macrosomia in Beijing has been effectively controlled, attributed to the promotion and continuous improvement of pregnancy nutrition clinics in Beijing (*10*). Pregnant women with high pre-pregnancy body mass index (BMI) or diagnosed with gestational diabetes mellitus (GDM) are recommended to receive professional dietary and exercise guidance in nutrition clinics, effectively controlling the incidence of macrosomia. However, the decline in macrosomia among multiparous women was not as rapid as among primiparous women, with the proportion in the P≥3 group consistently higher than the other two groups. Therefore, perinatal nutrition healthcare should be strengthened for women with high parities to reduce the incidence of macrosomia.

This study's strength is its exploration of characteristics in women with different parities using nearly all delivery information from Beijing between 2013 and 2022, providing a solid basis for developing targeted measures to control adverse pregnancy outcomes.

However, this study was subject to some limitations. First, it is solely based on data from Beijing and may not represent other provinces and cities. Second, the study lacks a comparison of high-risk factors. Future research should analyze and better understand the disease characteristics of women with different parities.

Conflicts of interest: No conflicts of interest.

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Methods and Applications

Development of a Multiplex Real-Time Quantitative PCR Assay for Detecting Vaginal Microbiota in Chinese Women — China, 2021–2022

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ABSTRACT

Introduction: The Nugent score, limited by subjectivity and personnel requirements, lacks accuracy. Establishing a precise and simple molecular test is therefore essential for detecting vaginal microbiota compositions and evaluating vaginal health.

Methods: We evaluated the vaginal health of Chinese women using quantitative polymerase chain reaction (qPCR) to target *Lactobacillus crispatus* (*L. crispatus)*, *L. iners*, *Gardnerella vaginalis* (*G. vaginalis)*, *Atopobium vaginae* (*A. vaginae)*, and *Megasphaera phylotype1*. bacterial vaginosis (BV)-related bacteria shared a fluorescent channel. Using 16S rDNA sequencing as a reference standard, we evaluated and validated the diagnostic accuracy of the qPCR assay.

Results: Both qPCR and 16S rDNA sequencing demonstrated 90.5% concordance in segregating vaginal community state type (CST), as visualized through heatmaps and PCoA. Spearman's correlation analysis revealed strong correlations between the two methods in calculating the RA of *L. crispatus* (CST I), *L. iners* (CST III), and BV-related bacteria (CST IV), with coefficients of 0.865, 0.837, and 0.827, respectively. Receiver operating characteristic analysis showed that qPCR had significant diagnostic accuracy for CST I, CST III, and CST IV (molecular BV), with area under the curve values of 0.967, 0.815, and 0.950, respectively, indicating strong predictive power.

Discussions: Vaginal health can be evaluated using a single qPCR amplification experiment, making the multiplex qPCR assay a highly accurate tool for this purpose.

The human vaginal microbiota is dominated by *Lactobacillus* species, with *L. crispatus* serving as a crucial indicator of reproductive health (*1*). Vaginal dysbiosis, characterized by a shift in microbiota composition from lactobacilli to pathogenic bacteria, commonly leads to bacterial vaginosis (BV) (*2*). BV is associated with an increased risk of negative reproductive health outcomes, including sexually transmitted infections and spontaneous preterm births (*3*–*4*). Traditional BV diagnosis relies on the Nugent score, which involves the examination of Gram-stained smears and a score greater than 7 indicating BV (*5*). However, this method requires experienced personnel and can be subjective, leading to reduced sensitivity and specificity. Recently, 16S ribosomal DNA (16S rDNA) sequencing has emerged as a tool for assessing vaginal health status by calculating community state types (CSTs). Notably, CST IV has been termed "molecular-BV" (*1*,*6*). However, the high cost and complexity of gene sequencing hinder its implementation in developing countries with large populations, such as China. Therefore, a simple and accurate diagnostic method for BV would be highly beneficial for both clinicians and patients.

Real-time quantitative polymerase chain reaction (qPCR) has revealed various dominant BV-related bacteria. *G. vaginalis* and *A. vaginae* were the most common species in BV with high abundance, and *Megasphaera* spp. was associated with BV independently (*7*). In this study, we developed a multiplex qPCR assay targeting the aforementioned BV-related bacteria and two *Lactobacillus* species (*L. crispatus* and *L. iners*), followed by 16S rDNA sequencing for validation. This assay allows vaginal health to be evaluated using a single qPCR amplification experiment, thereby facilitating early identification of patients at high risk of severe vaginal dysbiosis and prevention of future adverse sequelae.

METHODS

Study Participants and Specimen Collection

This study enrolled 84 participants at Beijing Ditan

Hospital, Capital Medical University, between October 2021 and November 2022. All participants were over 18 years old, with a median age of 38 (range 31.5–43), and had not used antimicrobials for two weeks prior to enrollment. Vaginal swabs were collected from the upper third of the vagina by an experienced gynecologist and used to determine Nugent scores via Gram staining. Additionally, swabs were immediately transported to the laboratory and stored at −80 °C for subsequent DNA extraction, qPCR, and 16S rDNA sequencing. This study received ethical approval from the Beijing Ditan Hospital Ethics Committee (No. 2021-22-01).

Multiplex qPCR Set-up

Total DNA was extracted using the MagaBio Bacterium DNA Fast Purification Kit (Hangzhou Bori Biological Technical, China) according to the manufacturer's instructions. Using a broad-coverage 16S rDNA gene sequence as an internal reference, PCR was performed for *G. vaginalis*, *A. vaginae*, *Megasphaera phylotype1*, *L. crispatus*, and *L. iners* according to a previously described methodology (*8*). Real-time qPCR was conducted on an ABI QuantStudio 1 Plus instrument (Thermo Fisher Scientific, USA) using multiplex Taq polymerase (Vazyme Biotech, China), with the three BV-associated bacteria sharing a fluorescent channel (Supplementary Table S1, available at https://weekly.chinacdc.cn/). The 25 µL qPCR mix contained 5 µL of DNA. Primer/probe concentrations for broad-coverage 16S rDNA were set at 1.5 µmol/L (forward), 1.0 µmol/L (reverse), and 0.5 µmol/L, and at 0.5 µmol/L and 0.25 µmol/L for other species. Primer and probe specificities were confirmed using PRIMER BLAST. The cycling conditions included a 2 min incubation at 37 °C and initial denaturation for 30 s at 95 °C, followed by 45 cycles of denaturation for 10 s at 95 °C and annealing/extension for 30 s at 60 °C. Samples were run in duplicate, with distilled sterile water as a negative control. Additionally, single-plex PCR was performed for the three BV-related bacteria and compared with the multiplex qPCR.

The cycle threshold (Ct) values obtained for each fluorescent channel (*L. crispatus*, *L. iners*, and BV-related bacteria) and the 16S rDNA gene sequences (internal reference) were designated as Ct1 and Ct2, respectively. The relative abundance (RA) of each species was calculated using $2^{-\Delta(Ct1-Ct2)}$ (*9*). Species with an RA exceeding 20% were considered dominant and used to determine the CST of the vaginal microbiota (*10*).

16S rDNA Sequencing

The V3–V4 regions of the 16S rDNA gene were amplified using primers 341F-805R and Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs). Two rounds of PCR were performed before sequencing on the Illumina HiSeq^{TM} platform. QIIME (version 2022.11.1, NAU, Flagstaff, USA) was used to analyze the data and identify operational taxonomic units and species. CSTs were determined based on the RA and distribution of dominant bacteria. CST I is dominated by *L. crispatus* and is usually linked to a healthy vaginal state. CST III is dominated by *L. iners* and can be found in both healthy and pathological states. CST IV is highly diverse with low levels of *lactobacilli* and is typically associated with BV.

Statistical Analysis

Chi-square and Fisher's exact tests were used to compare categorical variables (N/%) across groups. CST segregation by qPCR and 16S ribosomal DNA (16S rDNA) was assessed using principal coordinate analysis (PCoA) with Bray-Curtis distance. Spearman's correlation test was used to analyze the relationship between qPCR and 16S rDNA data. The diagnostic performance of qPCR for vaginal microbiota (CST I, III, and IV), including sensitivity, specificity, area under the curve (AUC), and positive and negative predictive values, was evaluated using receiver operating characteristic (ROC) curves. Statistical analyses were performed using SPSS (version 26.0, IBM, Armonk, NY, USA), GraphPad (version 9.0, GraphPad Software, San Diego, California), MedCalc (version 20.0, MedCalc Software, Mariakerke, Belgium), Easy application (*11*), and ImageGP (*12*). The two-tailed significance level was set at 0.05.

RESULTS

Basic Information of Patients and 16S rDNA-seq

16S rDNA sequencing revealed three CSTs at the species level. Of the 55 participants exhibiting *Lactobacillus* dominance, 28.6% (*n*=24) and 32.1% (*n*=27) exhibited *L. crispatus* (CST I) and *L. iners* (CST III) dominance, respectively. Four women had other *Lactobacillus* species, including two with *L. jensenii* and one each with *L. gasseri* and *L. delbrueckii*. CST IV, characterized by a lack of dominant species and a mix of *G. vaginalis*, *A. vaginae*, and other anaerobes, was identified in 34.5% (*n*=29) of the participants (Table 1).

Establishment of A Multiplex qPCR Set

The effectiveness of multiplex qPCR was assessed using the relative abundance (RA) of 16S rDNA amplicon sequencing as the standard. Figure 1A compares the amplification efficiency between single-plex and multiplex qPCR. Each row represents the amplification curve of each sample for *G. vaginalis*, *A. vaginae*, and Megasphaera phylotype 1 obtained using single-plex and multiplex qPCR. Both single-plex and

TABLE 1. Comparison of vaginal flora typing results based on qPCR and 16S rDNA V3V4 sequence analysis [*n* (%)].

Dominant species	CST	qPCR	16S rDNA	χ^2	*P*	Common cases
Lactobacillus crispatus	CST I	24 (28.6)	24 (28.6)			24 (32.9)
Lactobacillus iners	CST III	31 (36.9)	27 (32.1)			27 (32.9)
BV-related bacteria	CST IV	21 (25.0)	29 (34.5)	2.889	0.409	21 (28.8)
Other species	Other types	8 (9.5)	4 (4.8)			4 (5.5)
Total cases		84	84			76

Abbreviation: CST=community state type; qPCR=quantitative polymerase chain reaction.

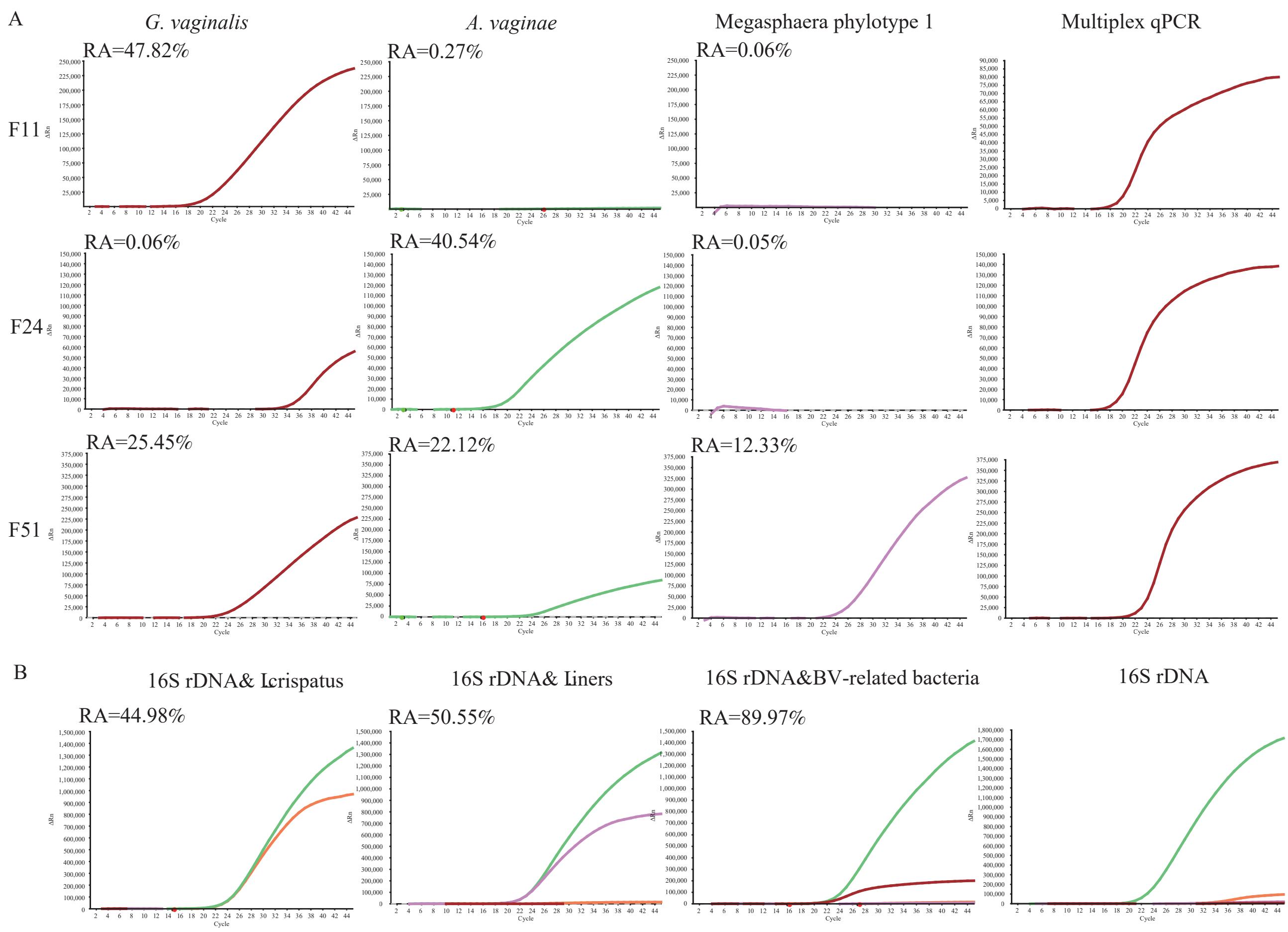

FIGURE 1. Comparison of amplification curves from multiplex qPCR and single-plex qPCR using 16S rDNA sequencing as the standard. (A) Three samples (F11, F24, F51) with absolutely higher RA of single BV-related species (*G. vaginalis*, *A. vaginae* and *Megasphaera phylotype1*) were selected to perform by single-plex qPCR with the same primer and probe of the multiplex qPCR. (B) Using RA calculated by 16S rDNA-seq, three samples with higher RA of single species and less abundance of the remaining species and one sample without these species were detected by the multiplex qPCR.

Note: For (A), the amplification curves were compared with those of the same sample detected by the multiplex qPCR.

Abbreviation: CST=community state type; RA=relative abundance; qPCR=quantitative polymerase chain reaction.

multiplex qPCR showed sigmoidal curves, reaching a plateau, indicating consistency with 16S rDNA sequencing data and validating the accuracy of multiplex qPCR in detecting these targets.

Figure 1B highlights the specificity of multiplex qPCR using the RA of 16S rDNA sequencing as the standard. In the first sample, *L. crispatus* (44.98% RA, CST I) was accurately amplified using multiplex qPCR (Ct=20.41), while the other four species were not detected. In the second sample, qPCR amplification (Ct=20.35) revealed *L. iners* dominance (50.55% RA, CST III), and other species were not detected. The third sample demonstrated *G. vaginalis* dominance (89.97% RA, CST IV) with amplification of both BV-related bacteria and universal primers (Ct=19.49), while *L. crispatus* and *L. iners* were not detected. In the fourth sample, characterized by a low RA for all targets, only the universal primer was amplified (Ct=18.84), aligning with the sequencing data.

Evaluation and Validation of Multiplex qPCR Assay's Accuracy

Table 1 presents a comparison of CSTs identified using qPCR and 16S rDNA, showing no significant difference between the methods (*P*=0.409). Among the 84 participants, 76 shared CSTs were identified, yielding a concordance rate of 90.5%. Heatmaps depicted the consistency of CST I and CST III across both methods and visually highlighted the 8 mismatches (Figure 2A–2B). Among the 8 samples with mismatched CSTs, F01, F07, and F11 exhibited opposite conclusions regarding CST classification by dominant microbiota, with CST III identified by multiplex qPCR and CST IV by 16S rDNA sequencing. In samples F36, F31, F02, F03, and F33, the dominant microbiota were *Shuttleworthia*, *Peptostreptococcus*, *Bifidobacterium breve*, *Streptococcus gallolyticus*, and *Bifidobacterium animalis*, respectively (Supplementary Table S2, available at https://weekly.chinacdc.cn/). These bacterial species were not within the detection range of the multiplex qPCR assay and were therefore not detected. PCoA demonstrated a clear segregation of the three CSTs using both qPCR and 16S rDNA (adonis *P*<0.001) (Figure 2C–2D). Additionally, a strong correlation was observed in the RA of *L. crispatus*, *L. iners*, and BV-related bacteria between the two methods, with Spearman's coefficients of 0.865, 0.837, and 0.827, respectively (Figure 2E–2G).

Table 2 presents the diagnostic performance of multiplex qPCR for *L. crispatus* (CST I), *L. iners* (CST III), and BV-related bacteria (CST IV), with optimal Ct cutoffs of 21.4, 22.9, and 22.8, respectively. ROC analysis demonstrated AUCs of 0.967, 0.815, and 0.950 for CST I, CST III, and CST IV, respectively (all *P*<0.001), indicating strong predictive capabilities (Supplementary Figure S1, available at https://weekly.chinacdc.cn/).

DISCUSSION

This study developed and validated a multiplex qPCR assay to evaluate vaginal health using a single amplification experiment. This assay addresses critical gaps in existing diagnostic approaches while enhancing accuracy and offering a more cost-effective solution for evaluating vaginal health.

The Nugent score has traditionally been used to assess the degree of clinical BV (*5*,*13*). However, its limitation to genus-level bacterial identification makes precise BV assessment challenging, hindering clinical diagnosis and treatment. In contrast, multiplex qPCR using 16S rDNA as a reference demonstrated 90.5% consistency, surpassing the Nugent score in accuracy. Excluding five cases (6.0%) beyond the detection scope, three discrepancies were observed for CST III and IV. This discrepancy may be attributed to the genomic variability of *L. iners*, which allows it to exist as either a symbiont or parasite in healthy, imbalanced, or diseased vaginal environments (*14*).

Previous studies have shown that single-plex qPCR to detect *G. vaginalis* or *A. vaginae* for predicting BV yielded limited diagnostic accuracy, with a sensitivity and specificity of 83.6% and 84.1%, respectively, for *G. vaginalis* and 87.1% and 90.7%, respectively, for *A. vaginae* (*15*). Conversely, a combined approach incorporating *G. vaginalis*, *A. vaginae*, and *Megasphaera phylotype1* significantly improved diagnostic performance, achieving a sensitivity of 92% and a specificity of 95% in diagnosing BV (*15*). Consistent with these results, we found that multiplex qPCR had strong diagnostic performance in predicting molecular BV, with a sensitivity of 89.7% and specificity of 94.5%. Notably, an innovative aspect of this multiplex qPCR method is its ability to detect three BV-related bacteria using a common fluorescent channel. Additionally, it incorporates two primary *Lactobacillus* species: *L. crispatus*, which is used to assess vaginal health, and *L. iners*, which is employed to evaluate the intermediate state of BV. Multiplex qPCR focuses on analyzing the composition of the vaginal microbiota as a whole rather than targeting individual BV marker organisms, thereby reducing both the time and costs associated with diagnosis.

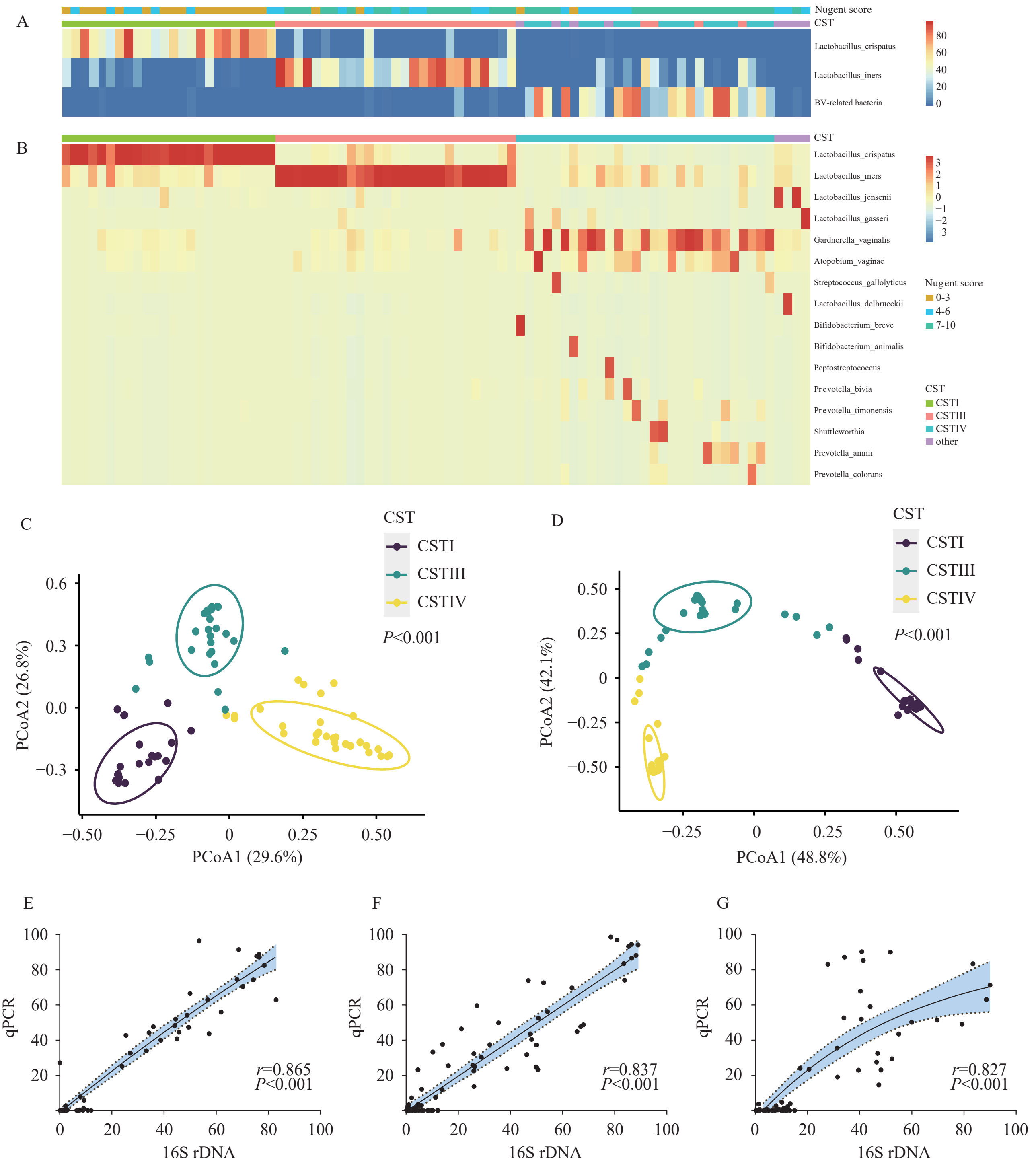

FIGURE 2. Comparison of CST categorized by multiplex qPCR and 16S rDNA sequencing. (A) Heatmap categorizing CSTs by dominant species using multiplex qPCR. (B) Heatmap categorizing CSTs by dominant species based on 16S rDNA sequencing. (C) PCoA based on the Bray-Curtis dissimilarity index from 16S rDNA sequencing. (D) PCoA based on the Bray-Curtis dissimilarity index from multiplex qPCR. (E) Correlation between multiplex qPCR and 16S rDNA sequencing in the RA of *Lactobacillus crispatus*. (F) Correlation between multiplex qPCR and 16S rDNA sequencing in the RA of *Lactobacillus iners*. (G) Correlation between multiplex qPCR and 16S rDNA sequencing in the RA of BV-related bacteria.

Note: *Lactobacillus crispatus* (CST I), *Lactobacillus iners* (CST III), and BV-related bacteria (CST IV).

Abbreviation: CST=community state type; RA=relative abundance; qPCR=quantitative polymerase chain reaction.

TABLE 2. Sensitivity, specificity and predictive values of bacterial signatures distinguishing the vaginal health condition and molecular BV by the multiplex qPCR.

AUC (95% *CI*)	Sensitivity (%) (95% *CI*)	Specificity (%) (95% *CI*)	PPV (%) (95% CI)	NPV (%) (95% *CI*)
Lactobacillus crispatus predicts CST I (21.4)*				
0.967 (0.904, 0.994)	100.0 (85.8, 100.0)	90.0 (79.5, 96.2)	80.0 (65.2, 89.5)	100.0
Lactobacillus iners predicts CST III (22.9)*				
0.815 (0.716, 0.892)	96.3 (81.0, 99.9)	59.7 (45.8, 72.4)	53.1 (45.0, 61.0)	97.1 (83.1, 99.6)
BV-related microbiota predicts CST IV (22.8)*				
0.950 (0.880, 0.986)	89.7 (72.6, 97.8)	94.6 (84.9, 98.9)	89.7 (74.1, 96.3)	94.5 (85.6, 98.1)

Abbreviation: AUC=area under the cure; BV=bacterial vaginosis; CST=community state type; *CI*=confidence interval; NPV=negative predictive value; PPV=positive predictive value; qPCR=quantitative polymerase chain reaction.
* Cutoff value.

This study provides valuable insights for accurately assessing vaginal health, but it has some limitations. First, this study is a single-center study and lacks multicenter validation, validation across multiple centers is necessary. Additionally, the small sample size of this cross-sectional study may have introduced bias. Therefore, future research should include larger longitudinal cohort studies to better understand causality.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. The multiplex qPCR target and primer/probe sequences.

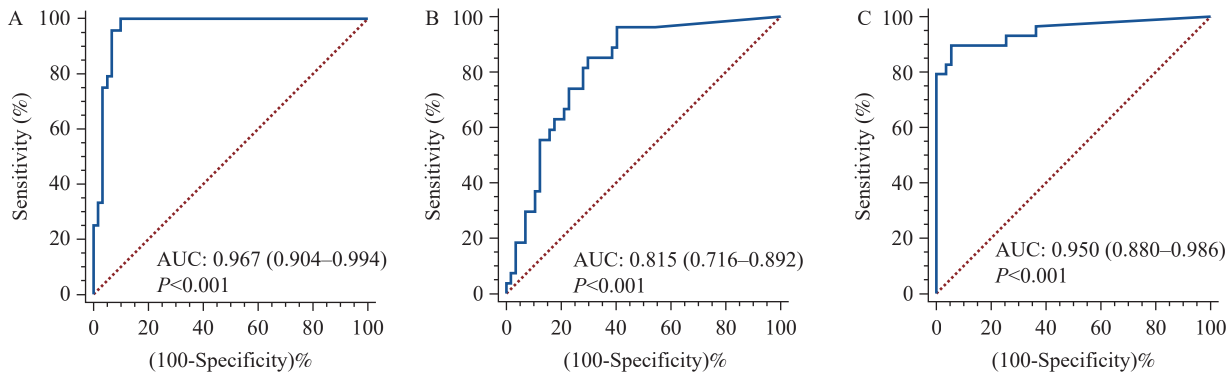
PCR target	Primer name	5′–3′ sequence*
16S rDNA	Forward	CGGTGAATACGTTCYCGG
	Probe	[VIC]CTTGTACACACCGCCCG
	Reverse	GGWTACCTTGTTACGACTT
Lactobacillus_iners	Forward	AGTCTGCCTTGAAGATCGG
	Probe	[FAM]CCAAGAGATCGGGATAACACCT
	Reverse	CTTTTAAACAGTTGATAGGCATCATC
Lactobacillus_crispatus	Forward	AACTAACAGATTTACTTCGGTAATGA
	Probe	[ROX]CCCATAGTCTGGGATACCACTT
	Reverse	AGCTGATCATGCGATCTGC
Atopobium vaginae	Forward	TAGGTCAGGAGTTAAATCTG
	Probe	[CY5]CTACCAGACTCAAGCCTGCC
	Reverse	TCATGGCCCAGAAGACCGCC
Gardnerella vaginalis	Forward	GCGGGCTAGAGTGCA
	Probe	[CY5]CTTCTCAGCGTCAGTAACAGC
	Reverse	ACCCGTGGAATGGGCC
Megasphaera phylotype1	Forward	GATGCCAACAGTATCCGTCCG
	Probe	[CY5]ACAGACTTACCGAACCGCCT
	Reverse	CCTCTCCGACACTCAAGTTCGA

Abbreviation: qPCR=quantitative polymerase chain reaction.
* Primers and probes were obtained from Beijing Tianyi Huiyuan Bioscience & Technology, Inc. (Beijing, China). Cyanine 5 (Cy5), Fluorescein (FAM), X-Rhodamin (ROX), and Victoria (VIC) were used as the 5′-coupled reporter fluorophores of the hydrolysis probes used in the multiplex PCR reaction, and the 3′-coupled with Tetramethylrhodamine (TAMRA) or Black Hole Quenchers (BHQ2) as quenchers.

SUPPLEMENTARY TABLE S2. The eight mismatch cases detected by qPCR and 16S rDNA V3V4 sequence analysis.

Sample ID	qPCR		16S rDNA	
	Dominant species	RA (%)	Dominant species	RA (%)
F01	*L. iners*	59.62	*G. vaginalis*	46.85
F07	*L. iners*	37.61	*G. vaginalis*	39.72
F11	*L. iners*	46.34	*G. vaginalis*	47.82
F36	*L. iners*	33.23	*Shuttleworthia*	27.16
F31	–	–	*Peptostreptococcus*	36.10
F02	–	–	*Bifidobacterium breve*	22.07
F03	–	–	*Streptococcus gallolyticus*	27.74
F33	–	–	*Bifidobacterium animalis*	24.75

Note: "–" means without the detection range of the multiplex qPCR assay.
Abbreviation: RA=relative abundance; qPCR=quantitative polymerase chain reaction.



SUPPLEMENTARY FIGURE S1. Using 16S rDNA sequencing as the gold standard, this figure assesses the predictive capability of multiplex qPCR for vaginal health in predicting CST I, CST III, and CST IV, respectively. (A) ROC curves for *Lactobacillus crispatus*; (B) ROC curves for *Lactobacillus iners*; (C) ROC curves for BV-related bacteria.

Abbreviation: CST=community state type; AUC=area under the curve; ROC=receiver operating characteristic; qPCR=quantitative polymerase chain reaction.

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