Trials

STUDY PROTOCOL

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Synbiotics in patients at risk for spontaneous preterm birth: protocol for a multi-centre, double-blind, randomised placebo-controlled trial (PRIORI)

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Abstract

Background Prematurity remains one of the main causes of neonatal morbidity and mortality. Approximately two thirds of preterm births are spontaneous, i.e. secondary to preterm labour, preterm prelabour rupture of membranes (PPROM) or cervical insufficiency. Etiologically, the vaginal microbiome plays an important role in spontaneous preterm birth (sPTB). Vaginal dysbiosis and bacterial vaginosis are well-known risk factors for ascending lower genital tract infections and sPTB, while a Lactobacillus crispatus-dominated vaginal microbiome is associated with term deliveries. Synbiotics may help to achieve and/or maintain a normal, Lactobacillus-dominated vaginal microbiome.

Methods We will perform a multi-centre, double-blind, randomised, placebo-controlled trial. Women aged 18 years or older with a singleton pregnancy are eligible for inclusion at $8^{0/7}$–$10^{6/7}$ weeks gestational age if they have one or more of the following risk factors for sPTB: previous sPTB at $24^{0/7}$–$35^{6/7}$ weeks, prior PPROM before $36^{0/7}$ weeks, or spontaneous pregnancy loss at $14^{0/7}$–$23^{6/7}$ weeks of gestation. Exclusion criteria are multiple gestation, cervix conisation, inflammatory bowel disease, uterine anomaly, and the use of pro-/pre-/synbiotics. Patients will be randomised to oral synbiotics or placebo, starting before 11 weeks of gestation until delivery. The oral synbiotic consists of eight Lactobacillus species (including L. crispatus) and prebiotics. The primary outcome is the gestational age at delivery. Vaginal microbiome analysis once per trimester (at approximately 9, 20, and 30 weeks) and delivery will be performed using metataxonomic sequencing (16S rRNA gene) and microbial culture. Secondary outcomes include PPROM, the use of antibiotics, antenatal admission information, and neonatal outcomes.

Discussion This study will evaluate the effect of oral synbiotics on the vaginal microbiome during pregnancy in a high-risk population and correlate the microbial changes with the gestational age at delivery and relevant pregnancy outcomes.

Trial registration ClinicalTrials.gov, NCT05966649. Registered on April 5, 2024.

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Keywords Preterm birth, Prematurity, PPROM, Vaginal microbiome, Pregnancy, Probiotics, Synbiotics, Lactobacilli, Next-generation sequencing, Randomised controlled trial

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Synbiotics in patients at risk for preterm birth: a multi-centre double-blind randomised placebo-controlled trial (PRIORI).
Trial registration {2a and 2b}.	ClinicalTrials.gov ID: NCT05966649
Protocol version {3}	Version 3.1 (April 2024)
Funding {4}	Belgian Health Care Knowledge Centre (KCE)
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Role of sponsor {5c}	ZOL AV acts as sponsor of the clinical trial, as defined in the Law of 2004, and has all responsibilities and liabilities in connection therewith and procures the mandatory liability insurance coverage in accordance with the Law of 2004.

Introduction

Background and rationale {6a}

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, remains the main cause of neonatal mortality and severe, potentially lifelong morbidity [1]. Moreover, the costs associated with the care for premature neonates, as well as long-term health problems, place an important economic burden on parents and health care systems. The overall PTB rate is around 10% worldwide, with large regional differences ranging from 4 to 16% [2]. Approximately two-thirds of all premature deliveries are non-iatrogenic or spontaneous, following preterm labour, preterm prelabour rupture of membranes (PPROM), or cervical insufficiency without prodromal labour [3–5].

Etiologically, spontaneous PTB (sPTB) is a multifactorial condition of which the exact cause and mechanism cannot be identified in most patients. However, it is hypothesised that intrauterine inflammation secondary to an ascending infection from the lower genital tract plays an important role in a significant proportion of sPTB cases [3, 6, 7]. The knowledge about the vaginal microbiome is evolving quickly. Besides microscopy, bacterial cultures, and polymerase chain reaction (PCR) tests, next-generation sequencing (NGS) is nowadays able to map a vaginal microbiome and provide more

insights in physiologic versus pathologic microbiome changes during pregnancy. Hence it is known that a disturbed vaginal microbiome, especially bacterial vaginosis (BV), early in pregnancy is an important risk factor for (subclinical) chorioamnionitis, PPROM, and sPTB [8–10]. On the other hand, a Lactobacillus—particularly L. crispatus—dominated vaginal microbiome in the first trimester is strongly predictive for term delivery [11]. While microbial diversity decreases throughout pregnancy in patients with term deliveries, a progressive depletion of lactobacilli and increasing diversity is observed in pregnancies complicated by PPROM and subsequent PTB, with a maximal diversity, and thus microbial instability, between 24 and 30 weeks of gestation [12]. This new evidence suggests that a progressively disturbed microbiome from early pregnancy on triggers the sPTB cascade and precedes clinically evident and culture/PCR-detectable infections. Consequently, instead of treating (asymptomatic) infections or secondary complications such as preterm labour and PPROM, interventions to prolong pregnancy duration in patients at risk for sPTB should start early.

A personal history of sPTB is a major risk factor for sPTB in subsequent pregnancies [4, 13–15]. However, interventions that interfere with the pathophysiology of sPTB in these high-risk patients are currently lacking. While antibiotics are effective in treating infections and BV in pregnancy, there is no effect on PTB rates [16, 17]. Recent literature suggests that supporting the vaginal microbiome with probiotics, rather than treating infections, could be a promising strategy [12]. Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (WHO definition [18], modified in the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement [19]), while a prebiotic is 'a substrate that is selectively utilised by host microorganisms conferring a health benefit' (ISAPP consensus statement [20]). Probiotic lactobacilli significantly increase vaginal Lactobacillus counts and stabilise the vaginal flora in patients with BV or dysbiosis without causing adverse effects [21, 22]. Previous studies with probiotics in pregnancy could not consistently demonstrate a favourable effect on pregnancy duration. However, clinical trials were very heterogenous in terms of patient population, primary outcome, study design, probiotic composition (bacterial species and strains), route of administration (oral or vaginal), timing, and duration of probiotic intake and were often underpowered to assess an effect on PTB rate or gestational age at delivery [23–37]. Based on the available literature, probiotics should probably be started early and continued throughout pregnancy to create a stable, Lactobacillus-(preferable L. crispatus-)dominated vaginal microbiome. Lactobacillus crispatus containing probiotics are promising, as L. crispatus is considered a biomarker of a healthy vaginal ecosystem and predictor for term birth [11, 38–40].

Objectives {7}

The aim of this study is to investigate the effect of oral synbiotics, a combination of probiotic bacteria and prebiotics, on the vaginal microbiome composition and on pregnancy duration in patients at risk for sPTB. The main hypothesis states that Lactobacillus crispatus containing synbiotics, when started early in pregnancy, support and maintain a healthy vaginal microbiome that is more resistant to ascending infections and associated with term delivery. It is expected that synbiotics can prolong pregnancy duration through favourable microbiome changes and that this higher gestational age (GA) at delivery translates into improved neonatal outcomes.

Trial design {8}

The PRIORI trial is a double-blind, randomised placebo-controlled trial wherein pregnant women at risk for sPTB will be recruited early in the first trimester of pregnancy and randomised in a 1:1 ratio into two parallel groups: an intervention (synbiotic) group and a control (placebo) group. The primary analysis of the primary endpoint (GA at delivery) is a superiority analysis comparing the intervention group with the control group.

An internal pilot study will be integrated in the full randomised controlled trial (RCT) to assess the effect of the oral synbiotics on the vaginal microbiome in the first 154 consecutively recruited patients. If the vaginal microbiome analyses after 4 weeks of treatment are in favour of the synbiotic, an independent data monitoring committee will decide to continue to the second phase (completion of the trial).

Methods: participants, interventions, and outcomes

Study setting {9}

The recruiting hospitals are mainly tertiary-level centres, both community and academic hospitals with a high-risk antenatal ward (also called maternal intensive care unit or MIC) and a neonatal intensive care unit (NICU) or N*. Only centres that have fulfilled all the duties with regard to study selection and training will be allowed to randomise patients. Recruitment will start in seven Belgian teaching hospitals: Ziekenhuis Oost-Limburg, University Hospitals Leuven, Ghent University Hospital, Citadelle Hospital CHU Liège, AZ Sint-Lucas Bruges, AZ Sint-Jan Bruges, and AZ Groeninge Kortrijk. An up-to-date list of all study sites can be found on the PRIORI website: https://www.prioritrial.be/en/deelnemende-ziekenhuizen.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Age 18 years or older 2. Singleton gestation 3. GA $8^{0/7}$–$10^{6/7}$ weeks at inclusion 4. At least one of the following risk factors for sPTB: - Prior sPTB at $24^{0/7}$–$35^{6/7}$ weeks - Prior PPROM before $36^{0/7}$ weeks - Prior spontaneous late pregnancy loss at $14^{0/7}$–$23^{6/7}$ weeks	1. Currently using pre-, pro-, or synbiotics 2. Multiple gestation 3. Preventive (type 1) cerclage will be planned in the first trimester 4. Inflammatory bowel disease 5. Congenital uterus anomaly 6. History of LLETZ/cervix conisation

GA gestational age, *sPTB* spontaneous preterm birth, *PPROM* preterm prelabour rupture of membranes, *LLETZ* large loop excision of the transformation zone

Eligibility criteria {10}

The study population consist of pregnant women at risk for sPTB based on their obstetric history. Inclusion and exclusion criteria are summarised in Table 1. Spontaneous preterm birth is defined as delivery at viable preterm gestation ($24^{0/7}$ until $35^{6/7}$ weeks) following preterm labour, PPROM, or cervical insufficiency. Spontaneous pregnancy late loss is defined as delivery at previable gestation ($14^{0/7}$–$23^{6/7}$ weeks) following preterm labour, PPROM, or cervical insufficiency. Written informed consent must be obtained from the participant or authorised surrogate before inclusion.

Who will take informed consent? {26a}

Written informed consent will be obtained from eligible patients by signing an informed consent form after detailed information is provided by a trained and delegated physician (maternal-foetal medicine specialist, gynaecologist or resident in obstetrics and gynaecology). The Principal Investigator retains overall responsibility for the informed consent of participants at their site and ensures that any person with delegated responsibility to participate in the informed consent process is duly authorised, trained, and competent according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

By signing the informed consent form, the study participants agree with collecting both maternal and neonatal clinical data and with taking and analysing biological specimens as described in the protocol (vaginal swabs, placental pathology, urine culture, and placental swabs in sPTB cases).

Interventions

Explanation for the choice of comparators {6b}

Patients in the control group will take an oral placebo that visually and physically matches the investigational product. All study participants will receive standard care (e.g. vaginal progesterone, serial cervical length measurements, etc.).

Intervention description {11a}

Pregnant women at risk for sPTB who are eligible for inclusion will be included and randomised into the intervention or control group at $8^{0/7}$–$10^{6/7}$ weeks of gestation. All study participants will start taking synbiotics or placebo, respectively, immediately after treatment allocation and continue until delivery. The daily dose is two capsules, one in the morning and one in the evening. Patients will be instructed about normal personal intimate hygiene and advised to avoid excessive genital cleaning or vaginal washings.

The investigational product (IP) is an oral synbiotic containing eight probiotic Lactobacillus strains, in total 2×10^{10} colony-forming units (CFU) per daily dose of two capsules, furthermore the prebiotics inulin, fructooligosaccharids (FOS), and D-mannose; see Table 2. The excipients are magnesium bisglycinate, magnesium stearate, and silicon dioxide. Capsules with enteric coating will be used to ensure delayed release for both the IP and placebo. The placebo capsules will only contain the excipients mentioned above. IP and placebo will be stored below 25°C.

Table 2 Composition of the investigational product

Active ingredient	Origin
Probiotic lactobacilli	
Lactobacillus crispatus	Human vagina
Lacticaseibacillus rhamnosus	Human faeces
Lactiplantibacillus plantarum	Fermented vegetables
Lactobacillus acidophilus	Human intestine
Limosilactobacillus fermentum	Child intestine
Lactobacillus gasseri	Human intestine
Lacticaseibacillus paracasei	Fermented food
Lacticaseibacillus casei	Human tissue
Prebiotics	
Inulin 20 mg	-
Fructooligosaccharids 20 mg	-
D-mannose 600 mg	-

Criteria for discontinuing or modifying allocated interventions {11b}

Previous studies demonstrated the safety of pro- and synbiotics in pregnancy [41, 42]. Therefore, we do not anticipate serious adverse events or complications secondary to taking either the IP or the placebo that could necessitate treatment discontinuation or modification.

Strategies to improve adherence to interventions {11c}

Patients will be given a diary that includes reporting the intake of the IP or placebo, a visit schedule, and contact information. The diary will be reviewed by a delegated team member and discussed with the patient on every study visit. Adherence to the intervention will be checked based on the patient's diary entries and the number of returned, unused capsules of IP or placebo.

Relevant concomitant care permitted or prohibited during the trial {11d}

At each study visit (including unscheduled visits such as hospital admissions), concomitant medication is checked and documented by the principal investigator (PI) or delegated team member. Because certain interventions may influence the study outcomes, standardised treatment protocols for indications that are relevant for the research question were made in collaboration with all participating centres. Recommendations were made for the use of vaginal progesterone (200 mg once daily for the indication of sPTB prevention), tocolysis, corticosteroids for foetal lung maturation, magnesium sulphate for neuroprotection, cervical cerclage or pessary placement, antifungal medication for symptomatic Candidiasis, and antibiotics for PPROM, Group B Streptococci (GBS) prophylaxis, symptomatic BV, and common sexually transmitted infections. The use of any pre-, pro-, or synbiotic other than the IP is not allowed during the study.

Provisions for post-trial care {30}

N/a. Post-trial care includes the routine postpartum follow-up and this is not influenced by trial participation. We do not anticipate any harm from trial participation.

Outcomes {12}

The primary outcome is the gestational age at delivery in weeks plus days, expressed as mean and standard deviation (SD) for both groups. Secondary outcomes include PTB rates in subcategories based on the GA at delivery (extreme PTB from $24^{0/7}$ until $27^{6/7}$ weeks, severe PTB from $28^{0/7}$ until $31^{6/7}$ weeks, and moderate to late PTB from $32^{0/7}$ until $36^{6/7}$ weeks of gestation) expressed as number (*n*) and proportion (%), PPROM rates (*n*, %) and GA at PPROM (weeks + days), vaginal microbiome analysis (see further), midstream urine culture at 16 weeks of gestation, GBS screening at 35 to 37 weeks of gestation, placental pathology, and neonatal outcomes (see further).

The vaginal microbiome will be examined once per trimester in order to correlate the effect of the oral synbiotic on the microbiome with pregnancy duration and duration of intake. Vaginal swabs will be taken at inclusion ($8^{0/7}$ to $10^{6/7}$ weeks), at $19^{0/7}$ to $21^{0/7}$ weeks, at $29^{0/7}$ to $31^{0/7}$ weeks, at delivery, and upon admission at the high-risk antenatal ward for threatened preterm birth (PPROM, preterm labour or short cervix). The vaginal microbiome will be analysed by bacterial culture and metataxonomic 16S rRNA gene sequencing. Because of potential interference with NGS analysis, patients on vaginal progesterone for PTB prevention are instructed to hold the dose of progesterone the evening before that study visit and to resume immediately after the study visit. Furthermore, the following swabs will be sampled during the PRIORI trial, frozen, and stored for metataxonomic sequencing with alternative funding: one vaginal swab for NGS at day 0, 3, 6, 9, 14, and 28 as long as the patient is admitted after PPROM, placental swabs in sPTB cases, and neonatal meconium swabs in PPROM cases. The results of the swabs mentioned above, sampled in the context of this study, will stay blind until the end of the trial and will not influence the patient's care.

During the internal pilot phase of the RCT, additional vaginal swabs will be taken during one extra study visit 4 weeks after the start of treatment. The primary outcomes of the internal pilot study are (1) the difference in total Lactobacillus abundance after 4 weeks of treatment compared to baseline by metataxonomic sequencing and (2) the vaginal detection of the Lactobacillus gasseri strain of the IP after 4 weeks of treatment by quantitative PCR (qPCR). The choice for L. gasseri as qPCR target is based upon the relatively low prevalence of natural L. gasseri dominance (< 10%), as compared to L. crispatus (around 40%) [43], in our European patient population (recently confirmed in the large-scale Belgian Isala project, Antwerp University), and the technical limitations in strain-specificity of qPCR.

Significant differences in pregnancy duration may be reflected in improved neonatal outcomes. Based on the Delphi consensus and in line with the Core Outcome Measures in Effectiveness Trials (COMET) initiative [44], we selected the following neonatal outcomes: neonatal mortality, birth weight, necrotising enterocolitis, bronchopulmonary dysplasia, intraventricular haemorrhage, encephalopathy of prematurity, infectious parameters (duration and number of antibiotic courses, early and late-onset sepsis), duration and type of respiratory support, surfactant administration, retinopathy, other neonatal morbidity, and duration of neonatal admission.

The economic impact and quality of life (QoL) will be assessed using the Work Productivity and Activity Impairment (WPAI) and EQ-5D questionnaire, respectively, during visit 2, 3, 4, 5, unscheduled visits (at admission and after 1 week), at delivery and during the neonatal follow-up period.

For continuous variables, mean and SD will be presented by study group and the difference (treatment effect) will also be presented with a 95% confidence interval. For binary variables, counts and percentages will be presented by the study group. The odds ratio, comparing the intervention group with the control group, and 95% confidence interval will also be presented.

Participant timeline {13}

The schedule of enrolment, interventions, and assessments can be found in the schematic diagram in Fig. 1.

Sample size {14}

The sample size calculation is derived from the primary outcome: gestational age at delivery (continuous variable). To detect a clinically relevant difference in pregnancy duration of 1 week between the intervention and control group with sufficient statistical power (i.e. 90%), assuming a SD of 3 weeks [27, 28], and with an alpha of 0.05, 382 patients are required in a 1:1 randomisation. These power calculations were based on a two-sided two-sample *t*-test. Information regarding the correlation between patients from the same centre, quantified by the intraclass correlation coefficient, is currently unavailable and thus could not be factored into the sample size calculation. However, it is anticipated that the intraclass correlation coefficient for gestational age at delivery is small, and therefore, the conducted power calculations are deemed applicable.

Anticipating that the primary outcome may not be available for a small proportion of the randomised patients due to reasons such as lost to follow-up or withdrawal of consent, a dropout rate of 5% is accounted for to maintain a power of 90%. Consequently, the total number of patients to be recruited for the trial is calculated as 402.

The sample size calculation was conducted using SAS for Windows, version 9.4.

Recruitment {15}

Patients will be recruited in seven Belgian teaching hospitals within a period of approximately 36 months. Depending on the recruitment speed, more sites will be activated to enrol 402 participants. The initial approach for pre-screening potential patients will be done by a member of the patient's existing clinical care team. Only physicians who are members of the PRIORI study team will inform the patient. If the treating physician is not a member of the PRIORI study team, he or she could refer the patient to a PRIORI investigator. The PRIORI investigator will confirm the eligibility of the patient and then the screening process can be started after a written informed consent is obtained.

Assignment of interventions: allocation

Sequence generation {16a}

An automated web-based system is used to randomly assign patients in a 1:1 ratio with variable block sizes, stratified for smoking ('yes' versus 'no' for smoking in the last 12 weeks before randomisation) according to the study centre.

Concealment mechanism {16b}

An automated web-based randomisation system will be used (randomized.net). The allocated treatment number will be checked by two delegated study members before the treatment (IP or placebo) is given to the patient.

Implementation {16c}

Only the PI or a qualified and delegated person (study nurse, midwife, or physician) to whom he/she has delegated this study task can enrol and randomise participants in the automated web-based system.

Assignment of interventions: blinding

Who will be blinded {17a}

As this is a double-blind randomised placebo-controlled trial, both the investigators and the patients will be blinded. The study team includes all care providers, site and sponsor staff, outcome assessors, and data analysts.

Procedure for unblinding if needed {17b}

Participants, site and sponsor staff, care providers, and data analysts will remain blinded from the time of

(See figure on next page.)

Fig. 1 Schedule of enrolment, interventions, and assessments. [1] Visit number and gestational age in weeks (w). [2] Admission on the high-risk antenatal ward for preterm labour, PPROM or short cervix. [3] New informed consent forms will be signed by the mother for the collection of neonatal data. [4] Height is only measured on visit 1 to calculate start Body Mass Index. [5] Microbial culture and metataxonomic sequencing. [6] Transvaginal ultrasound (TVUS). [7] Group B Streptococci (GBS) rectovaginal swab. [8] Quality of life questionnaire. [9] Work Productivity and Activity Impairment questionnaire

	STUDY PERIOD								
	Enrolment and allocation	Post-allocation							
TIMEPOINT[1]	*Visit 1 9±1w*	*Visit 1 9±1w*	*Visit 2 16±1w*	*Visit 3 20±1w*	*Visit 4 30±1w*	*Visit 5 36±1w*	*Maternal admission[2]*	*Delivery*	*Neonatal follow-up*
ENROLMENT									
Eligibility screen	X								
Informed consent	X							X[3]	
Dispense study treatment	X	X	X	X	X	X			
Allocation	X								
INTERVENTIONS									
Synbiotic group		←	—	—	—	—	—	→	
Placebo group		←	—	—	—	—	—	→	
ASSESSMENTS									
Demographics		X							
Medical/obstetric history		X							
IP compliance check			X	X	X	X	X	X	
Pregnancy complications			X	X	X	X	X	X	
Concomitant medication		X	X	X	X	X	X	X	
(Serious) Adverse Events		X	X	X	X	X	X	X	X
Body weight (and height[4])		X	X	X	X	X			
Vaginal swabs[5]		X		X	X		X	X	
Cervical length by TVUS[6]			X	X	X				
Urine midstream culture			X				X		
Biomarker PTB prediction test							X		
GBS screening[7]						X			
Placenta pathology								X	
Delivery info								X	
Swab meconium (PPROM)								X	
Swab placenta (PTB)								X	
Neonatal outcomes									X
EQ-5D questionnaire[8]			X	X	X	X	X	X	X
WPAI questionnaire[9]			X	X	X	X	X	X	X

Fig. 1 (See legend on previous page.)

treatment allocation until database lock. Though we do not foresee serious adverse events related to the IP, the study code will only be broken for valid medical or safety reasons. Unblinding of a patient can be performed by a physician of the study team. On receipt of the treatment allocation details, the PI or treating health care professional will continue to deal with the participant's medical emergency as appropriate. The PI/investigation team documents the breaking of the code and the reasons for doing so on the medical notes and CRF. It will also be documented at the end of the trial in any final study report. Unblinded data are to be kept strictly confidential until the time of unblinding of the trial and will not be accessible by anyone else involved in the trial with the following exceptions: the project manager of the company responsible for the labelling and packaging of the IP.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Individual patient data, included in the sponsor database and recorded in an electronic case report form (eCRF), will be handled in compliance with all applicable laws and regulations. The data collected will be pseudonymised and the data will only be used for the purpose(s) of this trial. All missing and ambiguous data will be queried. The study data will be transcribed from the source documents onto an eCRF by study staff within five working days of the participant's visit. Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the subjects' source documentation. Every effort should be made to ensure assessments susceptible to rater effects, which are to be recorded in the eCRF, are carried out by the same individual who conducted the initial screening assessment. The investigator must verify that all data entries in the eCRF are accurate and correct. All eCRF entries, corrections, and alterations must be made by the Investigator or other authorised study-site personnel. In case of a query, the investigator or an authorised member of the investigational staff must adjust the eCRF (if applicable) and complete the query.

PRIORI uses an eCRF to collect the data which will be used to perform statistical analysis for the trial. The CRF has been constructed to ensure (1) adequate data collection (only the data required by the protocol are captured in the CRF) and (2) proper audit trails to demonstrate the validity of the trial (both during and after the trial). An annotated CRF is developed with coding convention as will be used in the database. At the end of the trial, a copy of the CRF of each enrolled patient will be provided to the PI for archiving. The PI is responsible to keep records of all participating patients (sufficient information to link records e.g. CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages.

Swabs and requisition forms for NGS or PCR analysis will be labelled and frozen until shipment (once per 3 months) to the expert laboratory at Antwerp University. Swabs for classic microbial culture are analysed in the local lab.

Plans to promote participant retention and complete follow-up {18b}

Participants will be encouraged to remain on the assigned treatment and in close pregnancy monitoring for the total duration of the study. However, at any time during the study and without giving reasons, subjects may withdraw from the study at their own request or at the request of their legally acceptable representative. The subject will not suffer any disadvantage as a result of their withdrawal or discontinuation. In cases where subjects indicate they do not want to continue, investigators must determine whether this refers to discontinuation of study treatment, unwillingness to attend the follow-up visit, unwillingness to have telephone contact, unwillingness to have any contact with study staff, or unwillingness to allow contact with a third party (e.g., family member, doctor). In all cases, the reason for discontinuation (including 'at the subject's request') will be recorded in the eCRF and in the participant's medical records.

Data management {19}

Data management will agree with the 'EU General Data Protection Regulation'. All collected study data will be recorded and stored in the CRF created with the CASTOR© software. To protect the privacy of the participants, all collected data will be encoded. Following the creation of a new study record in the eCRF, a study-specific patient code will be created. The study code, e.g. 01-PRIORI-023, will consist of a code specific for the site of recruitment (i.e. 01, 02, etc.), followed by the abbreviation of the study (PRIORI), and an incremental three-digit number per centre (starting from 001 in order of inclusion). CASTOR© complies with all applicable medical data privacy laws and regulations: GCP, 21 CFR Part 11, EU Annex 11, the European Data Protection Directive, ISO9001, and ISO27001/NEN7510. The principal investigator will be responsible for data entry and the quality of the data at her hospital. The sponsor will be responsible for the data analysis. Detailed information regarding data handling and record keeping is provided in the data management plan.

Confidentiality {27}

Personal information will be collected, kept secure, and maintained in a way that conforms all regulation concerning the protection of privacy. Encoded, depersonalised data where the participant's identifying information is replaced by an unrelated sequence of characters will be created. The maintenance of the data and the linking code will be secured in separate locations using encrypted digital files within password-protected folders and storage media. Access will be limited to the minimum number of specific individuals necessary for quality control, audit, and analysis. The confidentiality of data will be preserved when the data are transmitted to sponsors and co-investigators. The data will be stored for at least 25 years and the sponsor is the data custodian.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Vaginal, placental, and neonatal meconium swabs for microbiome analysis will be sampled in duplicate: one swab for local testing, i.e. bacterial culture in the local lab of each study site, and one swab for central testing, i.e. molecular analysis in a specialised laboratory (University of Antwerp). Furthermore, the midstream urine culture and placenta pathology will be analysed locally as part of standard care. Biological specimens will be labelled appropriately. All results will stay blinded, except those that are considered standard care.

Swabs for next-generation sequencing will be stored in a − 20 °C freezer within 4 h after collection, until shipment to the central laboratory. Every working day, the freezer temperature will be monitored and large deviations (normal range − 15 °C until − 40 °C) will be notified to the study team. The samples will be shipped on dry ice by Inter Healthcare Transport once every 3 months.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Statistical analysis will be conducted using SAS for Windows, version 9.4 or higher.

The flow of patients will be described using a CONSORT-statement flow diagram.

Descriptive statistics will be presented for patient baseline characteristics to assess baseline comparability between the intervention and control group. For continuous variables, means and standard deviations will be reported or median and interquartile range (IQR) if the distribution is skewed. For binary and categorical variables, numbers and proportions will be given for each category.

The primary analysis of the primary endpoint, GA at delivery, is a superiority analysis comparing the intervention and control groups using a two-sided test at a 5% significance level. Mean GA and standard deviation will be reported for both groups. The treatment effect, along with a 95% confidence interval and p-value, will be evaluated using a linear mixed model. The statistical model will include a fixed treatment effect and random centre effect to correct for potential correlations between patients recruited in the same centre. The analyses will be performed for the intention-to-treat (ITT) population, employing multiple imputation techniques to address missing data. No covariate adjustment will be made in the primary analysis. If necessary, the transformation will be applied to the primary endpoint to achieve an approximately normal distribution, as visually assessed through diagnostic plots. Secondary analysis of the primary endpoint includes a frailty model for time-to-event outcomes.

For the secondary outcomes, the treatment effect will be investigated using a mixed model approach: a linear mixed model for continuous variables, a generalized mixed model (logistic/proportional odds) for binary/count outcome variables, and a frailty model for time-to-event outcomes. The treatment effect and 95% confidence interval will be obtained from this model. For continuous outcomes, the difference in means will be estimated and for binary outcomes, the odds ratio and for time-to-event outcomes the hazard ratio. No covariate adjustments will be made. Continuous variables with skewed distributions will be presented using the median and IQR, and treatment effect will be explored through endpoint transformation and/or nonparametric methods. All secondary efficacy analyses will be performed using the ITT population.

Interim analyses {21b}

N/A. Interim analyses will not be performed.

Methods for additional analyses (e.g. subgroup analyses) {20b}

N/A. Subgroup analyses will not be performed.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

The primary endpoint will be analysed on the ITT population and multiple imputation techniques will be used to account for the missing data. Furthermore, we will perform a per-protocol analysis. Patients with an overall compliance of less than 50% (defined as taking the recommended dose on less than 50% of the days during the treatment period), and participants who start taking pre-,

pro-, or synbiotics other than the study medication for more than 14 consecutive days, will be excluded in the per-protocol analysis.

Plans to give access to the full protocol, participant level-data, and statistical code {31c}

The PRIORI trial is registered on ClinicalTrials.gov with ID NCT05966649. We do not plan granting public access to the full protocol.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The trial management group includes those individuals responsible for the day-to-day management of the trial, such as the chief investigator, statistician, trial coordinator, and data manager. The role of the trial management group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to, and take appropriate action to safeguard participants and the quality of the trial itself.

The role of the Trial Steering Committee (TSC) is to provide the overall supervision of the trial. The TSC is composed of the chief investigator, a statistician, the trial coordinator, an independent expert, a neonatologist, representatives of other participating centres, up to two patient representatives, one representative of the sponsor, and one representative of the funder. The TSC will monitor trial progress and conduct and advise on scientific credibility. It will consider and act, as appropriate, and ultimately carries the responsibility for deciding whether a trial needs to be stopped on grounds of safety or efficacy. The TSC will meet on average three times per year in the first year and twice per year thereafter and send reports to the sponsor and funder. KCE shall have the right (but not the obligation) to be present at each TSC meeting.

Composition of the data monitoring committee, its role and reporting structure {21a}

The independent Data Monitoring Committee (iDMC) is an independent group of experts that will be responsible for the follow-up of the data of the internal pilot study. They will review the unblinded data periodically and recommend whether the results of the pilot study are favourable to proceed with the full study, based on preset, well-defined cutoffs of the pilot endpoints (change in Lactobacillus dominance and PCR detection of L. gasseri).

Adverse event reporting and harms {22}

Pregnancy complications will be collected on every study visit. Only specific data about gastrointestinal complaints will be collected as an AE, when there is a causal relationship with the IP: bloating, nausea, vomiting, diarrhoea, constipation, flatulence, abdominal pain, or intestinal cramps. Participants are instructed to report any new gastrointestinal symptom in the dairy, which will be reviewed by a delegated team member on the next study visit. Serious adverse events will also only be collected if they are likely related to the IP and should be reported within 24 h after awareness of the event; however, these are not anticipated since previous studies with synbiotics or probiotics have demonstrated safety in pregnancy [41].

Frequency and plans for auditing trial conduct {23}

The investigator will permit direct access to trial data and documents for the purpose of monitoring, audits, and/or inspections by authorised entities such as, but not limited to, the sponsor or its designees and competent regulatory or health authorities. As such, eCRFs, source records, and other trial-related documentation (e.g. the TMF, pharmacy records, etc.) will be kept current, complete, and accurate at all times. The frequency of audits has not been defined at this stage.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Substantial amendments that require review by the ethical committee (EC) will not be implemented until the EC grants a favourable opinion for the study. All correspondence with the EC will be retained in the Trial Master File/ Investigator Site File.

During the study, the valuable opinion of patient representatives will be asked whenever changes need to be made. For example, participants will be actively involved in the review of protocol amendments, changes in ICF, and their opinion and input will be valuable for recruitment. Patients from both Flanders and Wallonia and with a different cultural background are part of the TSC.

Dissemination plans {31a}

Upon completion of the trial, the data arising from the trial will be analysed and tabulated to create the Final Study Report, which can be accessed online as well as on ClinicalTrials.gov. Upon approval of the TSC, the investigators will publish the primary study results within 6 months after the statistical analysis. Funding by KCE will be acknowledged in publications.

The trial participants will be notified about the outcome of the trial by a newsletter containing a reference of the published manuscript.

The primary study results of the PRIORI study will be reported fully and made publicly available when the research has been completed. All researchers shall ensure that the outcome of the research is prepared as a research

paper for publication in a suitable peer-reviewed, preferably open-access, journal. The Consort Guidelines and checklist will be reviewed prior to generating any publications for the trial to ensure they meet the standards required for submission to high-quality peer-reviewed journals.

Discussion

The PRIORI study is designed to investigate the effectiveness of oral synbiotic supplements in supporting the vaginal microbiome and prolonging gestation in high-risk pregnant women. To our knowledge, this is the first randomised placebo-controlled trial wherein synbiotics are started before 12 weeks of gestation and that is powered to detect a clinically relevant difference in pregnancy duration in a population at risk for spontaneous preterm birth. Common limitations of previous clinical studies were the low-risk patient population, small sample size, the use of probiotic species (other than L. crispatus) that are less likely to improve vaginal health, and the late start of pro/synbiotics (e.g. only in the third trimester or after PPROM).

Furthermore, serial vaginal swabs for metataxonomic analysis allow to understand how the vaginal microbiome changes throughout pregnancy in patients receiving synbiotics versus placebo, as well as in those with preterm versus term deliveries. This is important to interpret clinical outcomes and to learn more about the pathophysiologic mechanism of sPTB.

To recruit a sufficiently large population that meets the eligibility criteria, patients will be recruited in seven Belgian hospitals, all with a high-risk antenatal ward and neonatal intensive care unit. If the recruitment speed would be lower than anticipated, additional hospitals can participate in this trial.

The IP is a synbiotic containing eight Lactobacillus strains, including L. crispatus. Multi-strain and multi-species probiotic mixtures may be more effective than single-strain probiotics because of the synergistic effects of multiple strains (e.g. increased inhibition of pathogens, production of substances that facilitate adhesion of other Lactobacillus strains) [45, 46]. Moreover, different lactobacilli have different modes of action and interactions with host microorganisms.

We choose the oral route of administration. Oral probiotics have shown to significantly increase vaginal Lactobacillus counts and restore the vaginal microbiome in patients with BV and vaginal dysbiosis without causing adverse effects [22, 47]. Like urogenital pathogens, lactobacilli ascend the vagina from the rectum [47, 48]. After oral intake, probiotic bacteria are able to colonise the colonic and rectal microbiome, which functions as a reservoir for both lactobacilli and pathogens ascending to the vagina [49–51]. Therefore, we hypothesise that parallel changes to the intestinal microbiome after oral intake may contribute to a more sustainable and stable vaginal microbiome. Furthermore, oral intake is simple and easy to implement into the patient's daily routine, which might be an advantage in terms of compliance compared to vaginal administration, certainly for an intervention that requires long-term and daily administration, in a patient population already using daily vaginal progesterone.

This trial has some limitations inherent to the protocol. First, the PRIORI trial is a pragmatic clinical study powered to detect a difference in pregnancy duration, but not (composite) neonatal outcome. There is no long-term follow-up of the neonate after discharge or during childhood. Nevertheless, multiple relevant neonatal outcomes, consistent with the COMET consensus [44], will be collected after birth and data collection will continue until discharge from the NICU, when applicable. Second, we do not plan a cost-effectiveness analysis. However, data about the total duration of antenatal and neonatal hospital admissions will be collected, which indirectly allow for economic comparisons.

Trial status

The current protocol version 3.1 was issued on April 5, 2024. Recruitment will start in May 2024 and will be completed by 2028.

Abbreviations

AE	Adverse event
BPD	Bronchopulmonary dysplasia
BV	Bacterial vaginosis
CFU	Colony-forming unit
CI	Chief investigator
COMET	Core Outcome Measures in Effectiveness Trials
CRF	Case report form
CSR	Clinical study report
EC	Ethics committee
eCRF	Electronic case report form
EQ-5D	EuroQol five dimension (questionnaire)
FOS	Fructooligosaccharids
g	Gram
GA	Gestational age
GBS	Group B Streptococcus
GCP	Good Clinical Practice
GMP	Good manufacturing practice
iDMC	Independent Data Monitoring Committee
IP	Investigational product
KCE	Belgian Healthcare Knowledge Centre
L.	Lactobacillus
LLETZ	Large loop excision of the transformation zone
mg	Milligram
MIC	Maternal intensive care
MPL	Mid-trimester pregnancy loss
NEC	Necrotizing enterocolitis
NGS	Next-generation sequencing
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
PI	Principal investigator
PPROM	Preterm prelabour rupture of membranes
PTB	Preterm birth

QoL	Quality of life
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
SAE	Serious adverse event
SES	Socio-economic status
sPTB	Spontaneous preterm birth
STI	Sexual transmitted infections
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
WPAI	Work Productivity and Activity Impairment questionnaire
ZOL AV	Ziekenhuis Oost Limburg Autonome Verzorgingsinstelling

Acknowledgements
N/A. Everyone who contributed to the study protocol and manuscript meets the criteria for authorship.

Authors' contributions {31b}
CVH is the Chief Investigator; she conceived the study, led the proposal and protocol development, and wrote the manuscript. KN is co-investigator; she conceived the study, designed the study protocol, provided clinical input, and wrote the manuscript. EP is the project manager; she designed the study protocol, provided support with grant application and clinical trial management, and wrote the manuscript. KT designed the study protocol, provided support with grant application and clinical trial management, and wrote the manuscript. FC is the co-Chief Investigator; he provided domain knowledge expertise and clinical input, and revised the manuscript. RD is co-investigator; he provided domain knowledge expertise, methodological and clinical input, and revised the manuscript. JVK is co-investigator; he provided domain knowledge expertise, methodological and clinical input, and revised the manuscript. ID is co-investigator; she provided domain knowledge expertise, methodological and clinical input, and revised the manuscript. VM is co-investigator; she provided domain knowledge expertise and revised the manuscript. ES is co-investigator; she provided domain knowledge expertise and revised the manuscript. DT provided domain knowledge expertise, methodological and clinical input, and revised the manuscript. WG provided domain knowledge expertise and clinical input, and revised the manuscript. HL provided domain knowledge expertise and clinical input, and revised the manuscript. LB provided statistical and epidemiological support, and revised the manuscript. DS provided domain knowledge expertise and clinical input, and revised the manuscript. MR provided domain knowledge expertise and clinical input, and revised the manuscript. SL provided domain knowledge expertise and clinical input, and revised the manuscript. CA provided domain knowledge expertise and clinical input, and revised the manuscript. EO provided domain knowledge expertise and clinical input, and revised the manuscript. All authors read and approved the final manuscript.

Funding {4}
This study (KCE20-1273) is funded by the Belgian Health Care Knowledge Centre (KCE) under the KCE Trials Programme.

Availability of data and materials {29}
Only the TSC has access to the final trial dataset in order to ensure that the overall results are not disclosed by an individual trial site prior to manuscript publication. Site investigators will be allowed to access the full dataset if a formal request describing their plans is approved by the TSC.

Declarations

Ethics approval and consent to participate {24}
Ethics Committee of University of Leuven (KU Leuven), Leuven, Belgium, number S67065. Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}
Not applicable.

Competing interests {28}
The authors declare that they have no competing interests.

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Received: 29 April 2024 Accepted: 2 September 2024
Published online: 17 September 2024

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