Vaginal microbiota as a predictor of preterm birth: an observational cohort study in a high-risk population

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Short title: Vaginal microbiota signatures of preterm birth

The authors report no conflict of interest.

At a glance

Why was this study conducted?

Despite the growing evidence of an association between vaginal microbiota composition and preterm birth, data remain elusive, because most studies were relatively small to examine cofounding factors. Moreover, longitudinal studies focusing on high-risk populations are lacking to transpose microbiological data into a clinical perspective.

Key findings

In a prospective cohort of over 1,800 pregnant women, using microbiological cultures of vaginal samples at admission and multivariable analyses adjusting for demographic and obstetrical factors, we identified lactobacilli depletion and enterobacteria as strong vaginal microbiota signatures of preterm labor and preterm rupture of membranes as compared to controls. Importantly, lactobacilli depletion constituted an independent risk factor for spontaneous preterm birth.

What does this add to what is known?

We confirm the implication of vaginal microbiota in preterm birth and highlight the importance of lactobacilli in this outcome. Accurate characterization of vaginal microbiota at admission for preterm labor as well as preterm premature rupture of membranes could improve the management of these complications of pregnancy.

Abstract

Background: Increasing evidence link vaginal microbiota composition and preterm birth. However, the impact of the vaginal microbiota on preterm labor and premature rupture of membranes and on their outcome remain elusive, mainly because most studies are relatively small-sized.

Objectives: To identify microbiota signatures of preterm labor, premature rupture of membranes, and spontaneous preterm birth.

Study Design: We conducted an observational, prospective, longitudinal cohort study from August 2018 until June 2023 in 3 maternity wards from university hospitals in the Paris, France, area. Women with singleton pregnancies were categorized in 3 groups including a control group, and 2 groups of increasing risk of preterm birth: preterm labor and preterm premature rupture of membranes. Demographic, clinical data, past medical and obstetrical history and pregnancy outcome were collected. Vaginal swabs were collected at admission and were analyzed using microbiological cultures. The association between bacterial species, the cohort groups, and spontaneous preterm birth was studied in univariate analyses. Adjusted odds ratio (aOR) and 95% confidence intervals (95% CI) were calculated in multivariable analyses.

Results: A total of 1,848 women were included, of whom 1,048 (56.7%) in the control group, 417 (22.6%) with preterm labor, and 383 (20.7%) with premature rupture of membranes. Together with demographic features such as ethnicity and obstetrical history, several vaginal microbiota signatures were identified in high-risk groups. In multivariable analysis, preterm labor was correlated with lactobacilli depletion (aOR 1.49, 95% CI 1.07-2.07), enterobacteria (aOR 1.91, 95% CI 1.40-2.62) and *Gardnerella vaginalis* (aOR 4.50, 95% CI 1.80-13.02), and premature rupture of membranes with lactobacilli depletion (aOR 2.10, 95% CI 1.52-

2.89) and enterobacteria (aOR 2.39, 95% CI 1.75-3.26). Finally, lactobacilli depletion was a risk factor for spontaneous preterm birth (aOR 1.68, 95% CI 1.07-2.68).

Conclusions: In this large cohort, *G. vaginalis* was associated with preterm labor, while lactobacilli depletion and enterobacteria were risk factors for preterm labor and premature rupture of membranes. In addition, lactobacilli depletion was correlated with spontaneous preterm birth. Further studies are needed to address the benefits of adapted antibiotics and probiotics to restore a healthy microbiota for preventing preterm birth.

Abstract word count: 342

Manuscript word count: 2970

Key words : Vaginal microbiota, dysbiosis, pregnancy outcomes, preterm birth, premature preterm rupture of membrane.

Introduction

Preterm birth (PTB), defined as birth before 37 weeks of gestation, is a frequent and severe adverse pregnancy outcome which concerns approx. 7% pregnancies in industrialized countries and accounts for 14 millions annual births worldwide.[1] As the leading causes of death in children under the age of 5 years, PTB and its sequelae represent one of the most prevalent conditions in the Global Burden of Disease analysis and are therefore considered as a major worldwide health issue.[2,3] Up to 40% of spontaneous PTB (sPTB) are preceded by preterm premature rupture of membranes (PPROM), increasing the risk of intrauterine infection and *in utero* demise,[4,5] and disruption of the vaginal microbiota is increasingly considered to be a contributing factor.[6,7]

The composition of the vaginal microbiota is highly dynamic, changing with age, hormonal fluctuations, gestational status, ethnicity, sexual behavior, and lifestyle.[8–10] Microbiota studies based on sequencing of vaginal samples from reproductive-age women have identified five types of vaginal community structures designated community state types (CSTs).[10] Four of these are dominated by one *Lactobacillus* species while the last one, designated CST-IV, is characterized by a more diverse microbiota dominated by anaerobic bacteria such as *Gardnerella vaginalis* and *Prevotella* spp. Although the distribution of these CSTs largely varies according to ethnicity,[11] it is generally believed that a healthy vaginal microbiota is dominated by lactobacilli with a low bacterial diversity.[12,13] Conversely, a decrease in lactobacilli abundance is correlated to an increase of microbiota diversity frequently referred to as vaginal dysbiosis and typically exemplified by the CST-IV. In the context of pregnancy, increasing evidence links preterm labor (PTL), PPROM, and PTB with low-lactobacilli microbiota characterized by a high diversity and an enrichment in *G. vaginalis* and various anaerobes, whereas microbiota dominated by lactobacilli and more

specifically by *Lactobacillus crispatus* (CST-I) have been found associated with term birth.[6,14–16]

Despite growing evidence of an association between vaginal microbiota and pregnancy outcomes, incorporating these observations into routine patient management remains a challenge. Studies that have attempted to address using conventional microbiological methods whether pregnancy outcomes could be correlated with specific microbial signatures other than vaginal dysbiosis are lacking.[17] In addition, the impact of the vaginal microbiota on PTL and PPROM and on their outcome remain elusive, mainly because most studies are relatively small-sized.[6]

Here, our goal was to use conventional microbial techniques to identify microbiota signatures of PTL and PPROM along with microbiological risk factors for sPTB in high-risk women hospitalized for PTL and PPROM.

Materials and methods

Study design

We conducted an observational, prospective, longitudinal cohort study from August 2018 until June 2023 in 3 maternity wards, of which 2 of level 3 and 1 of level 2b, from university hospitals in the Paris, France, area. The total annual number of births at these 3 centers was approximately 11,000. Women were categorized in 3 groups including a control group and 2 groups at high risk of PTB, referred to as the PTL and PPROM groups. The eligibility criteria for maternal participation in the study included all pregnant women aged >18 years who responded to the following inclusion criteria: *i)* PTL: hospitalization for threatened PTB with intact membranes; and *ii)* PPROM: rupture of membranes before 37 weeks of gestation and before the onset of labor contractions. The control group consisted of pregnant women who delivered at term with no history of PTL and no premature rupture of

membranes. These women were enrolled at the time of *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) antenatal screening between 34 and 38 weeks of gestation, as recommended by the French guidelines for the prevention of perinatal infectious disease.[18] Women who did not speak or understand French and multiple pregnancies were excluded.

Demographic, clinical data, past medical and obstetrical history, and pregnancy outcome were collected. Maternal place of birth was collected as a reflect of ethnicity, given that reporting ethnicity itself is forbidden in France.

Sampling and laboratory procedures

Vaginal swabs were sampled at admission at the hospital or at GBS antenatal screening and sent to the laboratory in Amies transport medium (ESwab™ system, Copan Diagnostics, Italy). Specimens were immediately processed for analysis by conventional culture methods using non-selective rich media incubated at 37°C in aerobic atmosphere with 5% CO_2. In addition, a Columbia agar with 5% horse blood, nalidixic acid and colistin (bioMérieux, Marcy l'Etoile, France) for the selective isolation of anaerobes and Gram-positive cocci and a Granada agar (Fisher Scientific, Illkirch, France) for the selective isolation of GBS were incubated at 37°C under anaerobic atmosphere. All plates were examined after 16h and 44h of incubation.

All different morphotypes of bacterial and yeast colonies were identified using matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Billerica, Massachussetts, USA). The density of vaginal colonization was estimated semi-quantitatively as follows: colonies on a single quadrant, low; colonies on more than two quadrants, high.

In addition, the overall vaginal microbiota composition was assessed by direct microscopic examination of the vaginal samples after Gram staining and determination of the Nugent score in 2 out of 3 maternity wards according to routine practices.[19]

Primary outcomes and studied factors

The primary outcomes were the microbiological signatures of PTL and PPROM and the microbiological risk factors for sPTB defined as spontaneous onset of labor before 37 weeks of gestation. The studied factors were the microbial species isolated in the vaginal samples at admission or antenatal GBS screening. A specific attention was paid to bacteria of the *Lactobacillus* genus, and to species potentially associated with adverse outcomes including enterobacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and others), *Staphylococcus aureus*, GBS, *Streptococcus anginosus*, *G. vaginalis*, strict anaerobes (*Prevotella* spp., *Bacteroides* spp., and others), and fungi. Unless otherwise specified, only the presence or absence of the bacterial species, not their quantity, was considered in the statistical analyses.

Microbiological data were adjusted to known sPTB risk factors and to confounding variables such as demographics (country of birth), body mass index (BMI), gravidity, parity and pregnancy-related risk factors (history of preterm delivery, gestational diabetes, hypertensive disease, and tobacco use).

Statistical analyses

All data were collected in a computerized database and analyzed using the GraphPad Software v8.3.0 (Dotmatics, Boston, Massachusetts, USA) and RStudio v1.1.447 (Posit, Boston, Massachusetts, USA). Firstly, we assessed the prevalence of the studied factors

according to the cohort groups as a proportion of the total number of participants in each group. Mann-Whitney tests were used when appropriate and categorical data were compared using chi-squared or Fisher's exact tests. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated in univariate analysis. Secondly, to study the microbiological signatures of PTL and PPROM and the microbiological risk factors for sPTB, multivariable logistic regression models were used for prognostic covariates. Microbiological signatures of PTL and PPROM were determined using the control group as a reference. Microbiological risk factors for sPTB were determined using women in the PTL and PPROM groups who delivered at term as the reference. Adjusted OR (aOR) were calculated using known sPTB risk factors and confounding variables in multivariable analyses. Two models were used to examine enterobacteria, with either enterobacteria as a single variable or the two most common species e.g., *E. coli* and *K. pneumoniae*, as independent variables. A P value < 0.05 was considered significant. Missing data were excluded.

Ethics declaration

The reported study was approved by the French ethics committee on November, 2017 (identifier 2017 – A02755-48; NCT03371056). All individuals enrolled in the cohort provided signed informed consent that included authorization for the collection of medical information and biological samples. All collected data were anonymized and integrated in a secured electronic database.

Results

Study population

A total of 1,848 women were included, of whom 1,048 (56.7%) in the control group, 417 (22.6%) in the PTL group, and 383 (20.7%) in the PPROM group. The characteristics of

the study population are summarized in Table 1. Compared to controls, PTL and PPROM were characterized by a higher proportion of women born in Sub Saharan Africa, history of preterm deliveries, and obesity. Eventually, 283 (57.6%) and 58 (13.4%) women with PTL and PPROM delivered at 37 weeks of gestation or more, respectively.

Vaginal microbiota signatures of PTL and PPROM

A Nugent score was determined for 1,435 (77.7%) vaginal samples and indicated that bacterial vaginosis was uncommon, below 5% whatever the cohort group considered (Table 2). Nevertheless, the distribution of the Nugent scores varied significantly between PTL, PPROM and controls, Nugent scores ≥ 7 being more frequent in PTL (4.8% vs. 1.9%, *P*=0.008) and intermediate scores being overrepresented in PPROM (18.7% *vs*. 12.8% in controls, *P*=0.01). Accordingly, microbial cultures of the vaginal samples revealed significant differences between the cohort groups, most importantly in the presence and abundance of lactobacilli, enterobacteria, and anaerobes, including *G. vaginalis* (Table 2). Conversely, the presence and abundance of *S. aureus*, GBS and *Candida* spp. did not vary according to the cohort groups.

Compared to controls, microbial signatures of PTL included a higher proportion of samples negative for lactobacilli and positive for enterobacteria and anaerobes (Figure 1). Enterobacteria were more frequent and also more abundant (Table 2), as assessed by the number of samples with numerous bacteria compared to the total number of positive sample (68.5% in PTL *vs*. 45.8% in controls, *P*=0.0001).

Microbial signatures of PPROM included a higher proportion of samples negative for lactobacilli and positive for enterobacteria but not for anaerobes (Figure 1). Significant differences were also observed considering their abundance (Table 2). Lactobacilli were more

frequently in low quantities (14.5% in PPROM *vs*. 3.8% in controls, $P<0.0001$) whereas enterobacteria were more abundant (62.8% in PPROM *vs*. 45.8% in controls, $P=0.004$).

Microbial risk factors for PTL and PPROM

Demographical and clinical characteristics such as ethnicity and BMI were associated with pregnancy outcomes. Since they are also presumed to impact microbiota composition, we tested their association with vaginal microbiota composition in our study population and confirmed their correlation with the Nugent score and with the presence and abundance of lactobacilli and enterobacteria (Supplementary Tables 1 and 2). The place of birth but not the BMI also correlated with anaerobes and *G. vaginalis* (Supplementary Table 1).

Next, to identify the microbiological factors associated with PTL and PPROM, we performed univariate (Table 3) and then multivariable logistic regression analyses adjusting for ethnicity and BMI into account, showing that PTL was strongly associated with maternal origin from French overseas territories and Sub Saharan Africa, with a history of preterm delivery, and also with the absence of lactobacilli (aOR: 1.49, 95% CI 1.07-2.07), the presence of enterobacteria (aOR: 1.91, 95% CI 1.40-2.62) and of *G. vaginalis* (aOR: 4.50, 95% CI 1.80-13.02) (Figure 2). For PPROM, the strongest risk factors were again maternal origin from French overseas territories and Sub Saharan Africa and also vaginal microbiota depleted in lactobacilli (aOR 2.10, 95% CI 1.52-2.89) and including enterobacteria (aOR 2.39, 95% CI 1.77-3.26) (Figure 2).

Overall, the absence of lactobacilli and the presence of enterobacteria in vaginal swab cultures were both correlated with PTL and PPROM, whereas *G. vaginalis* was only a signature of PTL. Similar results were obtained with the second model of multivariable logistic regression including *E. coli* and *K. pneumoniae* instead of enterobacteria (Supplementary Figure 1), showing that vaginal colonization by *E. coli* was strongly

associated with PTL (aOR 2.08, 95% CI 1.46-2.96) and PPROM (aOR 2.35, 95% CI 1.63-3.33).

Vaginal microbiota risk factors for spontaneous preterm birth

Finally, we sought to determine whether the vaginal microbiota at admission for PTL or PPROM could be predictive of sPTB, which affected 41.0% (119/290) and 95.4% (209/219) pregnancies in each group, respectively. In univariate analysis, we found that in addition to expected clinical factors such as gestational diabetes, the absence of lactobacilli and the presence of enterobacteria were associated with a higher risk of spontaneous preterm delivery (Table 4). In multivariable analysis, the absence of lactobacilli was the only microbiological risk factor for sPTB with an aOR of 1.68, 95% CI 1.07-2.68 (Figure 3).

Comment

Principal Findings

In this prospective cohort study of over 1,800 pregnant women, we identified using simple microbiological cultures of vaginal samples distinct microbiological signatures of PTL and PPROM. PTL was strongly associated with the presence of *G. vaginalis* and less significantly with the absence of lactobacilli, whereas PPROM was correlated with a depletion in lactobacilli and with the presence of enterobacteria. Importantly, the composition of microbiota on admission, particularly a depletion in lactobacilli, was predictive of sPTB.

Results in the Context of What is Known

Our results support previous findings regarding the importance of a healthy lactobacilli-rich microbiota for pregnancy outcome. Reduced *Lactobacillus* spp. abundance and increased vaginal bacterial diversity were identified as risk factors for PPROM in a study

that analyzed the vaginal microbiome of 86 women.[21] A meta-analysis conducted by Gudnadottir *et al.* in which the vaginal microbiome was assessed before the onset of labor reported an increased risk of preterm delivery in women with low-lactobacilli microbiota.[15] Lastly, in a recent longitudinal cohort study of 175 pregnant women comprising 40 PTB, the vaginal microbiome of pregnancies resulting in PTB was found to be genetically more diverse, a diversity which was driven by *Gardnerella* spp.[20] In our study, enterobacteria but not *G. vaginalis* were identified as a risk factor for PPROM. This discrepancy is likely related to the methodology used, as conventional culture techniques are less sensitive than metagenomics for the detection of anaerobes.

Clinical Implications

The role of bacterial vaginosis and *G. vaginalis* in PTL and PTB has long been suspected.[22,23] Increasing evidence now supports a direct role for *G. vaginalis* in the pathophysiology of PTL is now accumulating. *G. vaginalis* and other anaerobes such as *Prevotella* spp. are capable of degrading cervical mucus, promoting ascending intrauterine infections, local inflammation, uterine motility, and PTL.[24,25] It is now generally accepted that an antibiotic treatment, mainly based on metronidazole or clindamycin, should be given to women presenting with PTL and diagnosed with symptomatic bacterial vaginosis. In many countries, national guidelines also recommend screening for bacterial vaginosis and treatment of women with a history of PTB, although this strategy has not been shown to be effective in reducing the risk of PTB.[26–29] Here we show that *G. vaginalis* is a risk factor for PTL but more importantly, it is the absence of lactobacilli that appears to be predictive of sPTB. These observations, in line with numerous metagenomic studies highlighting the protective role of lactobacilli and in particular *L. crispatus*, *L. gasseri*, and *L. jensenii*,[16,30,31] argue for a comprehensive management of bacterial vaginosis in pregnancy aimed at restoring a healthy

microbiota. Such management would likely combine antibiotics targeting anaerobes with probiotics, as proposed for recurrent vaginosis.[28]

Our results also raise questions about the management of vaginal colonization by enterobacteria during PTL and PPROM. We identified *E. coli*, and more generally enterobacteria, as strongly associated with PTL and PPROM in univariate and multivariable analyses and also, although only in univariate analysis, for sPTB. However, in contrast to GBS, there are currently no consensus guidelines for the screening and the administration of antibiotics against enterobacteria during pregnancy, even in the case of PPROM. In the absence of evidence of infection, strategies vary widely between countries, ranging from no treatment to broad-spectrum antibiotics such as 3rd generation cephalosporins.[32] Our observations also support the administration of prophylaxis tailored to the pathogenic species present in the vaginal microbiota at admission for PTL and PPROM, although further studies are needed to determine the benefits of such a strategy.

Research Implications

Interventional studies to assess the efficacy and tolerability of antibiotics alone or in combination with probiotics to reduce the risk of PTB and PPROM in high-risk populations are still lacking. Randomized control trials should take into account that a particular probiotic strain may not be protective for all women according to their CST. The benefit of personalized targeted antibiotic prophylaxis directed against the specific pathogenic species *e.g.*, GBS, enterobacteria, *Candida* spp. and others, found in the vaginal flora in reducing the risk of PTB, especially in the case of PPROM, should also be investigated. There is also an important need to investigate the association between the vaginal microbiota and the risk of early-onset neonatal sepsis.[33]

Strengths and Limitations

This is a large cohort of over 1,800 patients, including over 400 PTL and nearly 400 PPROM along with low-risk controls. The sample size allowed adjustment for important confounders of the outcome measures, including demographic factors such as ethnicity, BMI, and history of PTB. The conventional culture approach is widely available in microbiology laboratories and thus applicable to routine clinical settings, in contrast to metagenomics. Hence, we were able to demonstrate that the vaginal microbiota is an independent risk factor for PTL, and PPROM, as well as for sPTB. However, there are limitations that need to be considered when interpreting our results. First, the microbiota analyses were performed using simple vaginal swab cultures. This leads to three main issues: (i) fastidious species such as *Atopobium* spp., or Mollicutes could not be studied; (ii) since culture is less sensitive than metagenomics, the reported species were most likely the dominant ones and minor species were overlooked; (iii) quantitative analysis could not be performed and ultimately only the presence or absence of major species was studied. The second major limitation is the lack of identification of lactobacilli at the species level. Consequently, the vaginal microbiotas were not categorized according to CSTs, and the protective role of the different *Lactobacillus* species could not be addressed.

Conclusions

In this large multicentric cohort study focusing on pregnancies at high risk of PTB, we used bacterial culture to highlight novel distinct vaginal microbiota signatures of adverse pregnancy outcomes. We identified *G. vaginalis* as the strongest risk factor for PTL, enterobacteria as a risk factor for PPROM, and lactobacilli depletion as a risk factor for PTL, PPROM, and sPTB. Further studies are needed to address the potential of interventions aimed at restoring a healthy microbiota in reducing these outcomes.

Transparency declaration

Ethics statement

The reported study was reviewed by the French ethics committee who granted approval on November, 2017 (identifier 2017 – A02755-48; NCT03371056). All individuals enrolled in the cohort provided signed informed consent that included authorization for the collection of medical information and biological samples. All collected data were anonymized and integrated in a secured electronic database.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Funding

This work was supported by the Programme d'Investissements d'avenir and bpifrance (Structuring R&D Project for Competitiveness—PSPC): # DOS0053477 SUB et DOS0053473 AR). L.L. is a doctoral fellow supported by the Association Nationale de la Recherche et Technologie (Cifre # 2019 / 1510). The funders had no role in the design and conduct of the study.

Acknowledgements

We thank all the contributors to this study, collectively called the InSPIRE Consortium. The full list can be found in the accompanying Supplementary Information.

Part of the results were previously presented in April 2023 at the ECCMID scientific meeting (Copenhagen, Denmark), in September 2023 at the ESPBC congress (Haarlem, The

Netherlands), and in January 2025 at the SMFM 2025 pregnancy meeting (Denver, Colorado, USA).

Access to data

All authors had full access to data. Data will be made available upon requests directed to the corresponding author.

Authors' contributions

Conceptualization: C.Po., L.M., and A.T. Methodology: J.R., P.-Y.A., L.M., and A.T. Formal analysis: L.Le., J.R., P.-Y.A., L.M., and A.T. Investigation: L.Le., J.R., and A.T. Resources: C.Pl., L.La, N.G., F.G., and L.M. Data curation: L.Le, J.R., C.Pl, L.La, N.G., and A.T. Writing – Original draft: L.Le and A.T. Writing – Review and Editing: all authors. Supervision: C.Pa, C.Po., L.M. and A.T. Funding acquisition: C.Pa, C.Po and L.M.

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Figure legends.

Figure 1. Vaginal microbiota composition at admission for preterm labor (PTL) and preterm rupture of membranes (PPROM).

Vaginal microbiota composition was characterized by vaginal swab cultures and compared between controls (n=1,048) and women admitted for PTL (n=417) or PPROM (n=383). The percentage of vaginal samples positive for selected bacterial group or species are represented. Error bars indicate the upper limit of the 95% confidence interval. ** $p<0.01$; *** $p<0.001$, ns: not significant.

Figure 2. Multivariable analysis of risk factors for preterm labor (PTL) and preterm premature rupture of membranes (PPROM).

Error bars indicate the upper and lower limit of the 95% confidence interval (CI); aOR: adjusted odds ratio.

Figure 3. Multivariable analysis of risk factors for spontaneous preterm birth.

Error bars indicate the upper and lower limit of the 95% confidence interval (CI); aOR: adjusted odds ratio.

535 **Table 1.** Cohort demographic and clinical characteristics.

	Control N=1,048	**PTL N=417**	**PPROM N=383**	***P* value**
Maternity ward, n (%)				NT
Center 1	677 (64.6)	114 (27.3)	192 (50.1)	
Center 2	371 (35.4)	203 (48.7)	137 (35.8)	
Center 3	0 (0)	100 (24.0)	54 (14.1)	
Age, median (IQR), years	33 (30-36)	32 (29-35)	33 (30-36)	<0.0001
Gestational age at inclusion, median (IQR), weeks	36 (35-36)	29 (25-32)	32 (27-35)	<0.0001
Maternal place of birth, n (%)				<0.0001
France	556 (53.2)	147 (35.9)	116 (30.7)	
France, overseas territories[a]	29 (2.8)	25 (6.1)	24 (6.3)	
Europe, other countries	79 (7.6)	22 (5.4)	20 (5.3)	
North Africa	199 (19.0)	84 (20.5)	106 (28.0)	
Sub-Saharan Africa	89 (8.5)	98 (24.0)	77 (20.4)	
Asia	35 (3.3)	10 (2.4)	19 (5.0)	
Others	58 (5.6)	23 (5.6)	16 (4.2)	
Missing	*3 (0.3)*	*8 (1.0)*	*5 (1.3)*	
Gravidity, n (%)				0.06
1	311 (29.7)	129 (30.9)	123 (32.1)	
2	336 (32.1)	122 (29.3)	92 (24.0)	
≥3	400 (38.2)	166 (39.8)	168 (43.9)	
Missing	*1 (0.1)*	*0 (0)*	*0 (0)*	
Parity, n (%)				0.001
0	469 (44.8)	220 (52.8)	190 (49.6)	
1	378 (36.1)	129 (30.9)	102 (26.6)	
≥2	200 (19.1)	68 (16.3)	91 (23.8)	
Missing	*1 (0.1)*	*0 (0)*	*0 (0)*	
History of preterm delivery, n (%)	109 (10.4)	95 (22.8)	67 (17.5)	<0.0001
Missing	*3 (0.3)*	*0 (0)*	*0 (0)*	
Body mass index, median (IQR)	22.4 (20.4 - 25.7)	23.0 (20.3 - 26.7)	24.5 (21.7 - 28.7)	<0.0001
Underweight (<18.5), n (%)	57 (5.4)	38 (9.2)	19 (5.1)	
Normal weight (18.5-24.9), n (%)	670 (64.0)	228 (55.2)	179 (47.6)	
Overweight (25-29.9), n (%)	211 (20.2)	90 (21.8)	102 (27.1)	
Obese (≥30), n (%)	109 (10.4)	57 (13.8)	76 (20.2)	
Missing	*1 (0.1)*	*4 (1.0)*	*7 (1.8)*	
Gestational diabetes, n (%)	194 (18.5)	69 (16.5)	82 (21.4)	0.21
Missing	*1 (0.1)*	*0 (0)*	*0 (0)*	
Hypertensive disease, n (%)	41 (3.9)	15 (3.6)	4 (1.0)	0.02
Missing	*1 (0.1)*	*0 (0)*	*0 (0)*	
Smoking during pregnancy, n (%)	56 (5.4)	36 (8.7)	25 (6.6)	0.06

	Control N=1,048	**PTL N=417**	**PPROM N=383**	***P* value**
Missing	*3 (0.3)*	*3 (0.7)*	*3 (0.8)*	
Fetal growth restriction, n (%)	27 (2.6)	29 (7.0)	27 (7.0)	<0.0001
Missing	*1 (0.1)*	*0 (0)*	*0 (0)*	
Gestational age at delivery, median (IQR), weeks	40.0 (39.0-40.7)	38.1 (35.1-39.3)	35.1 (30.6-36.4)	<0.0001
Extremely preterm (<28 weeks), n (%)	0 (0)	33 (7.9)	57 (15.0)	
Very preterm (28-31 weeks), n (%)	0 (0)	24 (5.8)	66 (17.3)	
Moderately preterm (32-36 weeks), n (%)	0 (0)	96 (23.0)	204 (53.5)	
Term (≥ 37 weeks), n (%)	1048 (100)	264 (63.3)	54 (14.2)	
Missing	*0 (0)*	*4 (1.0)*	*2 (0.5)*	
Mode of delivery				<0.0001
Vaginal, spontaneous	672 (64.4)	290 (71.2)	219 (57.6)	
Vaginal, induced	317 (30.4)	98 (24.1)	105 (27.6)	
Planned Caesarean	55 (5.2)	19 (4.7)	56 (14.8)	
Missing	*4 (0.4)*	*10 (2.4)*	*3 (0.8)*	
Interval between ROM and delivery, median (IQR), days	0 (0-1)	0 (0-1)	5 (1-17)	<0.0001
Missing	*0 (0)*	*36 (8.6)*	*13 (3.4)*	
Antibiotics between inclusion and delivery[b], n (%)	138 (13.2)	126 (30.4)	313 (81.7)	<0.0001
Missing	*0 (0)*	*2 (0.5)*	*0 (0)*	
Live births, n (%)	1046 (100)	411 (100)	378 (99.2)	NT
Missing	*2 (0.2)*	*6 (1.4)*	*2 (0.5)*	

[a] Including territories in South America (French Guiana), the Atlantic Ocean (Guadeloupe, Martinique, Saint-Martin, Saint-Barthélémy, Saint-Pierre-et-Miquelon), the Indian Ocean (Reunion Island, Mayotte), the Pacific Ocean (New Caledonia, French Polynesia, the Wallis and Futuna Islands), and the French Southern and Antarctic Territories.

[b] Including intrapartum antibiotic prophylaxis and excluding non-systemic antibiotic therapy.

IQR: interquartile range; NT: not tested; PPROM: preterm premature rupture of membranes; PTL: threatened preterm birth or preterm labor; ROM: rupture of membranes.

Table 2. Vaginal microbiology at inclusion.

Variable, n (%)	Control N=1,048	PTL N=417	*P* value[a]	PPROM N=383	*P* value[b]
Nugent score			0.01		0.048
Normal (0-3)	713 (85.3)	270 (85.7)		225 (79.2)	
Intermediate (4-6)	107 (12.8)	30 (9.5)		53 (18.7)	
Bacterial vaginosis (≥7)	16 (1.9)	15 (4.8)		6 (2.1)	
Missing	*212 (20.2)*	*102 (24.5)*		*99 (25.8)*	
Microbiological cultures					
***Lactobacillus* spp.**			<0.0001		<0.0001
Numerous	845 (80.8)	281 (67.5)		201 (54.9)	
Rare	33 (3.2)	16 (3.8)		34 (9.3)	
Absent	168 (16.0)	119 (28.6)		131 (35.8)	
Missing	*2 (0.2)*	*1 (0.2)*		*17 (4.4)*	
Enterobacteria, all species			<0.0001		<0.0001
Numerous	77 (7.3)	85 (20.4)		81 (21.2)	
Rare	91 (8.7)	39 (9.4)		48 (12.6)	
Absent	880 (84.0)	293 (70.3)		253 (66.2)	
Missing	*0 (0)*	*0 (0)*		*1 (0.3)*	
Escherichia coli			<0.0001		<0.0001
Numerous	52 (5.0)	59 (14.1)		51 (13.5)	
Rare	67 (6.4)	33 (7.9)		41 (10.8)	
Absent	929 (88.6)	325 (77.9)		286 (75.7)	
Missing	*0 (0)*	*0 (0)*		*5 (1.3)*	
Klebsiella pneumoniae			<0.0001		<0.0001
Numerous	13 (1.2)	21 (5.0)		22 (5.8)	
Rare	12 (1.1)	6 (1.4)		8 (2.1)	
Absent	1023 (97.6)	390 (93.5)		348 (92.1)	
Missing	*0 (0)*	*0 (0)*		*5 (1.3)*	
Staphylococcus aureus			0.07		0.64
Numerous	24 (2.3)	19 (4.6)		9 (2.4)	
Rare	25 (2.4)	10 (2.4)		6 (1.6)	
Absent	985 (95.3)	387 (93.0)		363 (96.0)	
Missing	*14 (1.3)*	*1 (0.2)*		*5 (1.3)*	
Group B *Streptococcus*			0.91		0.86
Numerous	125 (11.9)	53 (12.7)		43 (11.3)	
Rare	32 (3.1)	12 (2.9)		10 (2.6)	
Absent	891 (85.0)	352 (84.4)		328 (86.1)	
Missing	*0 (0)*	*0 (0)*		*2 (0.5)*	
Streptococcus anginosus			0.48		0.09
Numerous	26 (2.5)	15 (3.6)		17 (4.5)	
Rare	12 (1.1)	4 (1.0)		2 (0.5)	
Absent	1010 (96.4)	398 (95.4)		363 (95.0)	
Missing	*0 (0)*	*0 (0)*		*1 (0.3)*	
Anaerobes[c]			0.003		0.11
Numerous	113 (10.8)	72 (17.3)		53 (13.9)	

Variable, n (%)	**Control N=1,048**	**PTL N=417**	***P* value[a]**	**PPROM N=383**	***P* value[b]**
Rare	15 (1.4)	5 (1.2)		2 (0.5)	
Absent	919 (87.8)	340 (81.5)		326 (85.6)	
Missing	*1 (0.1)*	*0 (0)*		*2 (0.5)*	
Gardnerella vaginalis			<0.0001		0.06
Numerous	72 (6.9)	66 (15.8)		40 (10.5)	
Rare	10 (1.0)	4 (1.0)		2 (0.5)	
Absent	965 (92.2)	347 (83.2)		339 (89.0)	
Missing	*1 (0.1)*	*0 (0)*		*2 (0.5)*	
***Candida* spp.**			0.97		0.67
Numerous	149 (14.3)	61 (14.7)		53 (14.0)	
Rare	31 (3.0)	12 (2.9)		8 (2.1)	
Absent	865 (82.8)	341 (82.4)		318 (83.9)	
Missing	*3 (0.3)*	*3 (0.7)*		*4 (1.0)*	

[a] Statistical analysis comparing PTL to control.

[b] Statistical analysis comparing PPROM to control.

[c] including *Gardnerella vaginalis.*

PTL: preterm labor; PPROM: preterm premature rupture of membranes.

Table 3. Univariate analysis of factors associated with preterm labor and preterm premature rupture of membranes.

	PTL (N=417)			PPROM (N=383)		
	OR	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Place of birth						
France	1 (ref)	-	<0.0001	1 (ref)	-	<0.0001
France, overseas territories[a]	3.26	1.85-5.74	-	3.97	2.23-7.06	-
Europe, other countries	1.05	0.63-1.75	-	1.21	0.71-2.06	-
North Africa	1.60	1.17-2.18	-	2.55	1.87-3.48	-
Sub-Saharan Africa	4.16	2.97-5.85	-	4.15	2.88-5.97	-
Asia	1.08	0.52-2.23	-	2.60	1.44-4.71	-
Gravidity						
1	1 (ref)	-	0.57	1 (ref)	-	0.01
2	0.88	0.65-1.17	-	0.69	0.51-0.94	-
≥3	1.00	0.76-1.32	-	1.06	0.81-1.40	-
Parity						
0	1 (ref)	-	0.02	1 (ref)	-	0.002
1	0.73	0.56-0.94	-	0.67	0.51-0.88	-
≥2	0.72	0.53-1.00	-	1.12	0.83-1.52	-
History of preterm delivery	2.53	1.87-3.43	<0.0001	1.82	1.31-2.53	0.0003
Body mass index						
Normal weight (18.5-24.9)	1 (ref)	-	0.004	1 (ref)	-	<0.0001
Underweight (<18.5)	1.96	1.27-3.03	-	1.25	0.72-2.15	-
Overweight (25-29.9)	1.25	0.94-1.67	-	1.81	1.36-2.41	-
Obese (≥30)	1.54	1.08-2.1	-	2.61	1.86-3.65	-

	PTL (N=417)			PPROM (N=383)		
Gestational diabetes	0.87	0.64-1.1	0.37	1.20	0.90-1.60	0.22
Hypertension	0.92	0.50-1.67	0.78	0.26	0.09-0.73	0.006
Smoking during pregnancy	1.68	1.09-2.60	0.02	1.24	0.76-2.02	0.38
Vaginal swab cultures						
Absence of lactobacilli	2.09	1.60-2.74	<0.0001	2.91	2.22-3.82	<0.0001
Presence of						
- Enterobacteria	2.22	1.70-2.90	<0.0001	2.67	2.04-3.49	<0.0001
- *Escherichia coli*	2.21	1.64-2.98	<0.0001	2.51	2.86-3.40	<0.0001
- *Klebsiella pneumoniae*	2.83	1.62-4.94	0.0001	3.53	2.05-6.08	<0.0001
- *Staphylooccus. aureus*	1.51	0.94-2.42	0.09	0.83	0.46-1.50	0.54
- Group B *Streptococcus*	1.05	0.77-1.44	0.76	0.92	0.66-1.28	0.61
- *Streptococcus anginosus*	1.27	0.72-2.23	0.41	1.39	0.79-2.44	0.25
- Anaerobes[b]	1.63	1.19-2.21	0.002	1.21	0.86-1.70	0.27
- *Gardnerella vaginalis*	2.37	1.69-3.34	<0.0001	1.46	0.99-2.16	0.06
- *Candida* spp.	1.03	0.76-1.39	0.86	0.92	0.67-1.27	0.62

[a] Including territories in South America (French Guiana), the Atlantic Ocean (Guadeloupe, Martinique, Saint-Martin, Saint-Barthélémy, Saint-Pierre-et-Miquelon), the Indian Ocean (Reunion Island, Mayotte), the Pacific Ocean (New Caledonia, French Polynesia, the Wallis and Futuna Islands), and the French Southern and Antarctic Territories.

[b] including *Gardnerella vaginalis.*

CI: confidence interval; OR: Odds ratio; PPROM: preterm premature rupture of membranes; PTL: preterm labor.

Table 4. Risk factors for spontaneous preterm delivery.

Variable, n (%)	Term births N=181	Preterm births N=328	OR	95% CI	*P* value
Gestational age at admission, weeks					
32-36	64 (35.4)	135 (41.2)	1 (ref)	-	0.15
28-31	56 (30.9)	76 (23.2)	0.64	0.41-1.01	-
< 28	61 (33.7)	117 (35.7)	0.91	0.59-1.40	-
Place of birth					
France	76 (42.9)	108 (33.4)	1 (ref)	-	0.08
France, overseas territories[a]	12 (6.8)	17 (5.3)	1.00	0.45-2.21	-
Europe, other countries	12 (6.8)	19 (5.9)	1.11	0.51-2.43	-
North Africa	32 (18.1)	82 (25.4)	1.80	1.09-2.98	-
Sub Saharan Africa	30 (16.9)	65 (20.1)	1.52	0.90-2.57	-
Asia	5 (2.8)	18 (5.6)	2.53	0.90-7.12	-
Others	10 (5.6)	14 (4.3)	NT	NT	NT
Missing	*4 (2.2)*	*5 (1.5)*	-	-	-
Gravidity					
1	62 (34.3)	115 (35.1)	1 (ref)	-	0.31
2	60 (33.1)	89 (27.1)	0.80	0.51-1.25	-
≥3	59 (32.6)	124 (37.8)	1.13	0.73-1.76	-
Parity					
0	102 (56.4)	177 (54.0)	1 (ref)	-	0.85
1	53 (29.3)	99 (30.2)	1.08	0.71-1.63	-
≥2	26 (14.4)	52 (15.9)	1.15	0.68-1.96	-
History of preterm delivery	35 (19.3)	61 (18.6)	0.95	0.60-1.51	0.84
Body mass index					
Normal weight (18.5-24.9)	117 (65.0)	159 (49.2)	1 (ref)	-	0.005
Underweight (<18.5)	13 (7.2)	27 (8.4)	1.53	0.76-3.09	-
Overweight (25-29.9)	34 (18.9)	82 (25.4)	1.77	1.11-2.83	-
Obese (≥30)	16 (8.9)	55 (17.0)	2.53	1.38-4.64	-
Missing	*1 (0.6)*	*5 (1.5)*	-	-	-
Gestational diabetes	19 (10.5)	72 (22.0)	2.40	1.39-4.13	0.001
Hypertension	3 (1.7)	3 (0.9)	0.55	0.11-2.74	0.67
Smoking during pregnancy	12 (6.7)	23 (7.1)	1.07	0.52-2.20	0.86
Missing	*1 (0.6)*	*4 (1.2)*	-	-	-
Vaginal microbiological cultures					
Lactobacilli, absence	41 (22.7)	108 (33.6)	1.73	1.14-2.63	0.01
Missing	*0 (0)*	*7 (2.1)*	-	-	-
Presence of					

Variable, n (%)	Term births N=181	Preterm births N=328	OR	95% CI	*P* value
- Enterobacteria	45 (24.9)	111 (33.8)	1.55	1.03-2.32	0.04
- *Escherichia coli*	32 (17.7)	83 (25.3)	1.58	1.00-2.49	0.05
- *Klebsiella pneumoniae*	7 (3.9)	27 (8.2)	2.23	0.95-5.23	0.06
- *Staphylococcus aureus*	10 (5.5)	13 (4.0)	0.71	0.30-1.64	0.42
- Group B *Streptococcus*	28 (15.5)	41 (12.5)	0.78	0.46-1.31	0.35
- *Streptococcus anginosus*	9 (5.0)	20 (6.1)	1.24	0.55-2.79	0.60
- Anaerobes[b]	32 (17.7)	58 (17.7)	1.00	0.62-1.61	0.92
- *Gardnerella vaginalis*	30 (16.6)	49 (14.9)	0.88	0.54-1.45	0.62
- *Candida* spp.	28 (15.5)	56 (17.1)	1.13	0.69-1.85	0.64

[a] Including territories in South America (French Guiana), the Atlantic Ocean (Guadeloupe, Martinique, Saint-Martin, Saint-Barthélémy, Saint-Pierre-et-Miquelon), the Indian Ocean (Reunion Island, Mayotte), the Pacific Ocean (New Caledonia, French Polynesia, the Wallis and Futuna Islands), and the French Southern and Antarctic Territories.

[b] Including *Gardnerella vaginalis*.

CI: confidence interval. NT: not tested; OR: Odds ratio.

Supplementary Information

Supplementary Table 1. Vaginal microbiota characteristics according to the place of birth.

Supplementary Table 2. Vaginal microbiota characteristics according to body mass index.

Supplementary Figure 1. Second model of multivariable analysis of risk factors for preterm labor (PTL) and preterm premature rupture of membranes (PPROM).

In this model, *Escherichia coli* and *Klebsiella pneumoniae* were used instead of enterobacteria as variables. Error bars indicate the upper and lower limit of the 95% confidence interval (CI); aOR: adjusted odds ratio.

Supplementary File 1. Composition of the Inspire consortium.

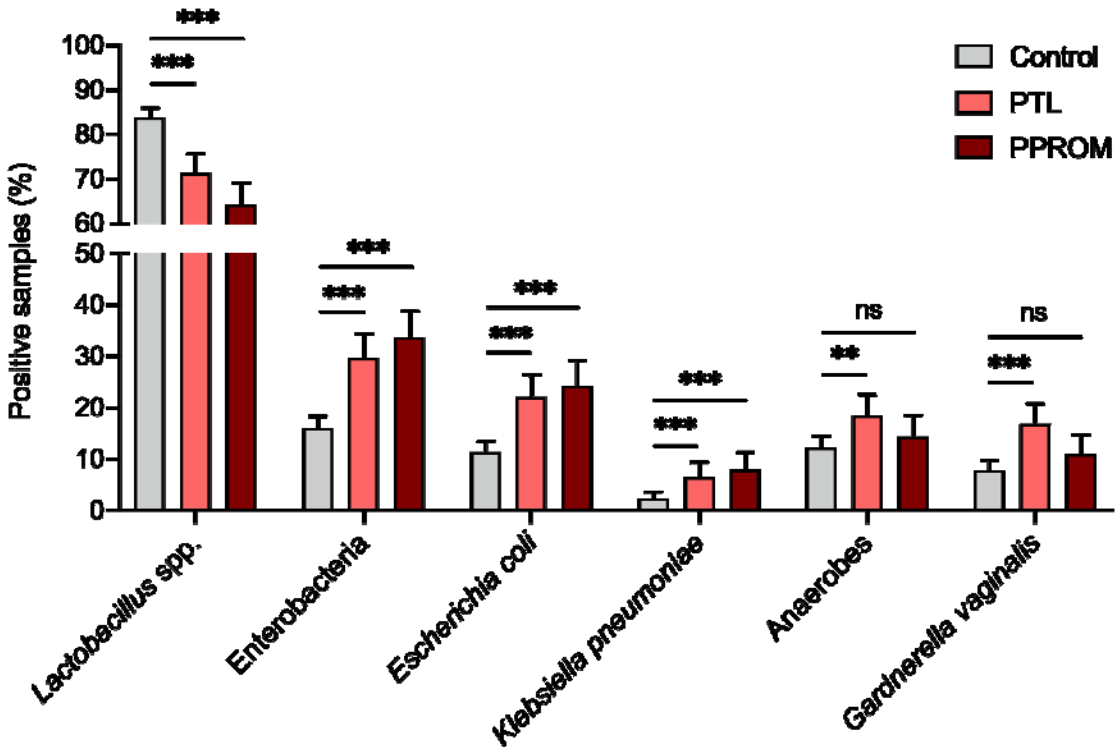

Figure 1. Vaginal microbiota composition at admission for preterm labor (PTL) and preterm rupture of membranes (PPROM).

Vaginal microbiota composition was characterized by vaginal swab cultures and compared between controls (n=1,048) and women admitted for PTL (n=417) or PPROM (n=383). The percentage of vaginal samples positive for selected bacterial group or species are represented. Error bars indicate the upper limit of the 95% confidence interval. ** $p<0.01$; *** $p<0.001$, ns: not significant.

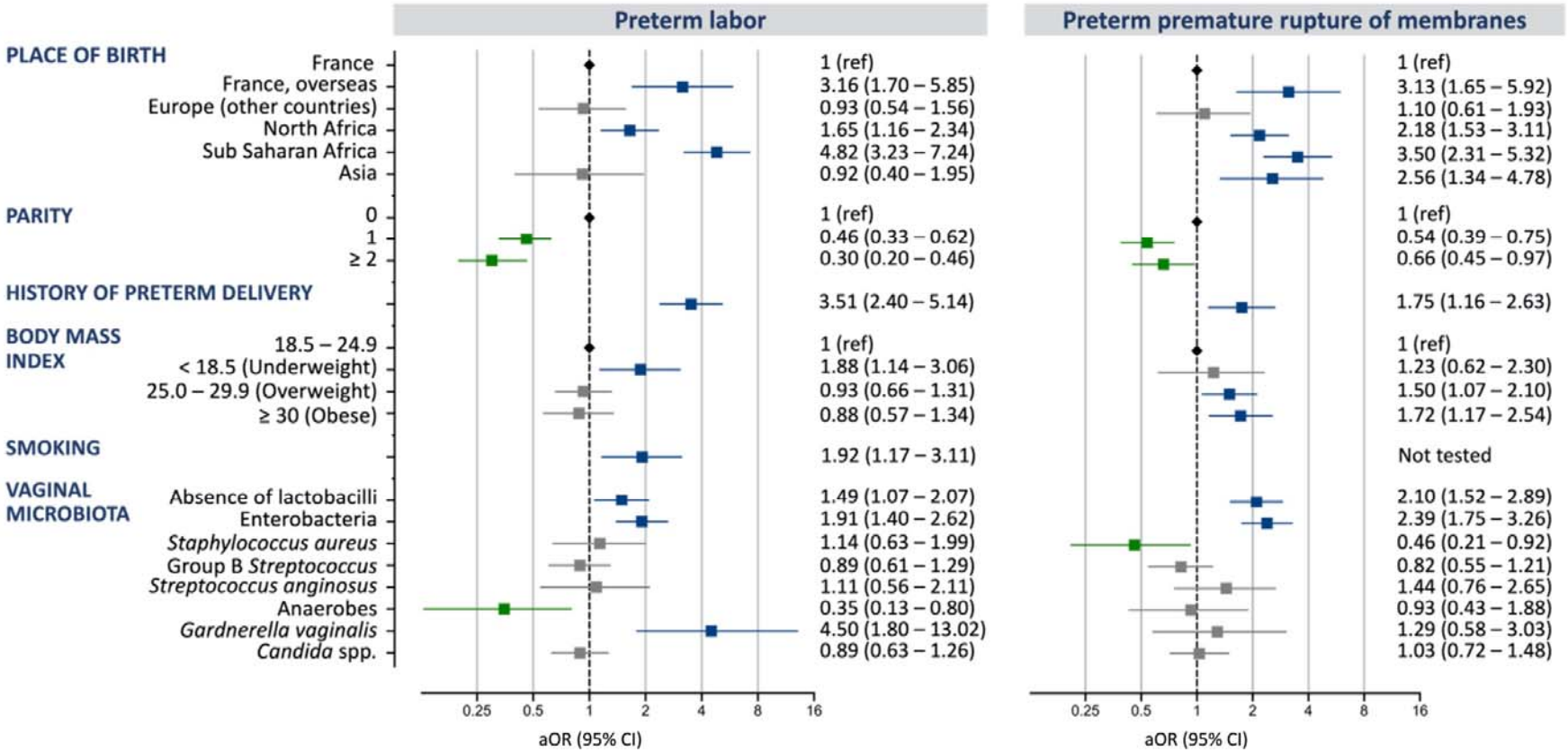

Figure 2. Multivariable analysis of risk factors for preterm labor (PTL) and preterm premature rupture of membranes (PPROM).

Error bars indicate the upper and lower limit of the 95% confidence interval (CI); aOR: adjusted odds ratio.

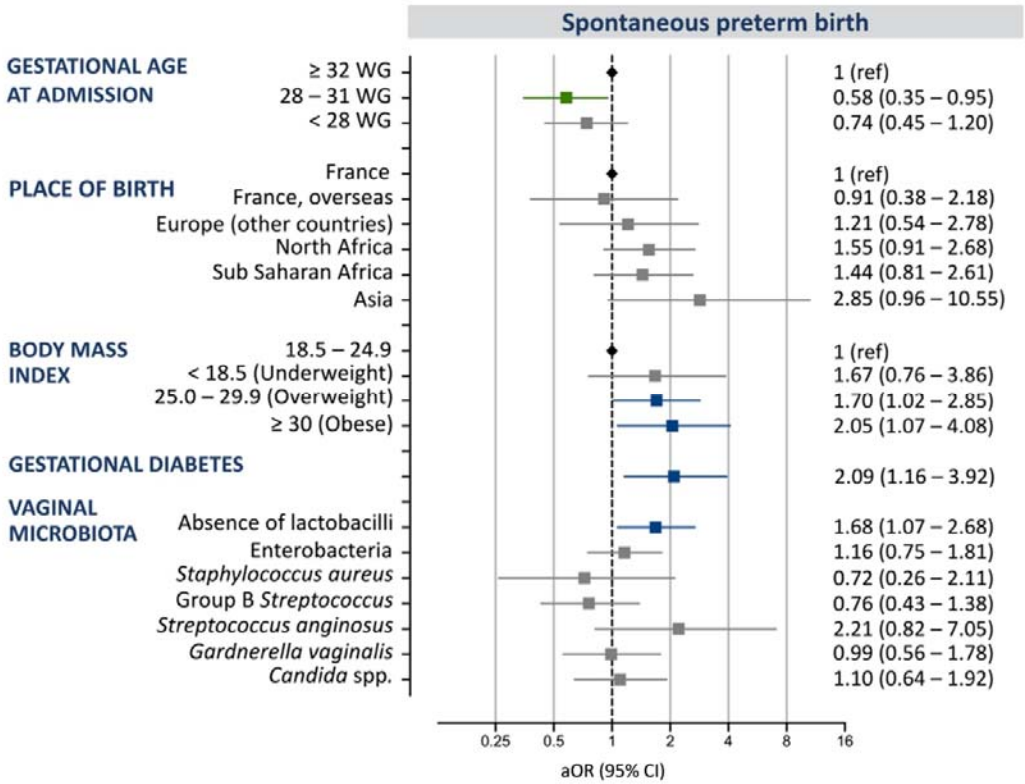

Figure 3. Multivariable analysis of risk factors for spontaneous preterm birth.

Error bars indicate the upper and lower limit of the 95% confidence interval (CI); aOR: adjusted odds ratio.