ProB Trial: Probiotics and the Prevention of Preterm Labour; A Randomised Controlled Trial Protocol

Corresponding Author:
Dr. Mohammad Othman, Consultant Obstetrician and Gynaecologist, School of Reproductive and Developmental Medicine, University of Liverpool, 84 Bradfield Road, M32 9LE - United Kingdom

Submitting Author:
Dr. Mohammad Othman, Consultant Obstetrician and Gynaecologist, School of Reproductive and Developmental Medicine, University of Liverpool, 84 Bradfield Road, M32 9LE - United Kingdom

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Author(s): Othman M

Background

Preterm birth defined as birth occurring after the gestational age of viability (23 weeks, 500 grams weight) and before 37 completed weeks (259 days) of pregnancy, is one of the most important problems in medicine today. Preterm birth is the single largest cause of mortality and morbidity for newborns. It accounts for 5% to 11% of births in the world but is responsible for 28% of all deaths within 28 days of birth and 50% of childhood neurological disabilities (1, 2).

Infection and preterm birth:
In the last 20 years infection has emerged as an important cause of preterm labour and delivery leading to more than 50% of all preterm deliveries world-wide (1, 3, 4, 5, 6, 7, 8, 9). In this context, the organisms involved may not be necessarily pathogenic; a change in vaginal flora may be enough to trigger the sequence of events leading to a preterm birth (1, 3, 8, 9, 10, 11, 12, 13). Changes in vaginal flora can increase the risk of adverse pregnancy outcomes through a variety of mechanisms. Metalloproteolytic enzymes and other bioactive microbial products act directly on cervical collagen leading to premature cervical shortening and ripening (6, 11, 14). Bacterial products can also weaken the fetal membranes and promote preterm premature rupture of the membranes (11). Pathologic microorganisms trigger the innate immune response to produce both prostaglandins E2 and F2a and cytokines such as Tumour Necrosis Factor alpha (TNF-a), Interleukin (IL1b, IL6, IL8) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) (4, 6, 15, 16, 17). Prostaglandins are potent stimulator of uterine contractions (16) whilst cytokines may lead to direct fetal tissue damage (e.g. fetal brain or lung) or may orchestrate preterm labour (8, 11).

Vaginal ecosystem:
Lactobacillus species, including Lactobacillus acidophilus, L.fermentum, L. crispatus, and L. jensenii are the dominant bacteria in the normal vaginal flora. Lactobacilli are gram positive, catalase negative, non-sporing rods. They ferment glycogen produced by the vaginal epithelium and this reaction leads to the formation of hydrogen peroxide. The presence of hydrogen peroxide producing strains of Lactobacilli in the vaginal flora is associated with a reduced incidence of abnormal flora including bacterial vaginosis (18, 19, 20, 21). The incidence of bacterial vaginosis in women without Lactobacilli is reported to be 56% compared with 32% in women colonized by non-hydrogen peroxide producing strains and only 4% in women who have hydrogen peroxide producing strains of Lactobacilli (17). It is postulated that reduced levels of lactobacilli allow the populations of other potentially pathogenic microorganisms to grow and trigger the inflammatory processes outlined above (7, 16, 18, 21, 22).

Probiotics and preterm labour:
Probiotics are defined as live microorganisms which, when administered in an adequate amount, confer a health benefit on the host (7, 18, 23, 24). They stimulate an immunomodulation process that includes the induction of mucus production, macrophage activation by lactobacilli signalling, stimulation of secretory IgA and neutrophils, inhibition of release of inflammatory cytokines, and stimulation of elevated peripheral immunoglobulins. It has also been shown that probiotics may modulate cytokine release resulting in large amounts of IL-10 and low levels of IL-12p70, IL-5 and IL-13 with the main source of IL-10 attributable to CD14+ (25, 26, 27, 28). Smits 2005 (45) has suggested that the beneficial effects of probiotics in the treatment of inflammatory diseases (such as Crohn’s) may be due to the probiotic cells targeting the C-type lactic DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN). Probiotics have been shown to displace and kill pathogens and modulate the immune response by interfering with the inflammatory cascade that leads to preterm labour and delivery (7, 25). The administration of Lactobacilli by mouth or intravaginally, or both have been shown to be safe and effective in reducing or treating urogenital infections in non-pregnant populations (7, 29, 30, 31)

Clinical trials of probiotics in pregnancy:
A Cochrane systematic review (32) was conducted and identified seven randomised clinical trials using probiotics for the prevention of preterm labour in women with bacterial vaginosis. One trial started in...
February 2005 and was terminated in 2007 because the tightly defined inclusion criteria were making recruitment very slow. Another trial started in 2006 and was terminated in 2009 because of limitation of funding. One trial with 381 women recruited was excluded because there were no data on clinical outcomes in the published article; we tried to contact the author with no response. The second trial was excluded because they used prebiotics not probiotics in the trial. Of the three trials included in the review, one enrolled 24 women after 34 weeks of pregnancy using oral fermented milk as probiotic, while the other study with 64 participants utilised commercially available yoghurt to be used vaginally by women diagnosed with bacterial vaginosis in early pregnancy. Third study enrolled 256 women in Finland. Participants were randomised into two experimental groups and one placebo control group. One of the experimental groups received placebo and dietary counselling while the other experimental group received probiotics (Lactobacillus rhamnosus GG and Bi-fidobacterium lactis Bb12 once daily) from the first trimester of pregnancy to the end of breastfeeding and dietary counselling. Effects on very preterm birth (less than 32 weeks) (risk ratio (RR) 0.65; 95% confidence interval (CI) 0.03 to 15.88) and preterm birth (less than 37 weeks) (RR 3.95; 95% CI 0.36 to 42.91) showed very wide CIs and no effect of statistical significance.

The trial reports focused on laboratory evidence of infection (lactobacillus count, type of abnormal vaginal flora, vaginal fluid pH, presence of clue cells in vaginal wet smear, number of leukocytes) rather than clinical signs of infection or preterm labour. Reduction in genital infection was therefore the only prespecified clinical outcome for which the data were available for both studies with pooled results showing 81% reduction in genital infection with the use of probiotics (Risk Ratio (RR) 0.19; 95% Confidence Interval (CI) 0.08, 0.48). We contacted the authors to provide us with the data on any clinical pregnancy related outcomes, but there were no other data than the published. Clinical pregnancy related outcomes include preterm birth before 28, 34, 37 weeks, preterm labour requiring hospital admission, neonatal mortality and severe morbidity. The preceding outcomes were going to help us to study the impact of probiotics on preterm labour and its complications.

**Rationale for further studies of probiotics in preterm labour:**

Probiotics have been found to be an effective treatment for Crohn’s disease. In Crohn’s disease, enhanced mucosa permeability may play a pivotal role in causing and perpetuating intestinal inflammation. Enhanced mucosa permeability may play a pivotal role in causing and perpetuating intestinal inflammation. Treatment for Crohn’s disease. In Crohn’s disease, probiotics have been found to be an effective treatment for preterm labour:

**Aims of the study**

This is feasibility, ‘proof of principle’ study with the following aims:

1. To confirm the association between pre-randomisation cervical macrophage count and risk of preterm birth
2. To evaluate whether the combination of oral and vaginal probiotics is acceptable intervention for pregnant women with history of preterm birth
3. To assess the feasibility of consenting procedures, randomisation and data collection in this group of women
4. To determine if probiotics can influence the cervical
macrophage count
5. To determine if post-probiotic cervical macrophage count is associated with timing of spontaneous delivery.

**Methodology**

**Design:** Hospital open label randomised controlled trial.

**Method of randomisation:**
Women will be randomised to one of the treatment groups using central randomisation service. There are no plans to stratify women by their baseline characteristics.

**Participants:** 50

**Inclusion criteria:**
1. Pregnant women between 12-16 weeks gestation
2. Certain gestational age confirmed by ultrasound
3. History of at least one spontaneous previous birth between 14 and 32 completed weeks
4. No pathogenic microorganisms on high vaginal and cervical swabs

**Exclusion criteria:**
1. History of cervical cerclage or history of cervical weakness as defined by need for elective cervical suture at before 16 gestation
2. Uterine congenital anomalies
3. Fetal congenital anomalies
4. Fetal death
5. Ethanol abuse
6. Drug addiction
7. Younger than 16 years old
8. Planned for antenatal care or delivery elsewhere
9. Regular user of probiotics (e.g. yogurt) unwilling to stop during the course of the trial

**Clinical Protocol:**
In the normal routine the booking visit, at 12 to 15+6 weeks all patients with history of previous delivery between 14 and 32 weeks will be assessed in the Preterm Labour Clinic. Visualization of the cervix, HVS, Endocervical swab, Trichomonas Vaginalis swab, Chlamydia swab, Ureaplasma and Mycoplasma swab will be carried out. After the initial assessment, patients will be informed about the trial and information sheet will be given to them. At the next visit between16 weeks and 18 weeks all patients will have trans-vaginal scan and the results of the swabs from the first visit will be reviewed. Patients diagnosed with an infection will be treated and will not be eligible to participate in this trial. Women free from any infection who agree to take part in this trial will be consented, have a vaginal swab taken for cytokines and Cytobrush for leukocytes and will be randomised to either the treatment group or control group. Both the experimental and control group will be managed as shown in Illustration 1.

**Intervention:**
Women randomised to the treatment arm will receive sufficient vaginal probiotic pessary and oral probiotic tablet to take as described once daily for a period of 6 weeks starting at 16-18 weeks gestation. Women in the control group will be managed as per standard clinical protocol. Any complications in both groups will be managed at the discretion of the attending clinicians.

**Consent:**
Potentially eligible women will be given written information about the study after initial assessment. Eligibility criteria will be confirmed 1-2 weeks later when written consent form will be obtained.

**Maternal outcomes:**
1. Neutrophil count (measured by ten fields ×400 magnification using a 10-mm×10-mm graticule Covering an area of 0.0625mm2 from the cervical swabs) at 24 and 28 weeks.
2. Cervical mucus inflammatory cytokines –IL6, IL8, TNF-α at 24 weeks.
3. Cervical mucous and vaginal IgA concentrations at 24 weeks.
4. Gestational age at delivery.
5. Preterm birth before 28 and 34 complete weeks of pregnancy.
6. Threatened preterm labour requiring administration of antenatal steroids and/or tocolysis.
8. Vaginal infection before birth confirmed by microbiological findings.
9. Incidence of chorioamnionitis on placental pathology
11. Assessment of compliance using the compliance diary.
12. Woman’s experiences using the compliance diary.

**Neonatal outcomes:**
1. Incidence of superficial colonisation with Candida species on admission to the neonatal unit
2. Incidence of superficial colonisation with Candida
3. Incidence of early onset neonatal sepsis (CRP ≥ 10mg/l within 72 hours of birth, ± positive blood cultures)
4. Incidence of invasive Candidal disease
5. Survival to discharge

Blinding:
Given the nature of the intervention (open label medications), it will not be possible to blind clinicians or women when clinical outcomes are assessed (including trans vaginal scan). However, laboratory based outcomes (neutrophil count, cytokines and IgA concentrations) will be assessed blindly and the specimens will be labelled using unique trial identifier only.

Data analysis:
The analysis will be descriptive with the aim of estimating the parameters for sample size calculation in a larger study. Data of patient drop out will not be included in the analysis.

Trial end point:
Trial will end by the birth of the last participating woman

Feasibility/ Sample size:
Normally, the high-risk preterm labour clinic at reputable specialised Hospital accepts 50 new referrals per annum at least who are eligible and that around two third will consent. Therefore, it is expected to recruit 50 women in 18 months and this will be the main target of this feasibility study. Twenty five women in each group will be sufficient to test feasibility and estimate parameters for the main trial.

Safety considerations:

Most of the current probiotic strains occur as normal commensal of the mammalian flora or are present in fermented food products already consumed for generations worldwide (Illustration 2). On this basis, the WHO and FAO concluded that probiotics can be generally recognized as safe. However, the organizations also state that probiotics may theoretically be responsible for three types of side effects (1) excessive immune stimulation in susceptible individuals, (2) systemic infections, and (3) deleterious metabolic activities (35). Adverse effects were mainly observed in immunocompromised patients, but vigilance regarding the detection of possible rare cases of infection due to probiotics should be exercised (36, 37, 46). No immunological side effect of any probiotic has been reported in man. However when administered parenterally cell wall components such as peptidoglycans from different gram-positive bacteria, including lactobacilli, can induce side-effects such as fever and arthritis (28, 37).

There is no evidence that ingested probiotic pose any risk of infection greater than that associated with commensal strains. In quantitative terms, the existing data suggest that the risk of bacteraemia is one case per million individuals. It is virtually impossible to propose a risk of death because of the common association of infections involving lactobacilli with fatal underlying conditions or the presence of polymicrobial infections. However, the risk is unequivocally in the “negligible” range (38, 39, 47). Immunocompromised patients generally are more vulnerable to infection with pathogens and have a higher incidence of opportunistic infections. However, there is no published evidence that consumption of probiotics increases the risk of opportunistic infection among such individuals (28, 38, 40, 47). In addition, 2 clinical studies have been conducted to assess the safety of probiotics in small groups of specific immunocompromised patients (e.g. patients with HIV infection), and the findings of these studies support the safety of probiotics consumed by such groups (41, 42). The risk of probiotic lactobacilli passage in blood, eventually by translocation, is important to determine. Bacterial translocation is defined as the passage of microorganisms from the gastrointestinal to extra-intestinal sites such as the mesenteric lymph nodes, liver, spleen and bloodstream. Indigenous bacteria are continuously translocating in low numbers but are rapidly killed in the lymphoid organs. Bacterial translocation is a major cause of severe infection in immunosuppressed, trauma and post-surgical patients. This may result from three mechanisms: intestinal bacterial overgrowth, increased permeability or damage of the intestinal mucosal barrier, and immunodeficiency (43, 44, 47). Rare cases of infection, including septicaemia and endocarditis caused by lactobacilli, bifidobacteria or other lactic acid bacteria have been reported with the incidence of less than 0.002% (38, 44). The survival of ingested probiotics at different levels of the gastrointestinal tract differs between strains. Some strains are rapidly killed in the stomach while others, such as Lactobacillus acidophilus, can pass through the entire gut at very high concentrations. Milk as a vehicle protects probiotics against gastric conditions. Excessive degradation of the intestinal mucus by probiotics may theoretically be detrimental. Some endogenous bacteria, including lactobacilli, and some strains of...
bacteroides have the ability to degrade mucus. No mucus degradation has been observed in vitro or in gnotobiotic rats to date (36, 37). The only observed metabolic side effect is gastric upset and this still uncommon side effect of oral probiotics with an incidence of 2%(37, 44).

From the above, adverse effects which we will be looking for are:
1. Local side effects
2. Allergy
3. Bacteraemia
4. Septicaemia
5. Endocarditis
6. Gastric upset.

Altering the vaginal flora could impact on the baby by modifying ascending infection or by modifying microbial colonisation of the baby during vaginal delivery. We expect probiotics to protect the baby. However, the predefined neonatal outcomes will also serve as makers of neonatal safety.

References

22. Wilks M, Wiggins R, Whiley A, Hennessy E,


42. Wolf BW, Wheeler KB, Ataya DG, Garleb KA. Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus. Food Chemical Toxicology 1998; 36: 1085–94.


Illustrations

Illustration 1

Illustration 1: trial flow chart
Illustration 2

Illustration 2: Probiotic micro-organisms

<table>
<thead>
<tr>
<th>Lactobacillus strains</th>
<th>Bifidobacterium species</th>
<th>Other strains</th>
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<tbody>
<tr>
<td>L. casei</td>
<td>B. bifidum</td>
<td>S. boulardii</td>
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<tr>
<td>L. rhamnosus</td>
<td>B. breve</td>
<td>S. thermophilus</td>
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<tr>
<td>L. acidophilus</td>
<td>B. infantis</td>
<td>P. acidilacti</td>
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<tr>
<td>L. bulgaricus</td>
<td>B. lactis</td>
<td>L. diacetylactis</td>
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<tr>
<td>L. fermentum</td>
<td>B. longum</td>
<td>E. faecium</td>
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<tr>
<td>L. johnsonii</td>
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<td>B. subtilis</td>
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<td>L. lactis</td>
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<td>O. formigenes</td>
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<td>L. paracasei</td>
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<td>L. plantarum</td>
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<td>L. reuteri</td>
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<td>L. salivarius</td>
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Illustration 3: Probiotic microorganisms
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