Safety, tolerability and acceptability of Lactobacillus crispatus CTV-05 (LACTIN-V) in pregnant women at high-risk of preterm birth

Erna Bayar
Imperial College London
David A MacIntyre
Imperial College London
Lynne Sykes
Imperial College London
Katie Mountain
Imperial College London
Yun Lee
Imperial College London
Thomas P Parks
Osel Inc
Peter P Lee
Osel Inc
Phillip R Bennett
p.bennett@imperial.ac.uk

Imperial College London

Research Article

Keywords: Preterm birth, vaginal microbiome, safety, acceptability, Lactobacillus crispatus, live vaginal biotherapeutics, LACTIN-V

Posted Date: March 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1418870/v1

Additional Declarations: Competing interest reported. T.P.P. and P.P.L. are respectively Founder and Executive Chairman and Director of Product Development of Osel Inc, who are developing live biotherapeutics for women's health and are the developers of LACTIN-V. T.P.P. and P.P.L. and D.A.M. and P.R.B. are named inventors on an application by Imperial College Innovations Limited for patent protection for the use of LACTIN-V for prevention of preterm birth.

Version of Record: A version of this preprint was published at Beneficial Microbes on February 23rd, 2023. See the published version at https://doi.org/10.3920/BM2022.0084.

Abstract

Background

The vaginal microbiota is a determinant for the risk of preterm birth (PTB). Dominance of the vaginal niche by *Lactobacillus crispatus* associates with term delivery. Vaginal administration of *Lactobacillus crispatus* as a live biotherapeutic in non-pregnant women leads to colonisation and reduced recurrence of bacterial vaginosis and urinary tract infections. This is a first in-pregnancy observational clinical study of live vaginal biotherapeutics (*Lactobacillus crispatus* CTV-05 (LACTIN-V, Osel)) in a cohort of pregnant women at high-risk of PTB. The primary aim was to explore safety, tolerability and acceptability of LACTIN-V in pregnancy.

Methods

Pregnant women at high-risk of PTB were offered a course of LACTIN-V at 14 weeks gestation for 5 consecutive days followed by weekly administration for 6 weeks. Participants were followed up at 15, 18-, 20-, 28- and 36-weeks' gestation and at delivery for assessment of adverse events, compliance, and tolerance. After study completion, women completed a questionnaire to gauge experience and acceptability. Assessment of adverse events was performed in those women completing LACTIN-V therapy and those who commenced LACTIN-V but later withdrew from the study.

Results

A total of 73 women were recruited, of whom 8 withdrew, leaving a final cohort size of 61. Overall patient reported compliance to the full course was high (56/60, 93%). Solicited adverse events were reported in 13 women (19%) including changes in vaginal discharge, odour, colour or consistency of urine, itching and vaginal bleeding. One unsolicited adverse event was reported as haematuria at 38 weeks gestation, but was not judged to be related to LACTIN-V and no serious adverse events occurred. One mild adverse event led to withdrawal from the study. A total of 31 women completed an experience and acceptability questionnaire. Women found LACTIN-V easy and comfortable to use and the majority (30/31, 97%) stated they would use LACTIN-V in future pregnancies. Eight women (8/31, 26%) reported that the schedule of use was difficult to remember. The rate of early PTB <34 weeks in this cohort was 3.3% compared to 7% in a historical cohort of 2190 women at similar background PTB risk.

Conclusions

With satisfactory uptake and good compliance, we demonstrate that LACTIN-V is safe and well accepted in pregnancy, with high tolerability. Further studies are needed to assess rates of *Lactobacillus crispatus* CTV-05 colonisation and clinical efficacy.

Trial Registration

The trial is registered at www.clinicaltrials.gov (NCT03992534). Date of registration was June 20, 2019

Background

The vaginal microbiota plays an important role in reproductive health and disease (1–3). In pregnancy, vaginal communities deplete in *Lactobacillus spp.* are associated with increased risk of preterm prelabour rupture of the fetal membranes (PPROM) (4, 5) and preterm birth (PTB) (6, 7). Conversely lactobacillus dominance, particularly by *Lactobacillus crispatus*, has been reported to be protective of PTB (7–9).

Depletion of *Lactobacillus* species and overgrowth of anaerobic bacteria is characteristic of bacterial vaginosis (BV). Randomised trials of antibiotics in BV during pregnancy to prevent PTB have had conflicting outcomes (10–12). A Cochrane review concluded there is insufficient evidence to support the screening and treatment of BV for the reduction of PTB (13). Moreover, harm with antenatal antibiotic use has been described. In one trial in women with previous PTB, clindamycin increased neonatal infectious morbidity and did not reduce PTB (14). Erythromycin when prescribed for the management of PPROM, has also been associated with chorioamnionitis, funisitis and early onset neonatal sepsis through its depletion of *Lactobacillus* spp. (5).

Live biotherapeutics, defined as live organisms designed and developed to treat, cure, or prevent a disease or condition in humans (15), have been explored as an alternative to antimicrobial compounds. Live biotherapeutics are differentiated from probiotics, which are regulated as dietary supplements and cannot make claims to treat or prevent disease (16). Evidence that probiotic use in pregnancy can improve outcomes is highly variable. A large cohort study of > 70,000 pregnant women reported that oral intake of lactobacilli containing probiotic milk was significantly associated with a lower risk of PTB (17). However, a Cochrane review of 12 studies examining oral probiotics in women at low-risk of poor pregnancy outcomes concluded that there was low quality evidence to suggest benefit from maternal probiotic use (18). Although earlier studies using culture-based approaches suggested that oral probiotics might increase vaginal lactobacillus dominance (19), subsequent bacterial DNA sequencing based studies indicate that lactobacilli administered orally do not colonise the vagina (20–22). To date, no clinical trials have assessed vaginally administered live biotherapeutics in pregnancy. *Lactobacillus crispatus* CTV-05 (LACTIN-V, Osel) is a live vaginal biotherapeutic containing a naturally occurring strain of *Lactobacillus crispatus.* Vaginal administration of LACTIN-V has shown vaginal colonisation of *Lactobacillus crispatus* CTV-05 and reduced recurrence of BV and urinary tract infections (UTIs) in non-pregnant women (23, 24).

In this study, we offered pregnant women at high-risk of PTB a course of LACTIN-V with the primary objective to explore its safety, tolerability and acceptability of use. The secondary objective was to observe the rate of spontaneous PTB in pregnant women using vaginal LACTIN-V.

Methods

Study design

This was an feasibility clinical study to access, safety, tolerance and acceptability of LACTIN-V use in a cohort of pregnant women at risk of preterm birth. The study was conducted between September 2019 and April 2021 at Queen Charlotte's and Chelsea Hospital, Imperial Healthcare NHS Trust. The study was performed in accordance with the Declaration of Helsinki Ethical approval was granted by the London-Surrey Borders Research Ethics Committee (IRAS 262987). Health Research Authority and Health and Care Research Wales Approval was given (REC reference 19/LO/1018). The trial is registered at www.clinicaltrials.gov (NCT03992534), registration date was 20 June 2019.

Participants

Participants referred to the Prematurity Prevention Service at Imperial Healthcare NHS Trust because of their increased risk of PTB were approached for study eligibility. Inclusion criteria were; a history of PTB, PPROM, large loop excision of the transformation zone of the cervix (LLETZ), cone biopsy, mid-trimester loss (>16 weeks) (MTL), short cervix (<25mm) in a previous pregnancy, Caesarean section at full cervical dilatation (FDCS), trachelectomy, previous cervical tear or congenital uterine anomaly; all of which are well-established risk factors for PTB (25-27). Women met the inclusion criteria if they were aged ≥18, between 11+0 and 15+6 weeks gestation and able to provide informed consent. Women living with human immunodeficiency virus (HIV) and those receiving antibiotics within 1 week of recruitment were excluded (28).

Study procedure

Women meeting the eligibility criteria were consented and enrolled during their first appointment at the prematurity prevention clinic. During this consultation, participants were instructed on the administration and timing of LACTIN-V use. Each pre-filled applicator contained *Lactobacillus crispatus* CTV-05 powder at a dose of 2×10^9 colony-forming units (CFU), preserved with inactive ingredients. The intervention included a total of 11 LACTIN-V doses, divided into the loading phase followed by a maintenance phase (Figure 1). The loading dose consisted of 5 consecutive daily doses of LACTIN-V and the maintenance dose consisted of 6 weekly doses of LACTIN-V.

Consenting women were provided with a carton containing 11 vaginal applicators. Participants were advised to store the product in a refrigerator and asked to abstain from sexual intercourse for 48 hours before each follow up visit and for the first 14 days of the study. A paper diary was given to each participant to record sexual activity, adverse events and any concomitant antibiotic use.

Participants were monitored via the prematurity prevention clinic with follow up performed at at 1, 4, 6, 14 and 22 weeks after recruitment corresponding to 15, 18-, 20-, 28- and 36- weeks gestation (Figure 1). Postnatally, participants were offered a questionnaire for the evaluation of LACTIN-V acceptability.

Assessed Outcomes

Women were asked to record solicited adverse events from the point of commencing LACTIN-V on their paper diary. At each follow up appointment, participants were specifically asked to describe any other, unsolicited adverse events. All solicited adverse events were considered to be related to LACTIN-V use. Clinical staff assessed unsolicited adverse events to determine the likelihood of their association with LACTIN-V. Adverse event severity was graded in accordance to the Division of AIDs of the NIH (29). As we studied a high-risk population, miscarriage, MTL, PPROM, chorioamnionitis and PTB were not deemed to be adverse outcomes, however we did assess the rates of PTB in the study cohort, compared to rates in a historical study cohort, to ensure that there was not an adverse effect upon preterm birth rates. The historical comparison cohort consisted of 2190 women seen within the prematurity prevention service at Queen Charlotte's Hospital over the preceding two decades for whom a full risk profile for PTB and pregnancy outcomes were available. Fetal adverse events were assessed by reviewing clinical notes for the incidence of fetal malformations, intra-uterine death and neonatal death. Tolerability was evaluated by the number of participants discontinuing LACTIN-V due to adverse events. Acceptability was determined by uptake and compliance and further explored through the exit questionnaire asking participants to rank ease of product use, storage, comfort, convenience, partner acceptance and future use.

Statistics

For the assessment of demographics, *P* values were calculated by Chi-squared testing, T-test or Mann-Whitney U, depending on distribution. A Chi-squared test with Yate's correction was performed for comparing the incidence of miscarriage, MTL, PTB, PPROM and chorioamnionitis between participants receiving LACTIN-V and the historical pregnancy control cohort. All statistical analyses were performed using the Graph Pad (version 9.3.1).

Results

A total of 123 women met the inclusion criteria and were approached for study participation. Of these, 73 (59%) accepted involvement and 50 (41%) declined (Fig. 2). Four women were lost to follow up as antenatal care was transferred elsewhere, or they chose to discontinue their care within the prematurity prevention service. A total of 8 women withdrew from the study. Seven withdrawing participants had commenced the LACTIN-V loading dose and one withdrawing participant completed both the loading and maintenance course of LACTIN-V, however wished to cease hospital attendance during the Covid-19 pandemic. The demographics of the included cohort (n = 61) are presented alongside the demographics of women from our historical control cohort which included women seen in the prematurity prevention service between 2002 and 2020 (Table 1).

Table 1

Demographic characteristics of study participants and clinical risk factor for PTB

Demographics are reported for participants receiving Lactin-V (n = 61) and for a historical control cohort (n = 2190) including women at similar risk of PTB. Data for age and BMI are presented as median (interquartile range). Abbreviations: IQR (interquartile range), BMI (body mass index), PTB (preterm birth), MTL (mid-trimester loss). Other: previous cervical cerclage, incidental cervical shortening, Caesarean-section at full cervical dilatation and congenital uterine anomalies *P* Values: T-test/Mann-Whitney U (depending on distribution), Chi-square with Yates' correction

	Lactin-V Cohort (n = 61)	**Historical Pregnancy Cohort (n = 2190)**	***P* value**
Age (IQR)	34 (3)	34 (7)	0.06
BMI (IQR)	26 (6)	24 (6)	0.89
Ethnicity (%)			
White	37 (61)	1188 (54)	0.39
Black	8 (13)	485 (22)	0.13
Asian	10 (16)	312 (14)	0.77
Other	6 (10)	205 (9)	0.92
Primary Risk Factor (%)			
PTB or MTL	29 (48)	1300 (59)	0.85
Cervical treatment	27 (44)	703 (33)	0.06
Other	5 (8)	187 (8)	0.89

Solicited and unsolicited maternal adverse events were assessed for all women commencing LACTIN-V, including the 61 participants completing the study, as well as the 8 participants who commenced LACTIN-V but withdrew (n = 69) (Table 2). There were 13 reported solicited adverse events (19%). For those completing the full course of LACTIN-V, 5 women described resolution of adverse events upon completion of the loading dose. All others reported self-resolving adverse events following completion of the maintenance dose. One unsolicited adverse event was reported in the form of haematuria at 38 weeks gestation. Clinical trial staff judged this to be unrelated to LACTIN-V due to the late onset of symptoms which were in keeping with a urinary tract infection. All adverse events were graded as mild and self-resolved. No adverse events were severe or life-threatening. Withdrawal reasons were recorded if volunteered by participants (Table 3). Only one withdrawal was associated with an adverse event, described as vaginal burning and itching associated with use, the onset of which was immediate and self-resolved within 15 minutes. Since antihistamine treatment was not required and functional activity remained unaffected, this adverse event was graded as mild. There were no cases of intra-uterine death or neonatal death in the 61 women completing LACTIN-V (Table 4). One fetal cardiac anomaly was

reported in our cohort (bronchopulmonary sequestration) and was determined by trial staff to be unrelated to LACTIN-V use, as LACTIN-V commenced after complete cardiac embryogenesis.

Table 2

Reported solicited and unsolicited adverse events (n = 69) Adverse events were reported for women completing the study (n = 61) as well as for 8 women who commenced Lactin-V but withdrew from study completion.

	Number of women (%)
Solicited adverse event	
Change in vaginal discharge	7 (10)
Change in vaginal odour	1 (1)
Change in colour/consistency or urine	1 (1)
Vulvar/vaginal itching	3 (4)
Vaginal bleeding	1 (1)
Unsolicited adverse event	
Macroscopic haematuria	1 (1)

Table 3

Withdrawal reasons for 8 participants. Reasons for withdrawal, if information was volunteered by participants

	Number of women
Reason	
No reason specified	2
Anxiety of clinical trial involvement during pregnancy	1
Taking too many medications	1
Vaginismus and preference to avoid vaginal route of administration	1
Burning and itch associated with use	1
Lost to follow up	2

Two thirds (59%) of women approached for study involvement, consented to participation. The majority of participants (60/61, 98%) were fully compliant to the 5-day loading dose, with one woman non-compliant to day 2 of LACTIN-V. Three women (5%) took an extra sixth daily dose of LACTIN-V during the loading phase. Compliance data for the maintenance phase of LACTIN-V is reported for 60 women, as one woman miscarried prior to commencing weekly LACTIN-V. In total, 56/60 (93%) women completed the 6-week course maintenance phase in full; four women (7%) forgot to administer one of the weekly

LACTIN-V doses. No participants omitted more than one of the weekly LACTIN-V doses. Overall compliance to the complete course of LACTIN-V was 93% (56/60).

Thirty-one women completed the study exit questionnaire, which explored ease of administration, comfort, schedule of use, storage, convenience, partner acceptance and future use (Fig. 3). Thirty (97%) women described the vaginal applicator as both easy and comfortable to use. Although 8 (26%) women described the regimen as hard to remember and required reminders, compliance remained high. Only 3 (10%) women found LACTIN-V messy to use and one woman reported difficulties storing LACTIN-V in the refrigerator. Two participants described their partners as uncomfortable with their use of LACTIN-V but felt this was more pronounced by partner exclusion from antenatal appointments during the Covid-19 pandemic. Most women (30/31, 97%) stated that they would use LACTIN-V in their next pregnancy if clinical benefits and safety were confirmed and 28 (90%) women would use LACTIN-V in non-pregnancy for the prevention of UTIs and BV if benefits were replicated in large trials.

Table 4

Fetal adverse events. Incidence of intra-uterine death and neonatal death reported for participants using Lactin-V and for women in our historical pregnancy control group.

	Lactin-V Cohort (n = 61)	**Historical Pregnancy Cohort (n = 2190)**
Intra-uterine death (%)	0 (0)	16 (0.7)
Neonatal death (%)	0 (0)	6 (0.3)

The incidence of PTB in the historical control cohort was 17.8% and 7.0% for < 37 and < 34 weeks respectively. The incidence of spontaneous PTB in women receiving LACTIN-V was lower; 9.8% (p = 0.15, Chi-squared with Yate's correction) and 3.3% (p = 0.38 Chi-squared with Yate's correction) for < 37 and < 34 weeks respectively (Table 5). We also assessed PTB rates according to risk factor; previous PTB (spontaneous PTB, PPROM or MTL) versus previous cervical excision (LLETZ/cone biopsy) (Table 6). The incidence of PTB < 34 weeks in women with previous cervical treatment in the historical control cohort was 5.0%. In the cohort of women receiving LACTIN-V the incidence of PTB < 34 weeks was 0%. A reduction in the incidence of PTB < 34 weeks was also observed in women with a previous PTB who received LACTIN-V (6.9%) compared to the historical control cohort rate (13.9%).

Table 5

Outcomes for participants receiving Lactin-V versus women from our historical pregnancy control cohort. *P* Values: Chi-square with Yates' correction

	Lactin-V	Historical Pregnancy Cohort	*P* value
	(n = 61)	(n = 2190)	
Cervical cerclage (%)	13 (21)	562 (26)	0.61
Number of women (%)			
Miscarriage < 16/40	1 (1.6)	23 (1.1)	0.85
Mid trimester loss > 16/40 and < 24/40	1 (1.6)	57 (2.6)	0.95
spontaneous PTB < 34/40	2 (3.3)	153 (7.0)	0.38
spontaneous PTB < 37/40	6 (9.8)	390 (17.8)	0.15
PPROM	4 (6.6)	160 (7.3)	0.98
Chorioamnionitis	0 (0)	22 (1.0)	

Table 6

Incidence of PTB according to risk factor. Gestational age of delivery reported according to risk factor in participants receiving Lactin-V versus women from our historical pregnancy control group.

Previous PTB or MTL	**< 37**	***P* value**	**< 34**	***P* value**
High-risk of PTB (n = 1300) (%)	354 (27.2)	0.16	181 (13.9)	0.42
High-risk of PTB + Lactin-V (n = 29) (%)	4 (13.8)		2 (6.9)	
Previous cervical treatment	**< 37**		**< 34**	
High-risk of PTB (n = 703) (%)	94 (13.4)	0.24	35 (5.0)	
High-risk of PTB + Lactin-V (n = 27) (%)	1 (3.7)		0 (0)	

Discussion

Two thirds of eligible women consented to study involvement which was in keeping with our expectations. The high uptake of LACTIN-V may represent a motivated group of women who acknowledge their increased risk of PTB. Although reasons for declining study participation were not recorded, clinical trials involving nutritional supplements in pregnancy report common barriers to include women being too busy and wishing to avoid invasive clinical samples (30). Participation in our study necessarily involve consenting to the collection of vaginal swab samples for research purposes which may have reduced enthusiasm for the study in some individuals, who might otherwise I've been prepared to use LACTIN-V.

We report 13 (19%) solicited adverse events, all of which were mild and self-resolved. The most common adverse event was a change in vaginal discharge (10%). Other factors influencing vaginal discharge in pregnancy should be considered. Pregnancy itself causes a physiological change in vaginal discharge from the first trimester, progressively increasing in amount throughout pregnancy (31). Secondly, vaginal progesterone, used by 26% of participants, is also known to alter vaginal discharge (32). Future in-pregnancy trials using randomization with placebo groups should be designed to assess whether adverse events such as altered vaginal discharge are exclusive to LACTIN-V.

Other clinical trials exploring vaginal administration of *Lactobacillus crispatus* in non-pregnant women have shown a similar safety profile. A pilot study evaluating the safety of *Lactobacillus crispatus* GAI 98322 for the prevention of UTIs (n=9) showed a significant reduction in the recurrence of UTIs without causing adverse events (33). The continuation of this trial is currently underway to further assess adverse events in a larger sample size (34). A randomized trial offering women *Lactobacillus crispatus* IP 174178 also showed no difference in adverse events between the intervention and placebo groups whilst displaying reduced BV recurrence and longer time to recurrence (35). More specifically, studies exploring the safety and efficacy of LACTIN-V in non-pregnant women have also displayed encouraging safety data. In keeping with our results, a phase 1 safety trial of LACTIN-V for BV prevention demonstrated the product to be safe and acceptable, with no difference in the frequency of adverse events between the LACTIN-V and placebo groups (36). The subsequent phase 2a trial detected vaginal colonisation in 61% of participants with LACTIN-V and re-demonstrated comparable adverse events in the LACTIN-V and placebo groups (37). A phase 1 trial of vaginal LACTIN-V for the prevention of UTIs also demonstrated vaginal discharge to be the most commonly reported adverse event, with no difference between the LACTIN-V group or the placebo group (38). The continued phase 2 study demonstrated high level vaginal colonization with *Lactobacillus crispatus* CTV-05 and a significant reduction in recurrence of UTIs in the LACTIN-V group in comparison to the placebo group (39). Similar to our results, a phase 2b clinical trial randomizing women to receive LACTIN-V or placebo after metronidazole treatment of BV, found the most common adverse events to include altered vaginal discharge and vaginal odour (23). In agreement with our data, no serious adverse events were reported. The number of both solicited and unsolicited events were much higher in both the LACTIN-V and placebo groups when compared to those reported in our trial; Cohen et al (2020) describe solicited events in 87% and 79% of the LACTIN-V and placebo group respectively. This difference may be attributed to the more frequent usage of LACTIN-V by Cohen et al, which included four consecutive daily doses during week 1 followed by twice-weekly doses for 10 weeks (23). [11] Additionally, in the trial by Cohen et al women with BV had more adverse events.

In our trial, only one withdrawal was associated with an adverse event, demonstrating LACTIN-V to be well-tolerated in pregnancy. Although one woman withdrew due to a history of vaginismus and preference to avoid vaginal administration, two participants with a history of vaginismus successfully completed the full course of LACTIN-V with no reported complaints. Compliance to LACTIN-V was impressive, with 93% of participants adherent to the complete regimen. In women at high-risk of PTB, the gestational timepoint providing women security is typically around 24 weeks, when the fetus reaches viability. This may explain high compliance levels in the study. Similarly, progesterone compliance in women at risk of first trimester

miscarriage is highest until 12-14 weeks gestation, reflecting an inclination of women to comply to treatment protocols until they feel their pregnancy is secure (40).

Our exit questionnaire indicated that most women found LACTIN-V easy and comfortable to use. Although 8 women (26%) described the dosage schedule difficult to remember, compliance was not compromised. The majority reported a desire to use LACTIN-V in their next pregnancy if safety and health benefits were confirmed. Consistent with this, a previously published study reported high satisfaction levels and willingness to use vaginal capsules containing *Lactobacillus crispatus* in the future (41).

Although our study was limited by a small sample size and was not powered to assess clinical outcomes, we observed a lower rate of PTB with LACTIN-V use when compared to our historical control cohort. The most profound observation in clinical outcomes in our study cohort was in women with a history of previous PTB, PPROM or MTL, in whom the incidence of prematurity (<34 weeks) was half that seen in the historical control cohort. PTB and PPROM have been associated with low relative abundance levels of vaginal *Lactobacillus* spp. (5, 42, 43). We have also previously shown that displacement of *Lactobacillus* dominated vaginal microbiota and increased bacterial diversity can activate local inflammation leading to premature cervical ripening (44, 45). Naturally occurring *Lactobacillus* species are thought to optimise the vaginal microbiome and restrict pathogen colonisation (46-48) through the production of antimicrobial compounds and lactic acid, which changes the local metabolome, lowers pH and exhibits anti-inflammatory properties (47, 49-55). In specific, *L. crispatus* CTV-05 has been shown to dampen down pro-inflammatory responses to uropathogens such as *Escherichia coli* (56). Our preliminary findings are promising, and may have application beyond preterm birth for example in first trimeter miscarriage and cervical intraepithelial neoplasia, each of which associates with a *Lactobacillus* deplete vaginal microbiota (57, 58). Randomised trials are now required to further explore the influence of *Lactobacillus crispatus* CTV-05 on PTB risk. It is also important that future studies explore whether LACTIN-V has microbiome modulating capability that could provide mechanistic understanding of clinical observations.

Conclusion

Among women at high-risk of PTB, use of LACTIN-V in pregnancy is safe, tolerable and well-accepted. The high study uptake and notable compliance highlight the feasibility of LACTIN-V use in pregnancy, thus qualifying LACTIN-V as a promising live vaginal biotherapeutic. Although not powered to assess clinical outcomes, we observed lower incidences of PTB with LACTIN-V compared to a historical control cohort. Studies are now required to explore *Lactobacillus crispatus* CTV-05 vaginal colonization dynamics in pregnancy and clinical efficacy.

List Of Abbreviations

PTB: Preterm birth

BV: Bacterial vaginosis

UTI: Urinary tract infection

LACTIN-V: *Lactobacillus crispatus* CTV-05

PPROM: Preterm prelabour rupture of membranes

LLETZ: Large loop excision of the transformation zone

FDCS: Caesarean section at full cervical dilatation

MTL: Mid-trimester loss

HIV: Human immunodeficiency virus

CFU: colony-forming units

CIN: cervical intraepithelial neoplasia

Declarations

Ethics approval

Ethical approval was granted by the London-Surrey Borders Research Ethics Committee (IRAS 262987). HRA and Health and Care Research Wales Approval was given (REC reference 19/LO/1018). The trial is registered at www.clinicaltrials.gov (NCT03992534).

Consent to participate

Written informed consent was obtained from all participants.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

PRB, DAM and TPP are named applicants on a patent application made by Imperial College for the use of *Lactobacillus crispatus* CTV-05 in the prevention of preterm birth (US 63/151,474). PPL is founder and

chairman, and TPP is Director of product development at Osel Inc. 320 Logue Ave, Mountain View, CA 94043. The remaining authors declare that they have no competing interests.

Funding

This study was funded by March of Dimes European Prematurity Research Centre at Imperial, Imperial College Healthcare NHS Trust, the Imperial NIHR Biomedical Research Centre and the, Tommy's National Centre for Miscarriage Research at Imperial.

The live biotherapeutic CTV-05 was supplied to the investigators by Osel Inc. 320 Logue Ave, Mountain View, CA 94043.

Authors' contributions

D.A.M. and P.R.B. conceived and designed the study. T.P.P. and P.P.L. contributed to study design and protocol development, but otherwise played no role in the conduct of the trial or analysis of results. E.B. and L.S. undertook patient recruitment, clinical sampling and coordination of metadata. Analysis and interpretation of data was performed by E.B., D.A.M., L.S., and P.R.B. The first draft of the manuscript was prepared by E.B. and all authors critically reviewed, read and approved the final manuscript.

Acknowledgements

We thank all study participants for their time and commitment to study involvement. We also thank all the staff of the Women's Health Research Centre, Imperial College NHS Trust. In particular, we thank our specialist research midwives M.A. and S.Z. who contributed to participant recruitment and clinical care, as well as R.M, laboratory manager of the Women's Health Research Centre. We also thank our patient and public involvement group at Imperial College London, who we worked in partnership with to optimise the experience of study participants.

References

1. MacIntyre DA, Sykes L, Bennett PR. The human female urogenital microbiome: complexity in normality. Emerging Topics in Life Sciences. 2017;1(4):363–72.
2. Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UM, Zhong X, et al. Temporal dynamics of the human vaginal microbiota. Science translational medicine. 2012;4(132):132ra52-ra52.
3. Pruski P, Lewis HV, Lee YS, Marchesi JR, Bennett PR, Takats Z, et al. Assessment of microbiota: host interactions at the vaginal mucosa interface. Methods. 2018;149:74–84.
4. Brown RG, Al-Memar M, Marchesi JR, Lee YS, Smith A, Chan D, et al. Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. Transl Res. 2019;207:30–43.
5. Brown RG, Marchesi JR, Lee YS, Smith A, Lehne B, Kindinger LM, et al. Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. BMC

medicine. 2018;16(1):1–15.

6. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proceedings of the National Academy of Sciences. 2015;112(35):11060-5.
7. Kindinger LM, Bennett PR, Lee YS, Marchesi JR, Smith A, Cacciatore S, et al. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. Microbiome. 2017;5(1):6.
8. Elovitz MA, Gajer P, Riis V, Brown AG, Humphrys MS, Holm JB, et al. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. Nat Commun. 2019;10(1):1305.
9. Payne MS, Newnham JP, Doherty DA, Furfaro LL, Pendal NL, Loh DE, et al. A specific bacterial DNA signature in the vagina of Australian women in midpregnancy predicts high risk of spontaneous preterm birth (the Predict1000 study). Am J Obstet Gynecol. 2021;224(2):206 e1- e23.
10. Lamont RF, Duncan SL, Mandal D, Bassett P. Intravaginal clindamycin to reduce preterm birth in women with abnormal genital tract flora. Obstetrics & Gynecology. 2003;101(3):516–22.
11. McDonald H, O'loughlin J, Vigneswaran R, Jolley P, Harvey J, Bof A, et al. Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial. BJOG: An International Journal of Obstetrics & Gynaecology. 1997;104(12):1391–7.
12. Hauth JC, Goldenberg RL, Andrews WW, DuBard MB, Copper RL. Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis. New England Journal of Medicine. 1995;333(26):1732–6.
13. Brocklehurst P, Gordon A, Heatley E, Milan SJ. Antibiotics for treating bacterial vaginosis in pregnancy. Cochrane Database of Systematic Reviews. 2013(1).
14. Vermeulen GM, Bruinse HW. Prophylactic administration of clindamycin 2% vaginal cream to reduce the incidence of spontaneous preterm birth in women with an increased recurrence risk: a randomised placebo-controlled double-blind trial. BJOG: An International Journal of Obstetrics & Gynaecology. 1999;106(7):652–7.
15. U.S. Department of Health and Human Services, Food and Drug Administration Guidance for Industry. Early clinical trials with live biotherapeutic products: Chemistry, manufacturing, and control information. wwwfdagov/downloads/Biologi%E2%80%A6/UCM292704pdf. 2016.
16. Charbonneau MR, Isabella VM, Li N, Kurtz CB. Developing a new class of engineered live bacterial therapeutics to treat human diseases. Nat Commun. 2020;11(1):1738.
17. Nordqvist M, Jacobsson B, Brantsæter A-L, Myhre R, Nilsson S, Sengpiel V. Timing of probiotic milk consumption during pregnancy and effects on the incidence of preeclampsia and preterm delivery: a prospective observational cohort study in Norway. BMJ open. 2018;8(1).
18. Grev J, Berg M, Soll R. Maternal probiotic supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database of Systematic Reviews. 2018(12).

19. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, et al. Oral use of Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol Med Microbiol. 2003;35(2):131–4.
20. Husain S, Allotey J, Drymoussi Z, Wilks M, Fernandez-Felix B, Whiley A, et al. Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 2020;127(2):275–84.
21. Yang S, Reid G, Challis JR, Gloor GB, Asztalos E, Money D, et al. Effect of oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the vaginal microbiota, cytokines and chemokines in pregnant women. Nutrients. 2020;12(2):368.
22. Yefet E, Colodner R, Strauss M, Gam Ze Letova Y, Nachum Z. A randomized controlled open label crossover trial to study vaginal colonization of orally administered Lactobacillus reuteri RC-14 and rhamnosus GR-1 in pregnant women at high risk for preterm labor. Nutrients. 2020;12(4):1141.
23. Cohen CR, Wierzbicki MR, French AL, Morris S, Newmann S, Reno H, et al. Randomized Trial of Lactin-V to Prevent Recurrence of Bacterial Vaginosis. N Engl J Med. 2020;382(20):1906–15.
24. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, et al. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis. 2011;52(10):1212–7.
25. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. The lancet. 2008;371(9606):75–84.
26. Williams C, Fong R, Murray SM, Stock SJ. Caesarean birth and risk of subsequent preterm birth: a retrospective cohort study. BJOG: An International Journal of Obstetrics & Gynaecology. 2021;128(6):1020–8.
27. Kyrgiou M, Athanasiou A, Paraskevaidi M, Mitra A, Kalliala I, Martin-Hirsch P, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. bmj. 2016;354.
28. Short C-ES, Taylor GP. Antiretroviral therapy and preterm birth in HIV-infected women. Expert review of anti-infective therapy. 2014;12(3):293–306.
29. U.S. Department of Health and Human Services NIoH, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. 2017. Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf.
30. Strömmer S, Lawrence W, Rose T, Vogel C, Watson D, Bottell JN, et al. Improving recruitment to clinical trials during pregnancy: a mixed methods investigation. Social Science & Medicine. 2018;200:73–82.
31. Khaskheli M, Baloch S, Baloch AS, Shah SGS. Vaginal discharge during pregnancy and associated adverse maternal and perinatal outcomes. Pakistan journal of medical sciences. 2021;37(5):1302.

32. Piette PC. The pharmacodynamics and safety of progesterone. Best Practice & Research Clinical Obstetrics & Gynaecology. 2020;69:13–29.
33. Uehara S, Monden K, Nomoto K, Seno Y, Kariyama R, Kumon H. A pilot study evaluating the safety and effectiveness of Lactobacillus vaginal suppositories in patients with recurrent urinary tract infection. International journal of antimicrobial agents. 2006;28:30–4.
34. Wada K, Uehara S, Ishii A, Sadahira T, Yamamoto M, Mitsuhata R, et al. A Phase II clinical trial evaluating the preventive effectiveness of lactobacillus vaginal suppositories in patients with recurrent cystitis. Acta Medica Okayama. 2016;70(4):299–302.
35. Bohbot J, Daraï E, Bretelle F, Brami G, Daniel C, Cardot J. Efficacy and safety of vaginally administered lyophilized Lactobacillus crispatus IP 174178 in the prevention of bacterial vaginosis recurrence. Journal of gynecology obstetrics and human reproduction. 2018;47(2):81–6.
36. Hemmerling A, Harrison W, Schroeder A, Park J, Korn A, Shiboski S, et al. Phase 1 dose-ranging safety trial of Lactobacillus crispatus CTV-05 (LACTIN-V) for the prevention of bacterial vaginosis. Sexually transmitted diseases. 2009;36(9):564.
37. Hemmerling A, Harrison W, Schroeder A, Park J, Korn A, Shiboski S, et al. Phase 2a study assessing colonization efficiency, safety, and acceptability of Lactobacillus crispatus CTV-05 in women with bacterial vaginosis. Sexually transmitted diseases. 2010;37(12):745–50.
38. Czaja CA, Stapleton AE, Yarova-Yarovaya Y, Stamm WE. Phase I trial of a Lactobacillus crispatus vaginal suppository for prevention of recurrent urinary tract infection in women. Infectious diseases in obstetrics and gynecology. 2007;2007.
39. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, et al. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clinical infectious diseases. 2011;52(10):1212–7.
40. Coomarasamy A, Harb HM, Devall AJ, Cheed V, Roberts TE, Goranitis I, et al. Progesterone to prevent miscarriage in women with early pregnancy bleeding: the PRISM RCT. Health Technology Assessment (Winchester, England). 2020;24(33):1.
41. Marrazzo JM, Cook RL, Wiesenfeld HC, Murray PJ, Busse B, Krohn M, et al. Women's satisfaction with an intravaginal Lactobacillus capsule for the treatment of bacterial vaginosis. Journal of Women's Health. 2006;15(9):1053–60.
42. Baldwin EA, Walther-Antonio M, MacLean AM, Gohl DM, Beckman KB, Chen J, et al. Persistent microbial dysbiosis in preterm premature rupture of membranes from onset until delivery. PeerJ. 2015;3:e1398.
43. Veščičík P, Musilová K, Stranik J, Štěpán M, Kacerovský M. Lactobacillus crispatus dominant vaginal microbita in pregnancy. Ceska gynekologie. 2020;85(1):67–70.
44. Kindinger LM, MacIntyre DA, Lee YS, Marchesi JR, Smith A, McDonald JA, et al. Relationship between vaginal microbial dysbiosis, inflammation, and pregnancy outcomes in cervical cerclage. Sci Transl Med. 2016;8(350):350ra102.

45. Chan D, Bennett PR, Lee YS, Kundu S, Teoh T, Adan M, et al. Microbial-driven preterm labour involves crosstalk between the innate and adaptive immune response. Nature Communications. 2022;13(1):1–15.
46. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal microbiota and reproductive health: the virtue of simplicity. Cell host & microbe. 2018;23(2):159–68.
47. Mastromarino P, Di Pietro M, Schiavoni G, Nardis C, Gentile M, Sessa R. Effects of vaginal lactobacilli in Chlamydia trachomatis infection. International Journal of Medical Microbiology. 2014;304(5–6):654–61.
48. Tomás MSJ, Duhart CIS, De Gregorio PR, Pingitore EV, Nader-Macías ME. Urogenital pathogen inhibition and compatibility between vaginal Lactobacillus strains to be considered as probiotic candidates. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2011;159(2):399–406.
49. Coman M, Verdenelli M, Cecchini C, Silvi S, Orpianesi C, Caspani M, et al. In vitro evaluation on HeLa cells of protective mechanisms of probiotic lactobacilli against Candida clinical isolates. Journal of applied microbiology. 2015;119(5):1383–90.
50. Spurbeck RR, Arvidson CG. Inhibition of Neisseria gonorrhoeae epithelial cell interactions by vaginal Lactobacillus species. Infection and immunity. 2008;76(7):3124–30.
51. Ciandrini E, Campana R, Casettari L, Perinelli DR, Fagioli L, Manti A, et al. Characterization of biosurfactants produced by Lactobacillus spp. and their activity against oral streptococci biofilm. Applied microbiology and biotechnology. 2016;100(15):6767–77.
52. Rybalchenko OV, Bondarenko VM, Orlova OG, Markov AG, Amasheh S. Inhibitory effects of Lactobacillus fermentum on microbial growth and biofilm formation. Archives of microbiology. 2015;197(8):1027–32.
53. Rizzo A, Fiorentino M, Buommino E, Donnarumma G, Losacco A, Bevilacqua N. Lactobacillus crispatus mediates anti-inflammatory cytokine interleukin-10 induction in response to Chlamydia trachomatis infection in vitro. International Journal of Medical Microbiology. 2015;305(8):815–27.
54. Rizzo A, Losacco A, Carratelli CR. Lactobacillus crispatus modulates epithelial cell defense against Candida albicans through Toll-like receptors 2 and 4, interleukin 8 and human β-defensins 2 and 3. Immunology letters. 2013;156(1–2):102–9.
55. Pruski P, Correia GDS, Lewis HV, Capuccini K, Inglese P, Chan D, et al. Direct on-swab metabolic profiling of vaginal microbiome host interactions during pregnancy and preterm birth. Nat Commun. 2021 Oct 13;12(1):5967.
56. Butler DS, Silvestroni A, Stapleton AE. Cytoprotective effect of Lactobacillus crispatus CTV-05 against uropathogenic E. coli. Pathogens. 2016;5(1):27.
57. Grewal K, Lee YS, Smith A, Brosens JJ, Bourne T, Al-Memar M et al. Chromosomally normal miscarriage is associated with vaginal dysbiosis and local inflammation. BMC Med. 2022 Jan 28;20(1):38.

58. Mitra A, MacIntyre DA, Ntritsos G, Smith A, Tsilidis KK, Marchesi JR et al. The vaginal microbiota associates with the regression of untreated cervical intraepithelial neoplasia 2 lesions. Nat Commun. 2020 Apr 24;11(1):1999.

Figures

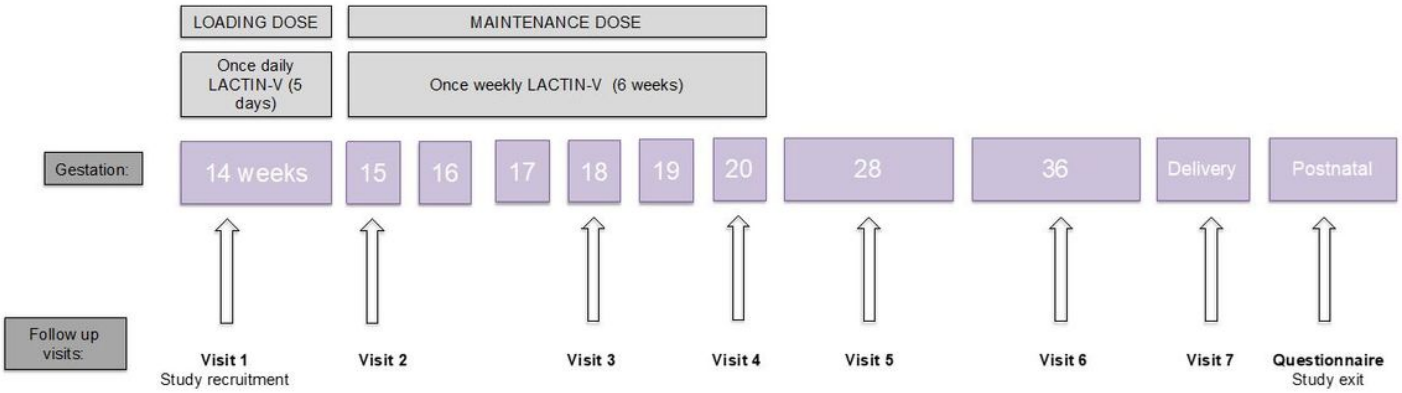

Figure 1

Study design: Lactin-V dosing regime in pregnant women at high-risk of spontaneous PTB Women at high-risk of PTB were offered a course of Lactin-V from as close to 14 weeks gestation as possible. Lactin-V was taken for 5 consecutive days followed by weekly administration for 6 weeks. Participants were followed up at various time points for assessment of adverse events, compliance, and tolerance.

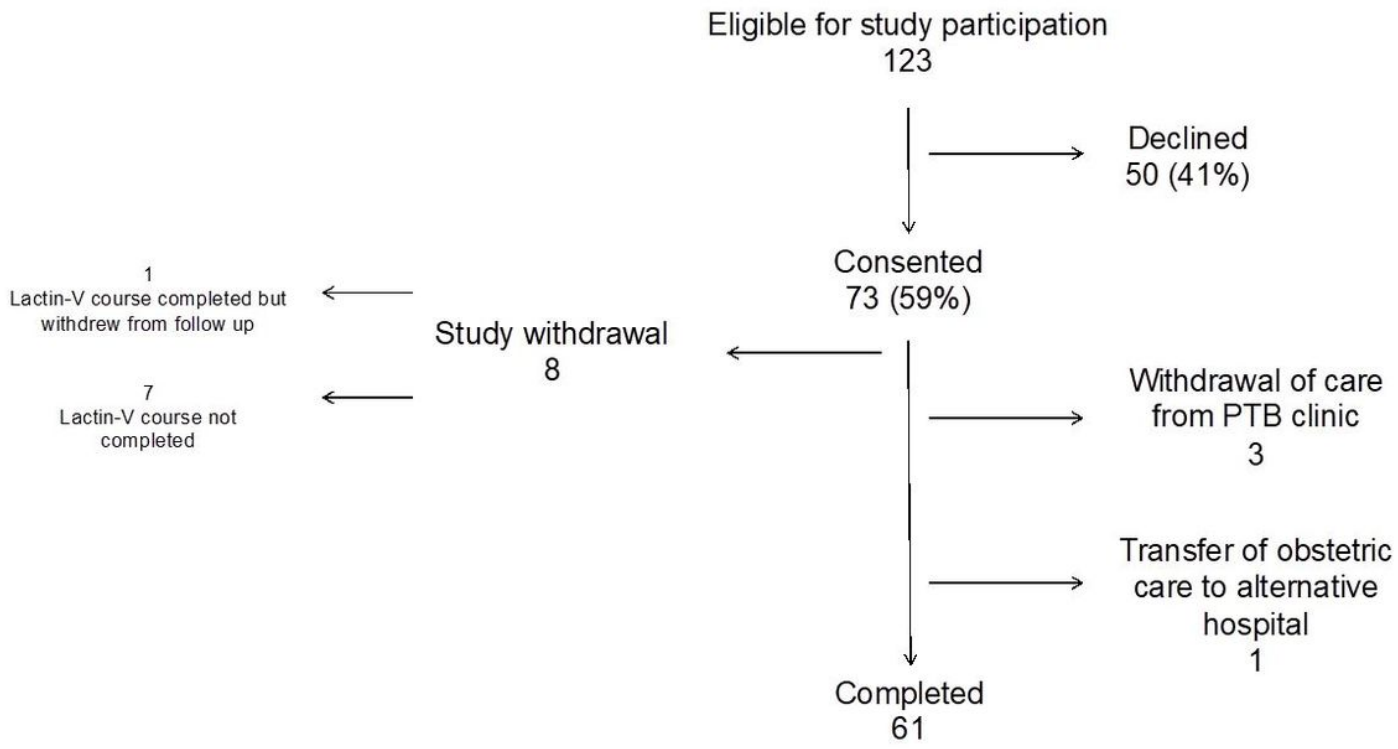

Figure 2

Flow diagram of study recruitment and completion rates. 123 women were eligible for study requirement and 73 women consented to study participation. Four women were lost to follow up and 8 withdrew from the study. Overall, 61 women completed the study.

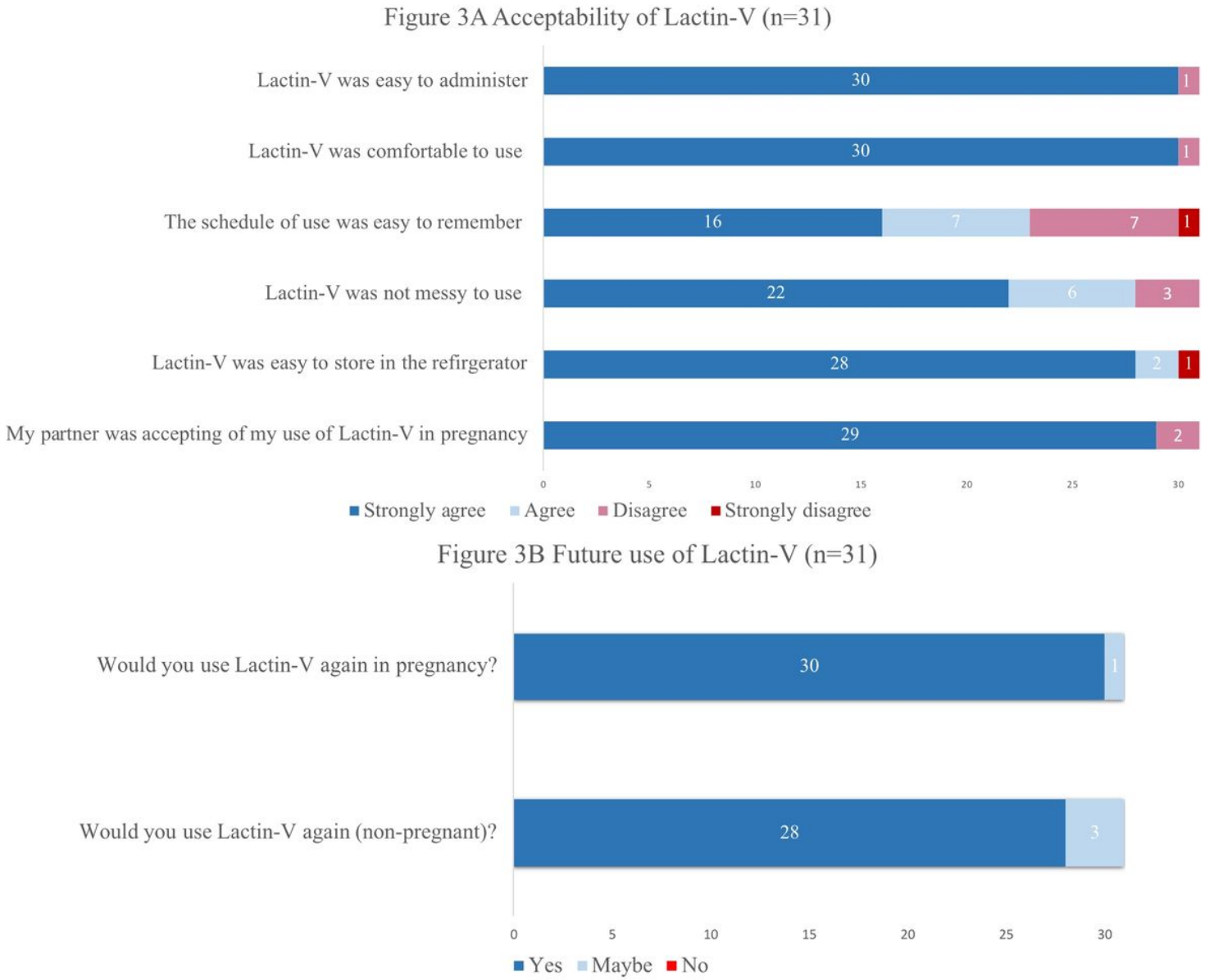

Figure 3

Questionnaire results exploring (A) acceptability and (B) likelihood of future use of Lactin-V. Thirty-one women completed the study exit questionnaire, which explored ease of administration, comfort, schedule of use, storage, convenience, partner acceptance and future use.