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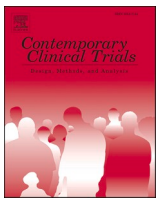

Preterm-birth-prevention with *Lactobacillus crispatus* oral probiotics: Protocol for a double blinded randomised placebo-controlled trial (the PrePOP study)

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ABSTRACT

Introduction: Effective spontaneous preterm birth (sPTB) prevention is an urgent unmet clinical need. Vaginal depletion of *Lactobacillus crispatus* is linked to sPTB. This trial will investigate impact of an oral *Lactobacillus* spp. probiotic product containing an *L. crispatus* strain with other *Lactobacilli* spp., on the maternal vaginal and gut microbiome in pregnancies high-risk for sPTB.

Methods: A double-blind, placebo-controlled, randomised trial will be performed at the National Maternity Hospital Dublin, Ireland. Inclusion criteria are women with history of sPTB or mid-trimester loss, cervical surgery (cone biopsy or two previous large-loop-excision-of-transformation-zone) or uterine anomaly. The intervention is oral supplementation for twelve weeks with probiotic or identical placebo. The probiotic will contains:

- 4 billion CFU *Lactobacillus crispatus* Lbv 88($2x10^9$CFU/Capsule)
- 4 billion CFU *Lactobacillus rhamnosus* Lbv 96($2x10^9$CFU/Capsule)
- 0.8 billion CFU *Lactobacillus jensenii* Lbv 116($0.4x10^9$CFU/Capsule)
- 1.2 billion CFU *Lactobacillus gasseri* Lbv 150($0.6x10^9$CFU/Capsule).

Investigators and participants will be blinded to assignment.

Results: The primary outcome is detectable *L. crispatus* in the vaginal microbiome after twelve weeks of treatment, measured using high-throughput DNA sequencing. A total of 126 women are required to detect a 25 % increase in detectable *L. crispatus*. Secondary outcomes include impact of intervention on the gut microbiome and metabolome, rate of sPTB and mid-trimester loss, neonatal outcomes and maternal morbidity.

Conclusions: This randomised trial will investigate ability of an oral probiotic containing *L. crispatus* to increase its abundance in the vaginal microbiome, both directly by horizontal transfer and indirectly via microbiome and metabolome of the gut.

1. Introduction

Preterm birth is the leading global cause of mortality for children under five [1]. Spontaneous preterm birth accounts for the majority of preterm births. Approximately 15 million babies are born preterm annually [2], defined as less than 37 weeks' gestation, and over 1 million of them die [3]. Those that survive can have significant co-morbidities, which continue to affect their lives from early childhood into

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adolescence and adulthood [4,5]. Preterm birth also has a substantial economic cost, estimated to be more than $26 billion annually in the United States alone [6]. Despite this, strategies to effectively predict and prevent spontaneous preterm birth are lacking. Alarmingly, the global rate of preterm birth has not decreased in the last decade [7] and remains the single greatest contributor to loss of human capital of any disease across the human lifecourse [8].

The maternal microbiome is increasingly recognised as a modifier of the inflammatory cascades that drive cervical shortening and spontaneous preterm birth. Unique microbial communities exist at numerous sites, or niches, within the body including the gut and vagina [9]. These complex communities and their associated genetic material are termed the microbiome. Microbial diversity is characterized by microbial richness and abundance [10]. Diversity manifests differently at each body site; the healthy gut generally exhibits high diversity, contrasting the healthy vaginal microbiome where *Lactobacillus* spp typically dominates [11]. The largest human microbial niche is the gut [12]. The gut microbiome plays a regulatory role on numerous key biological processes including immune-mediated inflammation, gluconeogenesis and hormone production, facilitated by production of short chain fatty acids during fermentation of food [13]. Gut and vaginal microbiota appear to be linked, with many gynaecological conditions associated with gut dysbiosis including persistent HPV and cervical cancer [14,15]. Gut microbiota in pregnancy has also been associated with gestational diabetes, with women experiencing excessive gestational weight gain display specific microbial changes in the gut prior to development of the clinical syndrome [16,17]. Although dysbiosis of the gut has been reported in women with preterm birth [18], the impact of the gut microbiome on pregnancy health and spontaneous preterm birth risk remains unclear [19,20].

The vaginal microbiome is often dominated by a single genus, *Lactobacillus* spp. These species produce high concentrations of lactic acid which exhibits both antimicrobial and immunomodulatory effects on the vaginal microenvironment [21]. Decreased relative abundance of *Lactobacillus* spp. leads to reduced lactic acid and increased vaginal pH [11], which is associated with increased microbial diversity and risk of pathogen colonization. A high diversity vaginal microbiota enriched with anaerobic and/or facultative bacteria is linked to gynaecological complications including bacterial vaginosis (BV) [22], recurrent urinary tract [23] and sexually transmitted infections [24], and HIV acquisition [25]. Microbe-induced inflammation in a dysbiotic vagina is also linked to adverse pregnancy outcomes, including spontaneous preterm birth [26,27] and miscarriage [28].

Within a healthy vaginal microbiome there are typically four prevalent *Lactobacillus* spp species including *L. crispatus*, *L. gasseri, L. jensensii and L. iners* [21]. Population-specific variations have been documented with Caucasian women tending to have higher prevalence of *L. crispatus* dominance [29,30], whereas women of Black or Hispanic ethnicity have increased prevalence of *L. iners* or high diversity compositions [11]. In pregnancy, a low abundance of *L. crispatus* is more common in instances of subsequent preterm birth [31,32]. Numerous meta-analyses have also demonstrated a link between lactobacilli dominance of the vaginal niche in pregnancy, particularly by *L. crispatus*, and lower subsequent events of preterm birth [33–36]. *L. crispatus* has also been reported to protect against preterm premature membrane rupture and early onset neonatal sepsis after delivery [37].

There are several potential mechanisms by which *L. crispatus* could confer protection against preterm birth and associated adverse pregnancy outcomes. Data shows that lower levels of pro-inflammatory cytokine expression are seen in *L. crispatus*-dominated environments, demonstrating species specific local immune action [38]. By contrast, *L. iners* fails to prevent the growth of potentially pathogenic microbes that have been linked to preterm birth [39,40]. *L. crispatus* is also highly exclusionary in the vaginal niche, while *L. iners* tolerates co-colonization of potentially pathogenic bacteria that could simulate pro-inflammatory pathway activation. At the vagina and cervix, these microbes induce an innate maternal immune response resulting in the release of inflammatory products including cytokines. Cytokines then stimulate prostaglandin release, which triggers uterine contractions and rupture of the fetal membranes, thus leading to preterm birth [26]. Consistent with this, cervical cerclage using multifilament suture has been previously shown to reduce vaginal *Lactobacilli* and increase vaginal pathogen colonization, driving activation of local inflammatory pathways associated with early cervical ripening [41].

Given the evidence linking the vaginal microbiome to spontaneous preterm birth, interventions that modulate the microbiome hold the potential to improve clinical outcomes. One of the proposed interventions being examined in recent years involves the administration of probiotics, defined as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host” [42]. With their proposed minimal side effect profile and postulated health benefits, probiotics of various formulations have become increasingly popular. Both oral and vaginal administration of probiotics are under investigation for efficacy in modulating the vaginal microbiome and, critically, improving clinical outcomes. Vaginal *L. crispatus* has been shown to improve abundance of *L. crispatus* in the vaginal microbiome in several RCTs outside of pregnancy [43–45]. Vaginal *L. crispatus* probiotics is improved rates of remission from bacterial vaginosis and has been linked to lower rates of preterm birth against historic controls, not against placebo controls and is not thought to impact the gut microbiome [44,46]. For oral *L. crispatus* probiotics, there has been conflicting evidence of efficacy in modifying vaginal microbiota [45,47]. Some studies of postmenopausal women with vaginal atrophy and high Nugent scores have found oral *Lactobacillus* spp supplements (ASTARTE product) improve Nugent scores [48], but a recent RCT of non-pregnant women found minimal impact on vaginal microbiota [45]. Critically however, oral *L. crispatus* has not been comprehensively evaluated in a high-risk spontaneous preterm birth cohort, who are known to have low *L. crispatus* abundance in early pregnancy [31,32] and a disordered gut microbiome and systemic inflammation involved in pathogenesis [18,49]. Oral *Lactobacillus* spp. has been shown in some instances to impact the gut microbiome [50], and has been linked to improving cervicovaginal HPV Clearance and HPV-related cytological anomalies through assumed immune-modulation [51,52]. Proposed benefits of an oral probiotic would include modulation of gut dysbiosis and prevention of the inflammation that is central to spontaneous preterm birth aetiology, as well as pragmatic benefits such as less invasive administration route, and ease of mass production with associated lower cost. Thus, a thorough evaluation is needed on the potential of oral *L. crispatus* supplementation to impact vaginal *L. crispatus* in a high-risk population to prevent spontaneous preterm birth, both directly and through gut microbial modification.

2. Materials and methods

2.1. Overview / Aims

We will perform a double blind, placebo-controlled, randomised trial investigating the efficacy of an oral probiotic supplement containing *L. crispatus* compared to placebo in the improvement of vaginal crispatus abundance in pregnancies at high-risk of spontaneous preterm birth.

2.2. Study population and eligibility criteria

The study population is women at high risk of spontaneous preterm birth attending a preterm birth prevention service. Many of these women have had a prior spontaneous preterm birth or mid pregnancy loss, with a recurrence risk of 20–30 % [53–56]. Other women attending have had excisional cervical surgery to treat cervical intraepithelial neoplasia, associated with persistent human papillomavirus (HPV). This may be either previous cone surgery (risk of spontaneous preterm birth 21 %) or two prior LLETZ procedures (risk of spontaneous preterm birth

13.2 %) [57,58]. Persistent HPV has been linked with absence of *L. crispatus* [14,15]. Other women attending have a uterine anomaly (risk of spontaneous preterm birth 3–22 % [59]). The rate of preterm birth prior to 37 weeks' gestation at this clinic is 15 % in addition to a mid-trimester loss rate of 1–2 % [60,61], which is double the national rate of preterm birth (7 %) [62]. Women attend the preterm birth clinic around 10–12 weeks of pregnancy, have their history assessed and examination performed including cervical length assessment with transvaginal ultrasonography. An individualised prevention strategy is formulated which may include vaginal progesterone, cervical cerclage, abdominal cerclage or cervical pessary. Frequency of clinic visits and cervical length surveillance is decided, typically performed every three weeks until 28 weeks of pregnancy. Criteria for determining eligibility for recruitment to the trial are detailed in Table 1.

2.3. Procedures, recruitment and randomisation

The study will be performed in the preterm birth surveillance clinic at the National Maternity Hospital, Dublin. All eligible women attending the clinic will be seen by clinicians trained in Good Clinical Practice at their first pregnancy visit to the clinic, at approximately 10–12 weeks' gestation. Patients will receive a patient information leaflet and a detailed verbal description of the proposed study. The aims of the study, methods, underlying evidence, anticipated benefits and potential hazards of the study will be explained. Subjects will be informed that participation is voluntary and withdrawing consent is possible at any stage, and declining participation will not affect their care. If deemed eligible and willing to participate, the researcher will go through the consent form to ensure the participant understands what is involved and given time to ask any questions. The patient will then be given time to read the written information and discuss with their support person before the study is re-discussed at their next clinic visit. The consent form will then be signed and dated by the participant and the researcher if they are ready to recruit. The trial has received institutional ethical approval and is registered with the ISRCTN (Registration number 11963471) [63].

2.4. Randomisation

Study randomisation will be conducted by a research statistician not involved in the trial. Using an online computerised randomisation service, the statistician will generate blocks of randomly allocated sequential study numbers for the two groups of capsules: intervention (probiotics) or placebo (control), randomised in a 1:1 ratio. Study capsules will be packed based on group allocation, with only study number denoted on the plain packaging. Participants will be consecutively allocated to each study number upon recruitment. Neither investigators nor participants will know study group designation, ensuring the trial is double blinded. Study supplements will be provided to the participant upon recruitment and randomisation to the study.

Table 1
Enrolment criteria for the trial.

Inclusion Criteria	Exclusion Criteria
Previous spontaneous preterm birth prior to 34 weeks' gestation	Age under 18 years
Previous spontaneous second trimester loss between 14- and 23-weeks' gestation	Non-fluent in English
Excisional cervical surgery including either two or more prior Large Loop Excision of Transformation Zone (LLETZ) or Cone Biopsy	Unable to provide informed consent due to lack of capacity
Uterine anomaly	Having a condition or taking medication, dietary supplement or food product that the investigators believe will interfere with the trial objectives

2.5. Intervention

Participants will be allocated to receive either the oral *Lactobacillus* spp. probiotic or placebo control. Identical capsules containing either the probiotic or placebo will be provided by industry partner, Novonesis. The product was selected for its high colony-forming unit per capsule content of *Lactobacillus crispatus*, but also its multistrain formulation due to the synergistic benefits of *Lactobacillus* spp. strains in the gut [64,65]. The formulation is as follows:

- *Lactobacillus* spp. probiotic – two capsules containing total dose of 10 billion colony-forming units (CFU) of the following *Lactobacillus* spp. strains:
  - o 4 billion CFU of L. *crispatus* Lbv 88 (2×10^9 CFU/Capsule)
  - o 4 billion CFU of L. *rhamnosus* Lbv 96 (2×10^9 CFU/Capsule)
  - o 0.8 billion CFU of L. *jensenii* Lbv 116 (0.4×10^9 CFU/Capsule)
  - o 1.2 billion CFU of L. *gasseri* Lbv 150 (0.6×10^9 CFU/Capsule)
- Placebo – two capsules containing maltodextrin and no probiotics, identical in colour, shape, appearance, taste and smell to the active agent

Supplementation will commence at recruitment (~12–16 weeks' gestation). The intervention will be continued until 4–6 weeks postpartum, given the potential ability of probiotics to colonise the neonatal gut and address maternal vaginal dysbiosis in the postnatal period [66,67]. Capsules are stored in the hospital pharmacy and dispensed by the pharmacy team, who verify participant identify, group allocation and keep a dispensing log. Patients are advised to consume two capsules each day, taken in the morning before food, and to store the capsules in the fridge. Apart from the assigned intervention, participants will also be receiving standard of care according to clinical protocols including vaginal progesterone, cerclage or cervical pessary.

2.6. Outcome measures

2.6.1. Primary outcome

The primary outcome will be detectable *Lactobacillus crispatus* in the vaginal microbiome in pregnant women at high risk of spontaneous preterm birth after at least twelve weeks of oral supplementation with a commercial LBV product containing *L. crispatus Lbv88*. The primary outcome measure will be an increase in the percentage of women with detectable *L. crispatus* in the vaginal microbiome by 25 % between baseline and twelve weeks of therapy in the intervention group, compared to placebo.

2.6.2. Pre-specified secondary outcomes and endpoints

Secondary outcomes will be rate of preterm birth related outcomes such as:

Preterm birth rate before 28-, 32-, 34- and 37-weeks' gestation (spontaneous, iatrogenic, total)

Rate of mid-trimester loss

Preterm prelabour rupture of membranes

Cervical length at 24 weeks gestation

Chorioamnionitis, measured by histological examination of placenta

Maternal morbidity

Maternal admission days for preterm labour.

Neonatal outcomes such as birth weight, admission to NICU, length of NICU stay, immediate complications of prematurity at delivery.

Longitudinal impact of the *Lactobacillus* spp. probiotic on the vaginal and gut microbial niches will also be examined with metagenomic and metabolomic analysis and will be reported separately from the primary trial results. The impact of maternal meta-data including dietary and lifestyle factors on the maternal microbiome and metabolome will be examined and reported separately to the primary trial results, given the proposed impact of metrics like diet and breastfeeding on both the maternal and infant gut microbiome [68,69].

2.6.3. Specimen and data collection

After recruitment the patient will remain enrolled in the trial and actively taking the assigned intervention until their postpartum visit (Fig. 1). Throughout pregnancy, the microbial communities will be characterized in the maternal vagina and gut, and changes in community composition will be identified. Microbe-host interactions will be determined by assessing microbe metabolic profiles, in addition to host specific immune responses including cytokine expression. Horizontal transmission of microbes to breast milk, and vertical transmission from mother to neonate will also be explored but will not be included in the primary outcome paper.

The samples collected at each data collection time point are detailed in Fig. 2 and Table 2. Data and specimens will be collected at five timepoints across the pregnancy course: at recruitment, after at least twelve weeks of intervention, at 36 weeks' gestation, at delivery and at 4–6 weeks postpartum (Fig. 2). The only reasons for not obtaining complete information will be that the patient is lost to follow up (delivered in another centre) or declines completion of follow up or specific specimen sets. At present, no long-term follow up is planned but permission will be sought during the recruitment consent process to approach patients for follow up research based on the initial results of the trial.

2.6.4. Compliance

All probiotic and placebo capsules will be provided in one-month supply batches to each trial participant at every visit. Compliance will be evaluated at each study visit, and when attending for each clinical consultation between study visits. Non-compliance will be defined as missing the assigned intervention for more than seven consecutive days. Pharmacy dispensing logs will be examined to determine consumption. Participants will be asked to honestly evaluate their compliance at the conclusion of their follow up.

2.7. Statistical analysis

2.7.1. Sample size

The study is designed as a superiority trial comparing effect of an oral *Lactobacillus* spp. probiotic versus placebo to improve detectable vaginal L. *crispatus*. In a study at the same clinic on a similar spontaneous preterm birth cohort ($n = 37$) compared to control group ($n = 20$), *L. crispatus* was present in the vaginal microbiome of 25.8 % of the high-risk women, compared to 50.9 % of normal risk groups. This has since been echoed in meta-analyses linking presence of L. *crispatus* to term delivery [33,70]. Based on this, we have extrapolated an effect size of 0.25 would result in a change in vaginal microbiome with a clinically meaningful impact. Using these data, a power of 0.8 and significance level of 5 %, a total of 126 women (63 in each arm) are needed to detect a 25 % increase in the relative abundance of L. *crispatus* in the vaginal microbiome of women in the intervention group compared to controls. We estimate an attrition rate of 20 % during the trial (~20 women) and will therefore recruit a total of 150 women but acknowledge this may be higher with the occurrence of mid-trimester loss or early preterm births.

2.7.2. Data analysis

Three populations will be described in statistical analysis: All randomised, intention to treat (all randomised having primary outcome vaginal microbial data) and per-protocol (all randomised who remained compliant and had no protocol violations). The primary outcome, detectable *L. crispatus* in vaginal microbiome, will be assessed by calculating the difference in detectable *L. crispatus* before and after twelve weeks of intervention and comparing these between intervention and placebo. Secondary clinical outcomes will be analysed with descriptive statistics and compared between active agent and placebo control. Software program including R Studio will be used for analysis and data modelling.

2.7.3. Trial management

The day to day running of the trial will be facilitated by the Trial Management Committee (TMC). Members of the TMC include the principal investigator, PhD students, post-doctoral research fellow, clinical lead of the preterm birth clinic and research assistants. The TMC will act on behalf of the sponsor and will ensure all sponsor responsibilities are carried out. The TMC will meet monthly. The Trial Steering Committee (TSC) will consist of principle investigators and co-investigators in addition to external collaborators and public and patient involvement. The role of the TSC is to provide overall supervision for the trial. The TSC will focus on progress of the study, protocol adherence, patient safety, consideration and integration of emerging evidence as the trial progresses. The TSC will meet quarterly.

Each enrolled subject will be assigned a subject identification number that will uniquely identify their record in the study database. Study data will be prospectively recorded on a secure password protected database RedCap, (Research Electronic Data Capture) with patient details anonymised.

2.7.4. Data safety monitoring committee

An independent Data Safety Monitoring Committee (DSMC) will be appointed for this trial. The function of the DMC is to protect the rights and wellbeing of participants throughout their screening, recruitment and follow up stages of the trial. The DMC will receive regular updates on the progress of the trial. At the mid-point of the trial after the primary outcome visit has been performed for sixty three participants, a safety assessment will be performed by the DMC. The purpose of these updates and interim analysis will be to monitor balance between arms to detect selection bias, and to review clinical safety data.

2.7.5. Public and patient involvement (PPI)

In parallel to this trial, a patient advisory council for research into spontaneous preterm birth has been established at the National Maternity Hospital. Members of this advisory council include lay persons with direct experience of spontaneous preterm birth, patient advocates in PPI and clinicians involved in delivering care in prevention of preterm birth. Feedback from the council on aspects of research in preterm birth prevention has been integrated into trial design. A lay person member of this council with direct experience of spontaneous preterm birth and expertise in Public and Patient Involvement has been appointed to the TSC. Trial design has been reviewed by this PPI member of TSC and adapted according to feedback. Updates of trial progress, experience of participants and care provided through the trial will be reviewed by the lay person. Analysis of data and results dissemination will also be discussed with both the TSC PPI member and the patient advisory council to improve accessibility of results to public and patients alike.

2.7.6. Ethics

Institutional ethics approval has been obtained from the National Maternity Hospital Ethics Committee (EC37.2020), and informed written consent will be obtained from study participants at trial enrolment.

3. Discussion

An estimated 15 million babies are born preterm every year, and over 1 million die. Babies that survive can have significant lifelong comorbidities affecting their lives into adulthood. Unsurprisingly, preterm birth can therefore have profound social, economic and medical implications for the infant, their family and wider society. Despite research efforts, the number of babies born preterm each year continues to rise, and innovative preventative methods are desperately needed. One burgeoning area of investigation is the maternal microbiome, a diverse array of microbes and their genetic material, living within different ecological niches in the body. The vaginal microbiome has been implicated in preterm birth, which arises when pathological microbes invade and alter local metabolic processes and stimulate host

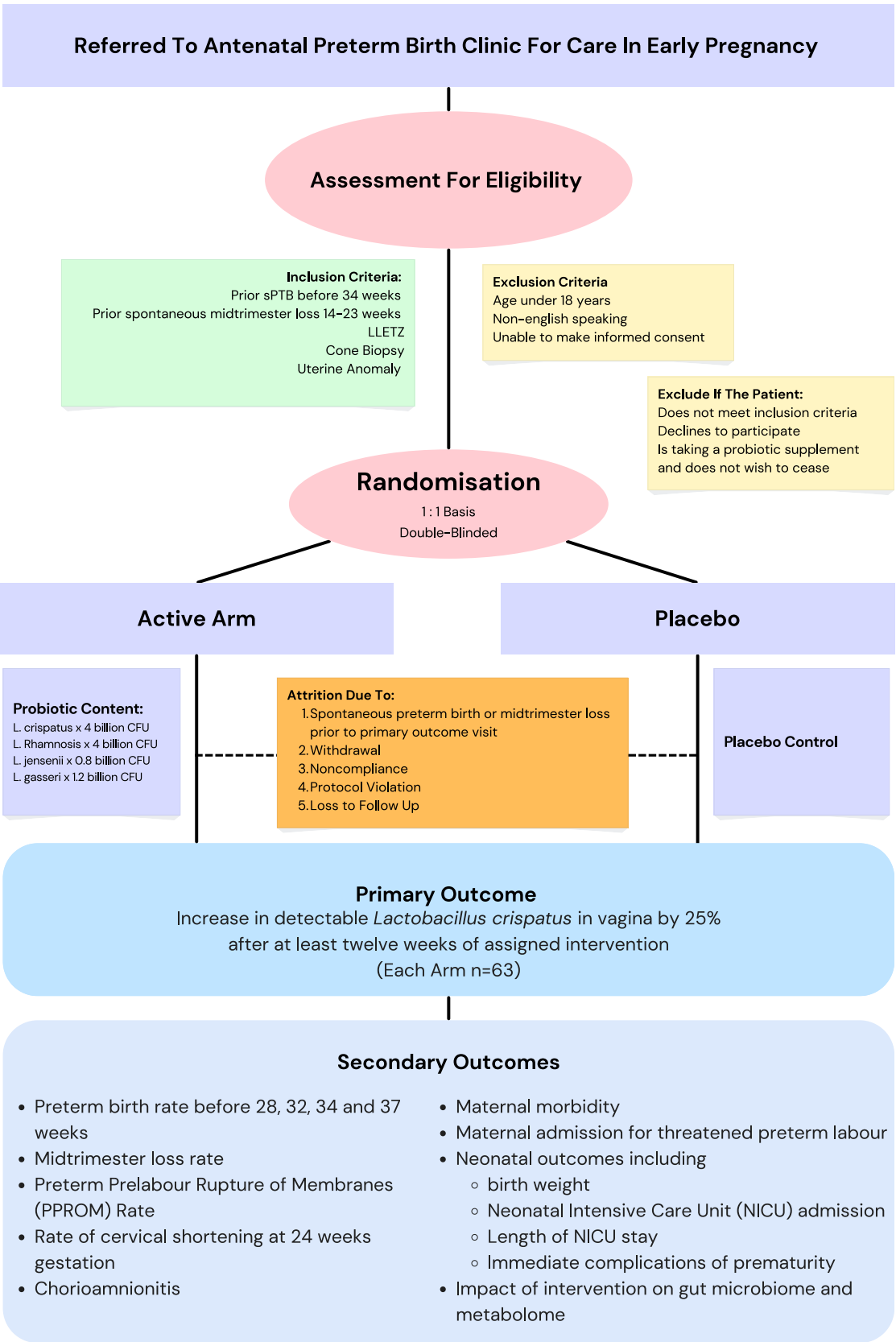

Fig. 1. Consort Flow Diagram for the PrePOP Clinical Trial.

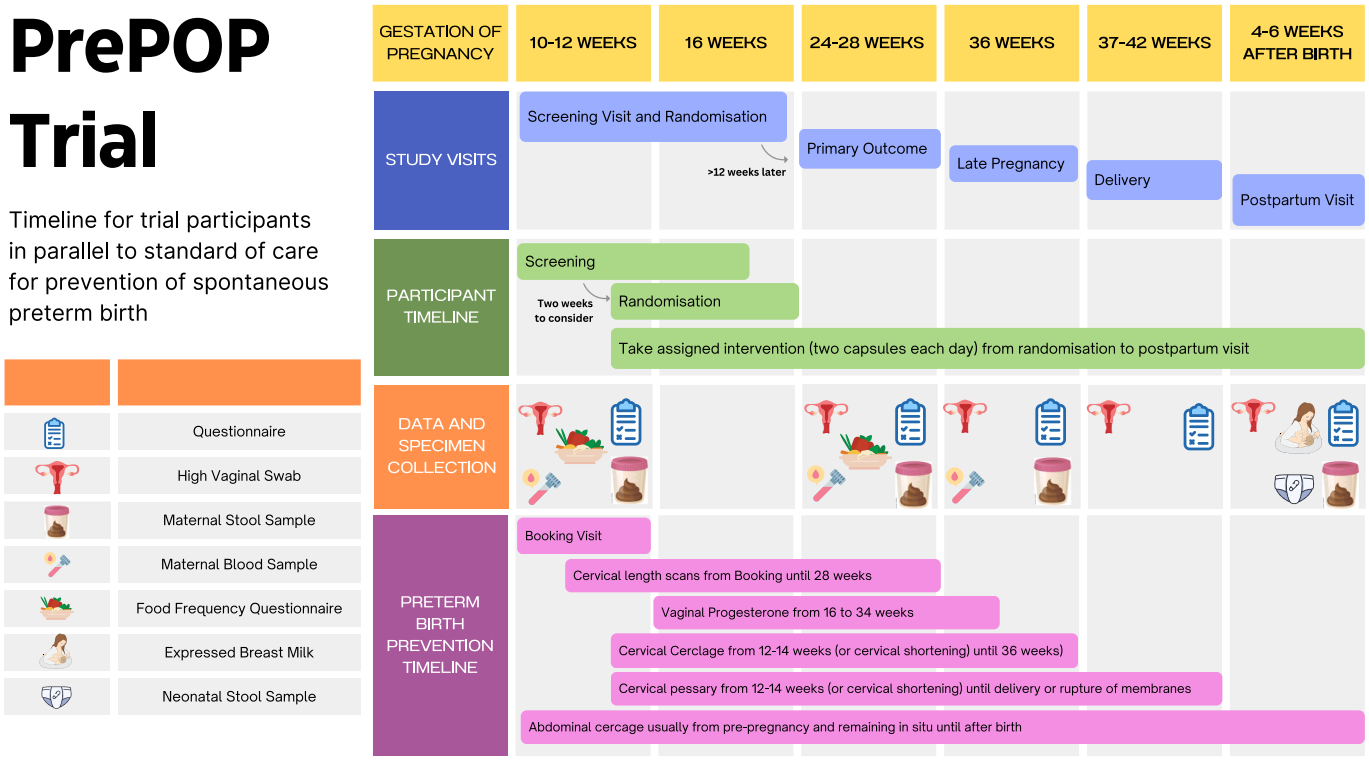

Fig. 2. Trial Timeline for participants, in parallel to standard of care for prevention of spontaneous preterm birth.

immune defenses. Augmentation of the microbiome with orally administered microbes in the form of a probiotic may effective promotes improved vaginal health via *Lactobacillus* spp. dominance in pregnancy. However, their role in preterm birth prevention remains to be seen. We aim to fully investigate the effect of probiotic supplementation on the vaginal microbiome in women at high risk of preterm birth using innovative molecular, immunological and metabolomic profiling techniques. The intervention is as a nutritional probiotic supplement and not a regulated product to prevent preterm birth. The study will assess the impact of the probiotic on vaginal microbiome, rather than on preterm birth rates.

Using prior experience with randomised controlled trials in pregnancy at our centre including the ROLO study, the Test study, the PEARS trial, and the MicrobeMom trial [34,71–73], analysis of barriers and criteria for success was conducted by the TSC and TMC. The main issues were: (i) attrition due to spontaneous preterm delivery and (ii) non-compliance. An attrition rate of 20 % was used for sample size calculation based on previous trial experience at this centre. Given the patient cohort is at high risk of delivery prior to primary outcome sample set, attrition will be based on rates of mid-trimester loss and spontaneous preterm birth within both recruitment arms. The rate of preterm delivery <34 weeks in this clinic is 4–7 % and mid-trimester loss rate is 1–2 % [60,74], which means attrition may be an additional 5–10 % higher than this 20 %. To combat this, close monitoring of preterm birth rate will be conducted throughout, and the trial will continue recruiting to secure 126 primary outcome visits, the threshold number to power the primary endpoint. To minimise noncompliance, every effort will be made by the research team to keep study visits and sample sets coordinated with clinical care. Study visits will be captured when a clinical consultation is scheduled. Vaginal swabs will be coordinated to occur prior to commencement of transvaginal scan to protect patient dignity and maintain convenience and comfort. Bloods will be taken during blood draws for other clinical reasons, tied into standard antenatal care such as anaemia or gestational diabetes screening. Integration of the clinical team to the Trial Management Committee is core to delivering the trial structure conveniently for this vulnerable patient cohort. Advice from the NMH Preterm Birth Advisory Committee and PPI member of the TSC has been sought to streamline screening recruitment and follow-up of participants throughout the trial, and approach of the research team will be structured around this feedback.

3.1. Dissemination

The preterm birth advisory council will be consulted to review accessibility and simplicity of results. Participants of this trial will be invited to provide feedback on trial results including results content, presentation and infographics, and will be invited to contribute to results dissemination. The results of the trial will be published in an established peer-reviewed journal, with at least one publication from the main results. Dissemination of results to current and future patients, public and academic audiences will take place via social media, hospital and sponsor websites, patient support platforms and academic conferences. Collaborating investigators, participants and advisory council members will be central to dissemination of trial results.

4. Conclusion

The purpose of the double blinded clinical controlled trial is to investigate the efficacy of an oral probiotic containing *L. crispatus* compared to placebo for improving detectable vaginal *L. crispatus* in pregnancies at high-risk of spontaneous preterm birth. The oral *Lactobacillus* spp. probiotic used is high in content of *L. crispatus,* more than other agents previously studied outside of pregnancy. Oral administration is a key element for this intervention to characterise the response of the gut microbiome and metabolome and its role in depletion of *L. crispatus* in the vagina and in the aetiology of spontaneous preterm birth. The primary outcome for the trial has deliberately been selected to be vaginal *L. crispatus* rather than preterm birth rate. If a positive effect is seen, a larger trial with spontaneous preterm birth as the primary outcome will follow. Preterm birth is a considerable burden on the individual, family and society. Interventions to reduce preterm birth hold potential to reduce this impact and to translate into considerable

Table 2
Data and Specimens collected for each participant at recruitment and through study follow up.

Data or Specimen Media	Specific data collected	Timepoint collected
Questionnaires	Demography, Medical history, Obstetric history	At recruitment visit
	Food Frequency Questionnaires including prebiotic and probiotic foods	At recruitment and primary outcome visits (12–16 and 24–28 weeks of pregnancy)
	Lifestyle including patterns of sleep and exercise, and mental health assessment	At recruitment and primary outcome visits (12–16 and 24–28 weeks of pregnancy)
	Intervention acceptability	Last study visit at 4–6 weeks postpartum
	Self-reported medications list	At each study visit
	Adverse effects screening	At each study visit
	Pregnancy outcome data including maternal and neonatal outcomes	At birth and at 4–6 weeks postpartum
Cervical length	Measured using transvaginal ultrasound	At Recruitment and at 24 weeks gestation
Maternal Pregnancy Samples	Vaginal mucosa swab (microbiome / immune & metabolic profile assessment)	At randomisation, primary outcome visit and at 36-weeks' gestation
	Blood sample (immune & metabolic profile assessment)	At randomisation, primary outcome visit and at 36-weeks' gestation
	Stool sample (microbiome and metabolome assessment)	At randomisation, primary outcome visit and at 36-weeks' gestation
	Vaginal pH	At randomisation, primary outcome visit and at 36-weeks' gestation
Maternal & Neonatal Delivery Samples	Vaginal mucosa swab (microbiome / immune & metabolic profile assessment)	At birth
Maternal and Neonatal Postpartum Samples	Vaginal mucosa swab (microbiome, immune & metabolic profile assessment)	At 4–6 weeks postpartum
	Maternal breast milk (microbiome assessment) if breastfeeding	At 4–6 weeks postpartum
	Neonatal stool (microbiome and metabolome assessment)	At 4–6 weeks postpartum
	Maternal stool sample (microbiome and metabolome assessment)	At 4–6 weeks postpartum

economic savings for health systems.

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CRediT authorship contribution statement

Gillian A. Corbett: Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Siobhan Corcoran:** Writing – review & editing, Resources, Project administration, Methodology, Investigation. **Conor Feehily:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Benedetta Soldati:** Writing – review & editing, Project administration, Investigation. **Anthony Rafferty:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition. **David A. MacIntyre:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Paul D. Cotter:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation. **Fionnuala M. McAuliffe:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Data availability

No data was used for the research described in the article.

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