

VAGINAL COMPOSITIONS AND METHODS AND USES THEREOF

FIELD OF THE INVENTION

5 The present invention relates to a vaginal pharmaceutical composition and the methods and uses thereof including: treating and preventing genital inflammation in a subject; modifying the level of one or more of *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, *Gardnerella vaginalis*, and *Lactobacillus iners* in the genital microbiome of a subject; and maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.

10

BACKGROUND OF THE INVENTION

When a woman's vaginal microbiome is unbalanced (non-optimal) it greatly impacts quality of life. Bacterial vaginosis (BV) is the most common clinical manifestation of a non-optimal vaginal microbiome affecting 30% of women of reproductive age globally. BV greatly impacts a woman's quality of life causing an abnormal and malodorous vaginal discharge and increases the risk of sexually transmitted infections (STIs) including human immunodeficiency virus (HIV) by 4.4-fold and spontaneous preterm birth, comprising 40–50% of the 14.8 million annual cases of global preterm births. BV is also associated with increase in endometritis, pelvic inflammatory disease, human papilloma virus (HPV), HPV cervical cancer, cervical intra-epithelial neoplasia, cervical cancer and infertility. BV promotes genital inflammation that disrupts the cervicovaginal mucosal epithelial barrier facilitating pathogen penetration and contributes to preterm birth. Current BV treatment relies on antibiotics which provide only short-term resolution and shifts the vaginal microbiome composition to unstable and unfavourable (or change to sub-optimal) *Lactobacillus-iners* dominance that may readily shift back to BV, resulting in a ~60% recurrence rate within 6 months.

Women's sexual and reproductive health is often neglected with little progress in BV treatment since the 1960's despite its global economic burden of \$US4.8 billion a year. There is a critical need for effective, non-antibiotic-based strategies to reduce genital inflammation, including genital inflammation caused by BV. A disrupted cervicovaginal microbiome, such as that observed in BV, is characterised by an increase in the load and diversity of anaerobic bacteria and a relative depletion of *Lactobacillus* spp. In contrast, an optimal vaginal microbiome is typically dominated by a single species of stable, non-inflammatory *Lactobacillus* spp. (i.e. *Lactobacillus crispatus*) that promotes favourable health outcomes. Depletion of vaginal *Lactobacillus* spp.

(particularly *L. crispatus*) has also been associated with recurrent urogenital tract infections (Gupta et al., 1998).

There are currently no approved treatments for preventing BV recurrence and there are no approved interventions that can select for optimal *L. crispatus* over the sub-optimal *L. iners*.

Accordingly, there remains a need for improved compositions for modulating the genital inflammation and/or genital microbiome. In particular, there remains a need for improved compositions for modulating cervicovaginal microbiome and/or cervicovaginal inflammation.

SUMMARY OF THE INVENTION

The present inventors have developed vaginal pharmaceutical compositions and devices comprising lactic acid that is iso-osmolal with the cervicovaginal fluid from a healthy subject. The present inventors have also developed vaginal pharmaceutical composition that are near iso-osmolal with the cervicovaginal fluid from a healthy subject. The present inventors have also developed methods and uses for such compositions, in particular methods and uses for modulating genital inflammation in a subject.

In an aspect, the present invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides a pharmaceutical composition comprising: a) lactic acid at about 1.40 % w/w to about 2.5% w/w; b) a mucoadhesive agent; c) a viscosity of about 50 to about 150 Pa.s; wherein the composition is iso-osmolal with cervicovaginal fluid. In an embodiment, the composition comprises lactic acid at about 1.64% w/w to about 2.25% w/w. In an embodiment, the mucoadhesive agent is hydroxyethylcellulose. In an embodiment, the composition comprises an osmolality of 300 to 410 mOsm/Kg. In an embodiment, the composition has a pH of about 3.2 to about 4.2.

In an aspect, the present invention provides a prescription composition comprising: a) lactic acid at about 1.40 % w/w to about 2.5% w/w; b) a mucoadhesive agent; c) a viscosity of about 50 to about 150 Pa.s; wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides an over the counter composition comprising: a) lactic acid at about 1.40 % w/w to about 2.5% w/w; b) a mucoadhesive

agent; c) a viscosity of about 50 to about 150 Pa.s; wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides a method of modulating genital inflammation in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of modulating cervicovaginal inflammation in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of modulating the genital microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of modulating the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of treating or preventing genital inflammation in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of treating or preventing cervicovaginal inflammation in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of maintaining or increasing the level of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein the subject.

In an aspect, the present invention provides a method of maintaining or increasing the level of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of maintaining or increasing the level of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of decreasing the level of *Lactobacillus iners* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of decreasing the level of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of decreasing the level of *Prevotella spp* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

5 In an aspect, the present invention provides a method of decreasing the level of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of maintaining or increasing the level of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

10 In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus gasseri* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

20 In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus jensenii* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus crispatus* to *Prevotella spp.* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

25 In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus crispatus* to *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

30 In an aspect, the present invention provides a method of increasing the ratio of *Lactobacillus crispatus* to *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject, the method comprising administering the composition as described herein to the subject.

35 In an aspect, the present invention provides a method of maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject, the method comprising administering the composition as described herein to the subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for treating or preventing genital inflammation in a subject.

5 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for treating or preventing cervicovaginal inflammation in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject.

10 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

20 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

25 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Prevotella spp.* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

35 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the

relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Prevotella spp.* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for increasing the ratio of *Lactobacillus crispatus* to *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.

In an embodiment, the medicament additionally comprises a mucoadhesive agent, a viscosity of about 50 to about 150 Pa.s and wherein the composition is iso-osmolal with cervicovaginal fluid.

5 In an aspect, the present invention provides an applicator or device having the composition as described herein.

In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

10 In an aspect, the present invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 4.5% w/w, wherein the composition has an osmolality of 300 to 800 mOsm/Kg. In an embodiment, the composition has an osmolality of about 500 to 800 mOsm/Kg. Such compositions are within the WHO osmolality recommendations for vaginal lubricants (below 1200 mOsm/Kg; Ayehunie et al., 2018).

15 In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 4.5% w/w, wherein the composition has an osmolality of about 300 to 800 mOsm/Kg. In an embodiment, the composition has an osmolality of about 500 to 800 mOsm/Kg.

20 Any embodiment herein shall be taken to apply *mutatis mutandis* to any other embodiment unless specifically stated otherwise. For instance, as the skilled person would understand examples of osmolality outlined above for the composition of the invention equally apply to methods and uses of the invention and *vice versa*.

25 The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

30 Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

35 **BRIEF DESCRIPTION OF THE ACCOMPANING DRAWINGS**

Figure 1. Shows the effects of lactic acid (LA)-containing gels on vaginal epithelial cell viability and integrity. The EpiVaginal tissue model (VEC-100) was used to assess one placebo and nine lactic acid-containing gels containing L-lactic acid alone, or D-lactic acid and L-lactic acid in combination at either a 1:1 (DL-lactic acid) or 1.5:1 (1.5DL-lactic acid) ratio respectively, at total lactic acid concentrations from 1.4-4.5% (w/w) (**A** and **C**). In addition, 11 over the counter (OTC) vaginal gels were analysed in parallel (**B** and **D**, gels as labelled). Cell viability (**A** and **B**) was assessed by MTT assay and epithelial barrier integrity (**C** and **D**) measured by trans epithelial electrical resistance (TEER) at 4 or 24 h after gel addition. **A** and **B** show mean and SD of n=2 experiments with two technical replicates per assay, while **C** and **D** show mean and SD from n=2 independent assays. Untr.: untreated, +ve: positive control (3% Nonoxynol-9), Plac.: placebo gel (HEC alone).

Figure 2. Shows the effects of lactic acid-containing gels on cytokine/chemokine production. The effect of nine lactic acid-containing gels, one placebo and 11 over the counter (OTC) vaginal gels (detailed in **Figure 1**) on the production of inflammatory factors from the EpiVaginal tissue model (VEC-100) was assessed after 24 h of treatment. Levels of MIP3 α (**A** and **D**), IL-1 β (**B** and **E**), IL-1RA (**C** and **F**), IL-1 α (**G** and **J**), IL-6 (**H** and **K**) and IL-8 (**I** and **L**) were measured in cell culture supernatants of tissues treated with (LHS panels) or OTC gels (RHS panels) by Luminex assay. Graphs show mean and SEM of n=6 replicates from two independent assays. Untr.: untreated, +ve: positive control (3% Nonoxynol-9), Plac.: placebo gel (HEC alone).

Figure 3. Shows the bactericidal activity of lactic acid-containing gels. The results shows DL-lactic acid gel (1% w/w) at pH 3.8 had potent bactericidal activity against *L. iners* and *G. vaginalis*, but not *L. crispatus* or *L. gasseri* after 1 hour incubation at 37°C under anerobic conditions compared to untreated bacteria incubated in medium without pH adjustment (untreated not pH adjusted). The decrease in *G. vaginalis* and *L. iners* viability mediated by the lactic acid containing gel was greater than placebo gel that was pH adjusted to 3.8 using HCl (Placebo 3.8). These data indicate that the bactericidal activity of lactic acid against *G. vaginalis* and *L. iners* is greater than gel acidified with HCL alone. No bactericidal activity was observed with the placebo gel at pH 5.5 (placebo 5.5)(*L. gasseri* was not tested). Bacteria treated with media acidified to pH 3.8 with HCl (water 3.8) was not as potent at killing *L. iners* compared to lactic acid (1% w/w DL-LA gel final). Error bars represent standard error of the mean from n=4 independent assays.

Statistical analysis was performed using the Mann-Whitney test where * $p < 0.05$ and ** $p < 0.01$

Figure 4. Shows the bactericidal activity of lactic acid-containing media. The results show L- lactic acid, D-lactic acid and racemic lactic acid (1% w/v) is bactericidal against *G. vaginalis* and *L. iners* but not *L. crispatus* at pH < 5.5. The density of water equals 1, accordingly 1% (w/v) of LA is similar to 1% (w/w) LA. Bacteria were incubated with lactic acid in medium adjusted to different pH values for 1 hour at 37°C under anaerobic conditions. Following incubation bacteria were plated to determine viability as determined by counting colonies. The bactericidal activity of lactic acid against *G. vaginalis* and *L. iners* increases in potency as the pH values decrease around and lower than the pKa of lactic acid (3.86). These data indicate that it is uncharged lactic acid mediating bactericidal activity against *L. iners* and *G. vaginalis* and not the lactate anion. In contrast *L. crispatus* retained viability under all conditions tested indicating that this bacterial species is resistant to the bactericidal effects of lactic acid. Error bars represent standard error of the mean from at least n=2 independent assays.

Figure 5. Shows the bactericidal and bacteriostatic activity of lactic acid against *G. vaginalis* and *L. iners*, respectively. Bacteria were cultured in S-Broth with 1% DL-lactic acid at pH 4.5 at 37°C under anaerobic conditions. Viability was evaluated at 24 h and 48 h, by quantifying colony forming units/ml (CFU/ml). Data are from N= 3 independent assays. Error bars represent standard error of the mean. (**) p-value = 0.002 as determined by 2 way ANOVA Tukey's multiple comparison test.

Figure 6. Shows that probiotic strains remain viable after 1 hr treatment with DL-lactic acid (DL-LA). Bacteria were treated with 1% DL-LA at pH 3.8 under anaerobic conditions for 1 h at 37°C. After treatment viability was quantified by calculating colony forming units/ml (CFU/ml). Data are from N= 3 independent assays. Error bars represent standard error of the mean. One way ANOVA Dunnett's multiple comparison test. P-value > 0.05 is reported as not significant.

Figure 7. Shows DL-lactic acid decreases the viability of *G. vaginalis* and *L. iners*, but not *L. crispatus* in a mixed bacterial culture. Mono and mixed cultures of *G. vaginalis*, *L. iners* and *L. crispatus* were incubated for 24 h with 1% DL-lactic acid at pH 4.5 under anaerobic conditions. After treatment cells were subjected to taxon-specific propidium monoazide quantitative PCR to specifically quantify viable *L. iners*, *L. crispatus* and *G.*

vaginalis. Error bars represent standard error of the mean from N=3 independent assay. Statistical analysis was performed using the 2-way ANOVA Dunnet's test. p-value 0.033 (*), 0.002 (**), <0.001(***). Left shows results of mix culture – viability PCR; Middle shows the results of single culture – viability PCR; Right shows the results of single culture – CFU/mL.

Figure 8. Shows that DL-lactic acid decreases the viability of *G. vaginalis* and *L. iners*, but not *L. crispatus* in a mixed bacterial culture. Mixed cultures of *G. vaginalis*, *L. iners* and *L. crispatus* were incubated for 24 h with 1% DL-lactic acid at pH 4.5 under anaerobic conditions. After treatment cells were subjected to taxon-specific propidium monoazide quantitative PCR to specifically quantify viable *L. iners*, *L. crispatus* and *G. vaginalis*. Data is shown as percentage of viable of total viable 16S RNA copies. Error bars represent standard error of the mean from N=3 independent assay. Statistical analysis was performed using the 1-way ANOVA Dunnet's test. p-value 0.033 (*), 0.002 (**), <0.001(***).

Figure 9. Shows that DL-lactic acid together with metronidazole (MTZ) 50 µg/ml decreases concentrations of viable *G. vaginalis* and *L. iners*, but not *L. crispatus*. Cultures of *G. vaginalis*, *L. iners* and *L. crispatus* were incubated for 24 h with 1% DL-lactic acid at pH 4.5, MTZ 50 µg/ml and combination under anaerobic conditions. After treatment viability was quantified by calculating colony forming units/ml (CFU/ml). Error bars represent standard error of the mean from at least N=3 independent assays. Statistical analysis was performed using the 2-way ANOVA Dunnet's test. p-value 0.033 (*), 0.002 (**).

DETAILED DESCRIPTION

General techniques and definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., protein, enzyme, bacteria).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated

element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein, the term “about”, unless stated to the contrary, refers to +/- 15%, +/- 10%, more preferably +/- 5%, even more preferably +/- 1%, of the designated value.

5 As used herein, the term “subject” refers to any animal. In one example, the animal is a vertebrate. In an embodiment, the subject is a mammal. In a preferred embodiment, the mammal is a human, a human companion animal or a human associated livestock. In a preferred embodiment, the subject is a human. In an embodiment, the subject is a non-human primate. In an embodiment, the non-human primate is a macaque.
10 In an embodiment, the subject is a model animal, such as rat, mouse or guinea pig. In an embodiment, the mammal may be a companion animal, such as a cat, dog or horse. In an embodiment, the mammal may be livestock such as pigs, cattle, horses, goats, sheep, and deer. In an embodiment, the subject is a human. In an embodiment, the subject is female. In an embodiment, the subject is male. In an embodiment, the subject has
15 increased genital inflammation or a condition or disease which causes increased genital inflammation. In an embodiment, the subject is female and the subject has increased cervicovaginal inflammation or a condition or disease which causes increased cervicovaginal inflammation. In an embodiment, the subject is a female with a sub-optimal cervicovaginal microbiome. In an embodiment, the subject is a female with
20 bacterial vaginosis.

As used herein, “osmolality” refers to the concentration of a solution expressed as the total number of solute particles per kilogram. It can be expressed as mOsm/kg of water and can be measured using an osmometer using the methods described herein. In an embodiment, osmolality is determined using the Osmolality test for assessment of
25 feminine hygiene gels and liquids using a cryoscopic osmometer (Gonotec Osmomat 030-D, Gemini BV, Apeldoorn, Netherlands) as described in Tyssen et al. (2022).

As used herein, “viscosity” refers to the measure of a fluids resistance to flow. The higher the viscosity the more resistance a fluid has to flow. The lower the viscosity the less resistance a fluid has to flow. In an embodiment, the composition as described
30 herein comprises a viscosity suitable for administration and retention/partial retention in the vagina. A pascal-second (Pa·s) is a derived metric SI (System International) measurement unit of dynamic viscosity. In an embodiment, viscosity is determined at 37°C using a Rheology TesRheology instrumentation Anton Paar MCR302 Rheometer. In an embodiment, viscosity is determined using an Anton Paar MCR92 Rheometer as
35 per manufacturers instruction. In an embodiment, viscosity is determined a ViscoQC rotational viscometer as per manufacturers instruction.

As used herein, “microbiome” refers to the microorganisms in a particular environment which can include the body or a part of the body of a subject. For example, the cervicovaginal microbiome refers to the community of microorganisms in the cervicovaginal part of the body. For example, the urogenital microbiome refers to the community of microorganisms in the urogenital part of the body.

As used herein a “non-optimal microbiome” (an example being bacterial vaginosis) refers to the microorganisms in a particular environment being at a non-optimal level or relative abundance for normal health (e.g. low level or relative abundance of beneficial microorganisms and/or a high level or relative abundance of pathogenic microorganisms). A non-optimal cervicovaginal microbiome is characterised by e.g. low level or relative abundance of beneficial microorganisms and/or a high level or relative abundance of pathogenic microorganisms and that are associated with an increase in genital inflammation that is typically subclinical due to the elevation of proinflammatory cytokines. A non-optimal cervicovaginal microbiome is described, for example, in McKinnon et al (2019) and can be identified in one example using methods for example, in McKinnon et al (2019) e.g. Amsel’s criteria, Nugent score, molecular assessment such as 16s RNA sequencing. In an embodiment, a non-optimal cervicovaginal microbiome is lactobacillus depleted (e.g. can be determined by molecular assessment). Bacterial vaginosis is a condition characterized by a non-optimal cervicovaginal microbiome which can be diagnosed by Amsel’s criteria and/or Nugent Score (Gram Stain) as described in McKinnon et al (2019).

As used herein, “sub-optimal microbiome” refers to the microbiome being dominated by *Lactobacillus iners* rather than with beneficial *Lactobacillus* spp. eg. *L. crispatus*. In an embodiment, the sub-optimal microbiome has a higher relative abundance or higher level of *Lactobacillus iners* compared to *Lactobacillus crispatus*.

As used herein, an “optimal microbiome that is dominated by beneficial *Lactobacillus* spp. e.g. *Lactobacillus crispatus*. In an embodiment, the optimal microbiome has a higher relative abundance or higher level of *Lactobacillus crispatus* compared to e.g. *Lactobacillus iners* or *Gardnerella vaginalis*.

As used herein, “cytotoxic” refers to a composition or component of a composition that can damage cells and/or cause them to die. In an embodiment, the composition or component is not cytotoxic to the cells of the cervicovaginal epithelial. In an embodiment, the MatTek model as described herein can be used to assess cytotoxicity wherein > or equal to 50% viability compared to untreated tissue is an indication of non-cytotoxicity.

As used herein, “genital/s” refers to the reproductive organs.

As used herein, “cervicovaginal” relates to the uterus, cervix and/or the vagina. The term can include one or more of the epithelial barrier, mucosa, microbiome and fluid. The vagina meets the external environment at the vulva, which also includes the labia, clitoris and urethra. The vagina is attached to the uterus through the cervix (including
5 endo and ectocervix), while the uterus is attached to the ovaries via the fallopian tubes. A reference to the cervicovaginal should be understood to include but not limited to the labium majora, labium minora, clitoris, perineum, vulva and urethral orifice.

As used herein, “integrity of the cervicovaginal epithelial barrier” refers to the connections between the cells of the cervicovaginal epithelial that restricts the passage
10 of pathogens deeper into the tissue and into the blood stream. A decrease in integrity refers to a disruption or decrease in the connections between the cells (e.g. a disruption between one or more tight junction, adherens and desmosomes proteins). The integrity of the epithelial barrier can be measured by, for example trans epithelial electrical resistance (TEER) as described in (Tyssen et al., 2022) or by FITC-labelled dextran flux
15 from the apical to basolateral layers (Hearps et al., 2017).

As used herein, “not substantially” refers to the detection of no difference or the detection of no statistically significant difference.

As used herein, the term “increase” or “increases” or “increased” or “increasing” refers to having a higher or greater level of a given parameter after application of the
20 compositions, methods or uses as described herein compared to the level of a given parameter at baseline.

As used herein, the term “reduce” or “reduces” or “reduced” or “reducing” refers to having a lower or lesser level of a given parameter after application of the compositions, methods or uses as described herein compared to the level of a given
25 parameter at baseline.

As used herein, the term “pharmaceutical composition” refers to a standard and purity suitable for medicinal use. In an embodiment, the pharmaceutical composition is a prescription pharmaceutical composition. Meaning that it is only available with a prescription. In an embodiment, the pharmaceutical composition is an over the counter
30 composition. As used herein, the term “over the counter” or “OTC” means that the composition is a medicine that is available without a prescription.

Vaginal pharmaceutical compositions

Current over the counter (OTC) vaginal gels containing lactic acid are available
35 and marketed for self-treatment of BV but most are hyperosmolal (more salt or solutes) in contrast to the cervicovaginal fluid from women with an optimal vaginal microbiome,

which causes mucosal irritation and decreased epithelial cell viability and barrier integrity *in vitro* (Ayehunie et al., (2018)). Hyperosmolal gels cause epithelial damage that promotes the transmission of sexually transmitted diseases including HIV. Consequently, the WHO recommends an osmolality limit of less than 1200 mOsm/kg for vaginal gels. However, these OTC vaginal gels have unfavourable inflammatory properties and/or effects on the integrity of the cervicovaginal epithelial barrier.

In some embodiments, the vaginal pharmaceutical compositions of the present invention have a similar osmolality or are iso-osmolal with the cervicovaginal fluid (300 to 800 mOsm/Kg).

10 In some embodiments, the vaginal pharmaceutical compositions of the present inventions have a similar osmolality to the cervicovaginal fluid (500 to 800 mOsm/Kg).

In some embodiments, the vaginal pharmaceutical compositions of the present inventions are iso-osmolal with the cervicovaginal fluid. As used herein, the composition being “iso-osmolal with cervicovaginal fluid” refers to the composition having an osmolality consistent with the osmolality of cervicovaginal fluid in a healthy subject. In an embodiment, the subject is human. In an embodiment, the composition comprises an osmolality of 300 to 410 mOsm/Kg. In an embodiment, the composition comprises an osmolality of about 330 to about 390 mOsm/Kg. In an embodiment, the composition comprises an osmolality of about 330 to about 380 mOsm/Kg. In an embodiment, the composition comprises an osmolality of about 380 to about 390 mOsm/Kg. In an embodiment, the composition comprises an osmolality of 330 to 390 mOsm/Kg. In an embodiment, the composition comprises an osmolality of 330 to 380 mOsm/Kg. In an embodiment, the composition comprises an osmolality of 380 to 390 mOsm/Kg.

25 In an aspect, the present invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides a vaginal pharmaceutical composition comprising a) lactic acid at about 1.40 % w/w to about 2.5% w/w; b) a mucoadhesive agent; c) a viscosity of about 50 to about 150 Pa.s; wherein the composition is iso-osmolal with cervicovaginal fluid.

30 As used herein, “lactic acid” refers to a molecule of the formula $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{H}$. It is also referred to as 2-Hydroxypropanoic acid. In an embodiment, about 18% to about 90% of the lactic acid is protonated. In an embodiment, about 18% to about 85% of the lactic acid is protonated. In an embodiment, about 18% to about 82% of the lactic acid is protonated. In an embodiment, about 30% to about 70% of the lactic acid is protonated. In an embodiment, about 40% to about 60% of the lactic

acid is protonated. In an embodiment, about 45% to about 55% of the lactic acid is protonated. In an embodiment, about 48% to about 52% of the lactic acid is protonated.

In an embodiment, when the pH is about 3.2 about 75% to about 88% of the lactic acid is protonated. In an embodiment, when the pH is about 3.2 about 82% of the lactic acid is protonated.

In an embodiment, when the pH is about 3.8 about 45% to about 60% of the lactic acid is protonated. In an embodiment, when the pH is about 3.8 about 53% of the lactic acid is protonated.

In an embodiment, when the pH is about 4.5 about 14% to about 24% of the lactic acid is protonated. In an embodiment, when the pH is about 4.5 about 18.5% of the lactic acid is protonated.

In an embodiment, the lactic acid is not at a supraphysiological concentration.

In an embodiment, the composition comprises lactic acid at about 1.4% w/w to about 2.5% w/w. In an embodiment, the composition comprises lactic acid at about 1.5% w/w to about 2.4% w/w. In an embodiment, the composition comprises lactic acid at about 1.55% w/w to about 2.35% w/w. In an embodiment, the composition comprises lactic acid at about 1.58% w/w to about 2.3% w/w. In an embodiment, the composition comprises lactic acid at about 1.6% w/w to about 2.3% w/w. In an embodiment, the composition comprises lactic acid at about 1.6% w/w to about 2.25% w/w. In an embodiment, the composition comprises lactic acid at about 1.64% w/w to about 2.25% w/w. In an embodiment, the compositions comprise lactic acid at about 1.64% w/w.

In an aspect, the present invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/v to about 2.5% w/v, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides a vaginal pharmaceutical composition comprising a) lactic acid at about 1.40 % w/v to about 2.5% w/v; b) a mucoadhesive agent; c) a viscosity of about 50 to about 150 Pa.s; wherein the composition is iso-osmolal with cervicovaginal fluid.

In an embodiment, the composition comprises lactic acid at about 1.4% w/v to about 2.5% w/v. In an embodiment, the composition comprises lactic acid at about 1.5% w/v to about 2.4% w/v. In an embodiment, the composition comprises lactic acid at about 1.55% w/v to about 2.35% w/v. In an embodiment, the composition comprises lactic acid at about 1.58% w/v to about 2.3% w/v. In an embodiment, the composition comprises lactic acid at about 1.6% w/v to about 2.3% w/v. In an embodiment, the composition comprises lactic acid at about 1.6% w/v to about 2.25% w/v. In an embodiment, the composition comprises lactic acid at about 1.64% w/v to about 2.25% w/v. In an embodiment, the

composition comprises lactic acid at about 1.64% w/v to about 2.25% w/v. In an embodiment, the composition comprises lactic acid at about 1.64% w/v.

In an embodiment, the composition comprises L-lactic acid. In an embodiment, the composition comprises D-lactic acid. In an embodiment, the composition comprises D-lactic acid and L-lactic acid. In an embodiment, the ratio of D-lactic acid to L-lactic acid is selected from: a) about 0.8 to 1 to about 1.5 to 1; c) about 1 to 1; and d) about 1.5 to 1. In an embodiment, the ratio of D-lactic acid to L-lactic acid is about 0.8 to 1 to about 1.5 to 1. In an embodiment, the ratio of D-lactic acid to L-lactic acid is about 1 to 1. In an embodiment, the ratio of D-lactic acid to L-lactic acid is about 1.5 to 1.

In an embodiment, the mucoadhesive agent can be any mucoadhesive agent suitable for vaginal administration that does not promote cervicovaginal inflammation. In an embodiment, the mucoadhesive agent can be any mucoadhesive agent suitable for vaginal administration that, in combination with the other components of the composition provides a viscosity of about 50 to about 150 Pa.s.

In an embodiment, the mucoadhesive agent is selected from one or more of: hydroxyethylcellulose (HEC) or a derivative thereof, a carbomer/Carbopol®, methylhydroxypropyl cellulose, a hydrogel, and hydroxypropyl methylcellulose. In an embodiment, the mucoadhesive agent is HEC or a derivative thereof. In an embodiment, the mucoadhesive agent is HEC. In an embodiment, the mucoadhesive agent is a carbomer/Carbopol®. In an embodiment, the mucoadhesive agent is methylhydroxypropyl cellulose. In an embodiment, the mucoadhesive agent is a hydrogel. In an embodiment, the mucoadhesive agent is hydroxypropyl methylcellulose.

In some embodiments, the mucoadhesive agent is a polyacrylic acid (carbomer)/Carbopol® polymer such as carbomer homopolymer type A, carbomer homopolymer type B, Carbopol® 974, Carbopol® 971, Carbopol® 940, Carbopol® 941, Carbopol® 934, Carbopol® 980 and Carbopol® 981. In an embodiment, the carbomer is homopolymer type A. In an embodiment, the carbomer is carbomer homopolymer type B. In an embodiment, the carbomer is Carbopol® 974. In an embodiment, the carbomer is Carbopol® 971. In an embodiment, the carbomer is Carbopol® 940. In an embodiment, the carbomer is Carbopol® 941. In an embodiment, the carbomer is Carbopol® 934. In an embodiment, the carbomer is Carbopol® 980. In an embodiment, the carbomer is Carbopol® 981.

In an embodiment, the mucoadhesive agent is a non-ionic polymer.

In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 0.1% to about 20% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 0.1% to about 10% w/w.

In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 0.1% to about 5% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 0.5% to about 5% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 0.5% to about 3% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 1% to about 3% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 1% to about 2.5% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 1% to about 2% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 1% to about 1.5% w/w. In an embodiment, the composition comprises the mucoadhesive agent at a concentration of about 1.32% w/w.

In an embodiment, the composition has a pH of about 3.2 to about 4.5. In an embodiment, the composition has a pH of about 3.2 to about 4.5. In an embodiment, the composition has a pH of about 3.2 to about 4.2. In an embodiment, the composition has a pH of about 3.3 to about 4.1. In an embodiment, the composition has a pH of about 3.4 to about 4. In an embodiment, the composition has a pH of about 3.5 to about 3.9. In an embodiment, the composition has a pH of about 3.7 to 3.8.

In an embodiment, the pH of the composition was adjusted with sodium hydroxide or hydrochloric acid.

In an embodiment, the pH of the composition has a pH of about 4.2 to about 4.5 and an osmolality of 500 to 800 mOsm/Kg.

In an embodiment, the pH of the composition has a pH of about 4.5 and an osmolality of 500 to 800 mOsm/Kg.

In an embodiment, the composition additionally comprises one or more of: a paraben, sodium lactate and sodium chloride. In an embodiment, the paraben is methylparaben and/or propylparaben. In an embodiment, the paraben is methylparaben. In an embodiment, the paraben is propylparaben.

In an embodiment, the paraben is present at a concentration of about 0.01% to about 0.3%, or about 0.01 to about 0.2%, or about 0.02% to about 0.18%, or about 0.18%, or about 0.02% w/w. In an embodiment, the paraben is present at a concentration of about 0.01 to about 0.3% w/w. In an embodiment, the paraben is present at a concentration of or about 0.01 to about 0.2% w/w. In an embodiment, the paraben is present at a concentration of or about 0.02% to about 0.18% w/w. In an embodiment, the paraben is present at a concentration of about 0.18% w/w. In an embodiment, the paraben is present at a concentration of about 0.02% w/w. In an embodiment, the paraben is methylparaben

and is present at about 0.18% w/w. In an embodiment, the paraben in propylparaben and is present at about 0.02% w/w.

In an embodiment, the composition does not comprise a paraben.

In an embodiment, the composition is not cytotoxic to cervicovaginal epithelial
5 cells.

In an embodiment, the composition does not disrupt the integrity of the cervicovaginal epithelial barrier.

In an embodiment, the composition maintains or increases the integrity of the cervicovaginal epithelial barrier.

10 In an embodiment, the composition comprises a preservative selected from one or more of: glyceryl monocaprylate, glyceryl monolaurate, phenoxyethanol, benzyl alcohol, a quaternary ammonium compound and a paraben as described herein. In an embodiment, the quaternary ammonium compound is selected from one or both of benzalkonium chloride or benzethonium chloride.

15 In an embodiment, the composition comprises an antioxidant selected from one or more of: ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, tocopherol and tocopherol acetate. In an embodiment, the antioxidant comprises ascorbic acid. In an embodiment, the antioxidant comprises ascorbyl palmitate. In an embodiment, the antioxidant comprises butylated
20 hydroxyanisole. In an embodiment, the antioxidant comprises butylated hydroxytoluene. In an embodiment, the antioxidant comprises propyl gallate. In an embodiment, the antioxidant comprises tocopherol. In an embodiment, the antioxidant comprises tocopherol acetate.

In an embodiment, the composition as described herein does not comprise a
25 component or an excipient that induces genital inflammation in a subject. In an embodiment, the composition does not comprises a component or excipient selected from one or more or all of: ethylenediaminetetraacetic acid (EDTA), glycerine/glycerol, polyethylene glycol (PEG), citric acid, organic aloe barbadensis leave juice, xanthan gum, natural flavour, potassium sorbate, potassium bitartrate, acetic acid, butyric acid,
30 succinic acid, boric acid, propylene glycol, caprylyl glycol, levulinic acid, P-anisic acid, xylose, clary sage oil, phenethyl alcohol, thymus mastichina herb oil, triethanolamine, hydrogenated castor oil ethoxylated, ethylparaben, propionic acid, sodium benzoate, chlorhexidine gluconate and sorbic acid. In an embodiment, the composition does not comprise EDTA. In an embodiment, the composition does not comprise
35 glycerine/glycerol. In an embodiment, the composition does not comprise PEG. In an embodiment, the composition does not comprise citric acid. In an embodiment, the

composition does not comprise organic aloe barbadensis leave juice. In an embodiment, the composition does not comprise xanthan gum. In an embodiment, the composition does not comprise natural flavour. In an embodiment, the composition does not comprise potassium. In an embodiment, the composition does not comprise potassium sorbate. In an embodiment, the composition does not comprise potassium bitartrate. In an embodiment, the composition does not comprise acetic acid. In an embodiment, the composition does not comprise butyric acid. In an embodiment, the composition does not comprise succinic acid. In an embodiment, the composition does not comprise boric acid. In an embodiment, the composition does not comprise propylene glycol. In an embodiment, the composition does not comprise caprylyl glycol. In an embodiment, the composition does not comprise levulinic acid. In an embodiment, the composition does not comprise P-anisic acid. In an embodiment, the composition does not comprise xylose. In an embodiment, the composition does not comprise clary sage oil. In an embodiment, the composition does not comprise phenethyl alcohol. In an embodiment, the composition does not comprise thymus mastichina herb oil. In an embodiment, the composition does not comprise triethanolamine. In an embodiment, the composition does not comprise hydrogenated castor oil ethoxylated. In an embodiment, the composition does not comprise ethylparaben. In an embodiment, the composition does not comprise propionic acid. In an embodiment, the composition does not comprise sodium benzoate. In an embodiment, the composition does not comprise chlorhexidine gluconate. In an embodiment, the composition does not comprise sorbic acid. In an embodiment, the composition does not comprise potassium and calcium. In an embodiment, the composition does not comprise magnesium and manganese. In an embodiment, the composition does not comprise potassium, calcium, magnesium and manganese.

In an embodiment, the compositions as described herein are suitable for treating or preventing genital inflammation in a subject. In an embodiment, the inflammation is cervicovaginal inflammation.

In an embodiment, the composition does not significantly increase the level of pro-inflammatory cytokines in the subject.

In an embodiment, the composition does not substantially increase the expression of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β (interleukin 1 beta), IL-6 (interleukin 6), IL-12p40 (interleukin 12p40), IL-12p70 (interleukin 12p70), tumor necrosis factor (TNF)- α , TNF- β , TNF-related apoptosis inducing ligand (TRAIL), interferon (IFN)- γ . In an embodiment, the composition does not substantially increase the expression of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β (interleukin 1 beta) and IL-6

(interleukin 6). In an embodiment, the composition does not substantially increase the expression of pro-inflammatory cytokine IL-1 α . In an embodiment, the composition does not substantially increase expression of the pro-inflammatory cytokine IL-1 β . In an embodiment, the composition does not substantially increase the expression of pro-inflammatory cytokine IL-6.

In an embodiment, the composition reduces the expression of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β (interleukin 1 beta), IL-6 (interleukin 6), IL-12p40 (interleukin 12p40), IL-12p70 (interleukin 12p70), tumor necrosis factor (TNF)- α , TNF- β , TNF-related apoptosis inducing ligand (TRAIL), interferon (IFN)- γ . In an embodiment, the composition reduces the expression of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β (interleukin 1 beta) and IL-6 (interleukin 6). In an embodiment, the composition reduces the expression of pro-inflammatory cytokine IL-1 α . In an embodiment, the composition reduces expression of the pro-inflammatory cytokine IL-1 β . In an embodiment, the composition reduces the expression of pro-inflammatory cytokine IL-6.

In an embodiment, the composition does not significantly increase the level of pro-inflammatory chemokines in the subject. In an embodiment, the composition does not substantially increase the expression of a pro-inflammatory chemokines selected from: MIP3 α (macrophage inflammatory protein-3 alpha), IL-8 (interleukin 8), IP-10 (IFN—gamma-inducible protein 10), and RANTES (regulated upon activation, normal T-cell expressed and presumably secreted). In an embodiment, the composition does not substantially increase expression of the pro-inflammatory chemokine MIP3 α . In an embodiment, the composition does not substantially increase expression of the pro-inflammatory chemokine IL-8. In an embodiment, the composition does not substantially increase expression of the pro-inflammatory chemokine RANTES.

In an embodiment, the composition reduces the expression of a pro-inflammatory chemokines selected from: MIP3 α (macrophage inflammatory protein-3 alpha), IL-8 (interleukin 8), IP-10 (IFN—gamma-inducible protein 10), and RANTES (regulated upon activation, normal T-cell expressed and presumably secreted). In an embodiment, the composition reduces expression of the pro-inflammatory chemokine MIP3 α . In an embodiment, the composition reduces expression of the pro-inflammatory chemokine IL-8. In an embodiment, the composition reduces expression of the pro-inflammatory chemokine RANTES.

In an embodiment, the composition increases the expression of the anti-inflammatory factor interleukin-1 receptor antagonist (IL-1RA).

In an embodiment, the compositions as described herein are suitable for preventing a urogenital infections (i.e. urinary tract) in a subject.

In an embodiment, the composition is not cytotoxic to urogenital epithelial cells.

In an embodiment, the composition does not disrupt the integrity of the urogenital
5 epithelial barrier.

In an embodiment, the composition maintains or increases the integrity of the urogenital epithelial barrier.

In an embodiment, the composition as described herein does not comprise a component or an excipient that induces urogenital inflammation in a subject.

10 In an aspect, the invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 4.5% w/w, wherein the composition
15 has an osmolality of 300 to 800 mOsm/Kg.

In an aspect, the invention provides a vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/v to about 2.5% w/v, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the invention provides a vaginal pharmaceutical composition
20 comprising lactic acid at about 1.40 % w/v to about 4.5% w/v, wherein the composition has an osmolality of 300 to 800 mOsm/Kg.

Inflammation

As used herein, "inflammation" refers to a local response to cellular injury
25 characterised by one or more: vasodilation, increased vascular permeability, cellular infiltration, changes to the biosynthetic, metabolic and catabolic profiles of affected organs, and activation of the cells of the immune system. Without limiting the present invention to any one theory or mode of action, the inflammatory response is a complex response characterised by a series of physiological and/or immunological events which
30 are induced to occur by the release of a cytokine cascade in response to any one of a variety of stimuli including, but not limited to, tissue injury, infection, an immune response (such as to a pathogen or an innocuous agent – as occurs with allergies) or disease (such as tumour formation or an autoimmune response). Inflammation serves as a biological mechanism initiating the elimination of noxious agents and/or damaged
35 cells. In an embodiment, the inflammation is subclinical inflammation. In an

embodiment, the inflammation is associated with a non-optimal or a sub-optimal microbiome.

As used herein, the term “modulating inflammation” refers to increasing or decreasing inflammation in a subject.

5 Inflammation (including subclinical inflammation) can be assessed using any method known to a person skilled in the art including measurement of the levels of one or more of: pro-inflammatory cytokines, pro-inflammatory cytokines and anti-inflammatory factors. In an embodiment, the inflammation can be measured based on inflammatory cytokine and/or chemokine profiles by K-means clustering, described for
10 example in Alisoltani et al., 2020.

In an embodiment, the inflammation is associated with an increase in the level of pro-inflammatory chemokines.

In an embodiment, inflammation is assessed by measuring the level of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β
15 (interleukin 1 beta), IL-6 (interleukin 6), IL-12p40 (interleukin 12p40), IL-12p70 (interleukin 12p70), tumor necrosis factor (TNF)- α , TNF- β , TNF-related apoptosis inducing ligand (TRAIL), and interferon (IFN)- γ .

In an embodiment, inflammation is assessed by measuring the level of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β
20 (interleukin 1 beta) and IL-6 (interleukin 6), wherein an increased level indicates inflammation.

In an embodiment, inflammation is assessed by measuring the level of one or more pro-inflammatory chemokines selected from: MIP3 α (macrophage inflammatory protein-3 alpha), IL-8 (interleukin 8), IP-10 (IFN—gamma-inducible protein 10), and
25 RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), wherein an increased level indicates inflammation.

In an embodiment, inflammation is assessed by measuring the level of one or more anti-inflammatory factors, wherein a reduced levels indicates inflammation. In an embodiment, the anti-inflammatory factor is interleukin-1 receptor antagonist (IL-1RA).

30 In an embodiment, the condition to be treated or prevented is a cause or a result of genital inflammation. As used herein, “genital inflammation” refers to inflammation that disrupts the mucosal epithelial barrier of the genitals, increases the expression of one or more pro-inflammatory cytokines and/or increases the expression of one or more pro-inflammatory chemokines in the subject. Disruption of the mucosal epithelial barrier is
35 undesirable as it can enable pathogen penetration. Increased genital inflammation in a subject increases adverse sexual and reproductive health outcomes. In an embodiment,

the genital inflammation is cervicovaginal inflammation. In an embodiment, the genital inflammation is vaginitis (e.g. viral vaginitis, atrophic vaginitis, non-infectious vaginitis and vulvovaginitis). In an embodiment, the genital inflammation is vulvovaginitis. In an embodiment, the genital inflammation is vaginosis. In an embodiment, the genital inflammation is male accessory gland infection (MAGI). In an embodiment, the genital inflammation is balanitis.

In an embodiment, the genital inflammation comprises one or more of the following symptoms: itchy vagina; sore vagina; feminine discomfort, vaginal discomfort, vaginal irritation, vaginal discharge; vaginal dryness; pain when peeing; pain when having sex; light vaginal bleeding; sore, swollen or cracked skin around the vagina.

In an embodiment, the vaginitis comprises one or more of the following symptoms: itchy vagina; sore vagina; feminine discomfort, vaginal discomfort, vaginal irritation, vaginal discharge; vaginal dryness; pain when peeing; pain when having sex; light vaginal bleeding; sore, swollen or cracked skin around the vagina.

A person skilled in the art would appreciate that many of the above symptoms can be assessed using the Female Genital Grading Table for Use in Microbicide Studies (Division of Aids Adverse Event Grading Tables (2007)).

In an embodiment, the cervicovaginal inflammation is the cause or result of a condition selected from one or more of following conditions: BV, Chlamydia trachomatis, gonorrhoea, human papillomavirus, human immunodeficiency virus, trichomonas infection, vaginitis, pelvic inflammatory disease, ureaplasma, mycoplasma (e.g. mycoplasma genitalium), syphilis, allergy, campylobacter, improper hygiene, douching or other vaginal practices, vaginal penetration of the penis (with or without a condom) and herpes simplex virus. In an embodiment, the cervicovaginal inflammation is the cause or result of BV. In an embodiment, the cervicovaginal inflammation is the result of Chlamydia trachomatis. In an embodiment, the cervicovaginal inflammation is the result of gonorrhoea. In an embodiment, the cervicovaginal inflammation is the result of human papillomavirus. In an embodiment, the cervicovaginal inflammation is the result of human immunodeficiency virus. In an embodiment, the cervicovaginal inflammation is the result of trichomonas infection. In an embodiment, the cervicovaginal inflammation is the cause or result of ureaplasma. In an embodiment, the cervicovaginal inflammation is the result of syphilis. In an embodiment, the cervicovaginal inflammation is the result of allergy. In an embodiment, the cervicovaginal inflammation is the cause or result of campylobacter. In an embodiment, the cervicovaginal inflammation is the result of mycoplasma. In an embodiment, the cervicovaginal inflammation is the result of improper hygiene. In an embodiment, the

cervicovaginal inflammation is the result of douching or other vaginal practices. In an embodiment, the cervicovaginal inflammation is the result of herpes simplex virus. In an embodiment, the inflammation is BV associated inflammation.

5 In an embodiment, the condition is the cause or result of a non-optimal microbiome.

In an embodiment, the condition is the cause or result of a sub-optimal microbiome.

10 In an embodiment, condition as described herein disrupts one or more of: the cervicovaginal pH, the cervicovaginal microbiome, the properties of the cervicovaginal mucosa, the properties of the cervicovaginal fluid and the integrity of the cervicovaginal epithelial barrier.

15 In an embodiment, the inflammation is urogenital tract inflammation (inflammation associated with a urinary tract infection). As used herein, a urinary tract infection (UTI) is an infection in any part of the urogenital system. In an embodiment, the urinary tract infection is a recurrent urinary tract infection.

Methods and uses

In an embodiment, the composition as described herein is for treating or preventing genital inflammation as described herein.

20 In an embodiment, the composition as described herein is for treating or preventing genital odour. As used herein, the terms "treating" or "treatment" refers to at least partially obtaining a desired therapeutic outcome. In an embodiment, treatment comprises reducing or eliminating at least one symptom of a specified disease or condition.

25 As used herein, the term "prevention" or "prophylaxis" refers to stopping or hindering the development of at least one symptom of a specified disease or condition. Prevention need not be complete and does not imply that a subject will not eventually develop the symptom of the specified disease or condition.

30 In an embodiment, the composition as described herein is for use in modulating the microbiome. In an embodiment, the microbiome is the genital microbiome. In an embodiment, the microbiome is the cervicovaginal microbiome.

35 In an embodiment, the composition as described herein is for use in favourably optimising the cervicovaginal microbiome in subject (pushing the microbiome away from a diseased state towards the microbiome of a healthy subject/pushing the microbiome from non-optimal or sub-optimal to an optimal microbiome). In an embodiment, the composition as described herein is for use in favourably optimising the

urogenital microbiome in a subject. In an embodiment, favourably optimising the microbiome comprises increases or decreasing the level or relative abundance of select microorganisms.

As used herein, “increase the level” refers to an increase in the number of a specific microorganism compared to a baseline level.

As used herein, “relative abundance” refers to the percentage of a microbiome made up a specific microorganism. A person skilled in the art will appreciate the relative abundance can be assessed

A person skilled in the art will appreciate that symptoms of genital inflammation and genital odour can be assessed using the Female Genital Grading Table for Use in Microbicide Studies (Division of Aids Adverse Event Grading Tables (2007)).

In an embodiment, the composition is for use in maintaining or increasing the level of *Lactobacillus crispatus* in the cervicovaginal microbiome. In an embodiment, the composition is for use in maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in maintaining or increasing the level of *Lactobacillus crispatus* in the urogenital microbiome. In an embodiment, the composition is for use in maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the urogenital microbiome.

In an embodiment, the composition is for use in reducing the level of *Gardnerella vaginalis* in the urogenital microbiome. In an embodiment, the composition is for use in reducing the relative abundance of *Gardnerella vaginalis* in the urogenital microbiome.

In an embodiment, increasing the level of *Lactobacillus crispatus* in the urogenital microbiome reduces the level of *Escherichia coli* in the urogenital microbiome.

In an embodiment, increasing the relative abundance of *Lactobacillus crispatus* in the urogenital microbiome reduces the relative abundance of *Escherichia coli* in the urogenital microbiome.

In an embodiment, increasing the level of *Lactobacillus crispatus* in the urogenital microbiome reduces the level of *Gardnerella vaginalis* in the urogenital microbiome.

In an embodiment, increasing the relative abundance of *Lactobacillus crispatus* in the urogenital microbiome reduces the relative abundance of *Gardnerella vaginalis* in the urogenital microbiome.

In an embodiment, the composition is for use in maintaining or increasing the level of *Lactobacillus gasseri* in the cervicovaginal microbiome. In an embodiment, the composition is for use in maintaining or increasing the relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in maintaining or increasing the level of *Lactobacillus jensenii* in the cervicovaginal microbiome. In an embodiment, the composition is for use in maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome.

5 In an embodiment, the composition is for use in decreasing the level of *Lactobacillus iners* in the cervicovaginal microbiome. In an embodiment, the composition is for use in decreasing the relative abundance of *Lactobacillus iners* in the cervicovaginal microbiome.

10 In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus gasseri* to *Lactobacillus iners* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus jensenii* to *Lactobacillus iners* in the cervicovaginal microbiome.

15 In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus crispatus* to *Gardnerella vaginalis* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus crispatus* to *Prevotella spp.* in the cervicovaginal microbiome.

20 In an embodiment, the composition is for use in increasing the ratio of *Lactobacillus crispatus* to *Fannyhessia vaginae* in the cervicovaginal microbiome.

In an embodiment, the composition is for use in maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.

In an embodiment, the composition is for increasing the integrity of the cervicovaginal epithelial barrier in a subject.

25 In an embodiment, the composition is for maintaining the integrity of the cervicovaginal epithelial barrier in a subject.

In an embodiment, the composition is for reducing vaginal discomfort.

In an embodiment, the composition is for reducing vaginal dryness.

In an embodiment, the composition is for reducing vaginal itch.

30 In an embodiment, the composition is for reducing excessive vaginal discharge.

In an embodiment, the composition is for reducing vaginal odour.

In an embodiment, the composition is for reducing one or more symptoms of vaginitis.

35 In an embodiment, the composition is for maintaining a naturally acidic vaginal pH. In an embodiment, the pH is less than or equal to about pH 4.5. In an embodiment, the pH is about 3.5 to about 4.5.

In an aspect, the

present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for treating or preventing genital inflammation in a subject.

5 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for treating or preventing cervicovaginal inflammation in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject.

10 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

20 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Prevotella spp.* in the cervicovaginal microbiome in a subject.

30 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the level of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

35 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the

relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Prevotella spp.* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for decreasing the relative abundance of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for increasing the ratio of *Lactobacillus crispatus* to *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/v in the manufacture of a medicament for treating or preventing genital inflammation in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for treating or preventing cervicovaginal inflammation in a subject.

5 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome in a
10 subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.

15 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the level of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

20 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the level of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the level of
25 *Prevotella spp.* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the level of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

30 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the level of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome in a
35 subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.

5 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the relative abundance of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the relative
10 abundance of *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the relative abundance of *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v
15 to about 2.5% w/v in the manufacture of a medicament for decreasing the relative abundance of *Prevotella spp.* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for decreasing the relative abundance of *Fannyhessia vaginae* in the cervicovaginal microbiome in a subject.

20 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintain or increasing the relative abundance of *Lactobacillus vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v
25 to about 2.5% w/v in the manufacture of a medicament for increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for increasing the ratio of
30 *Lactobacillus crispatus* to *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/v to about 2.5% w/v in the manufacture of a medicament for maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.

35 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for reducing vaginal discomfort.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for reducing vaginal dryness.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for reducing vaginal itch.

5 In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for reducing excessive vaginal discharge.

In an aspect, the present invention provides use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for reducing one or more
10 symptoms of vaginitis.

Other therapeutics

A person skilled in the art will appreciate that the compositions, methods, uses and devices as described herein may comprise one or more other therapeutics.

15 In an embodiment, the therapeutic is a small molecule. The term “small molecule” as used herein, refers to a chemical compound or molecule having a molecular weight below 2000 Daltons, preferably below 1500 Daltons, more preferably below 1000 Daltons, still more preferably below 750 daltons, yet more preferably below 500 Daltons. In an embodiment, the small molecule is not a polypeptide.

20 In an embodiment the therapeutic is a biotherapeutic. As used herein, “biotherapeutic” refers to a pharmaceutical made from and/or comprises living cells or organisms.

In an embodiment, the therapeutic is an antibiotic. In an embodiment, the antibiotic is metronidazole. In an embodiment, the antibiotic is clindamycin. In an
25 embodiment, the antibiotic is tinidazole. In an embodiment, the antibiotic is secnidazole. In an embodiment, the antibiotic is trimethoprim. In an embodiment, the antibiotic is sulfamethoxazole. In an embodiment, the antibiotic is cephalexin.

In an embodiment, the therapeutic is an anti-retroviral. In an embodiment, the antiretroviral is selected from: dapivirine, tenofovir, nevirapine, emtricitabine,
30 cabotegravir, rilpivirine, and saquinavir.

In an embodiment, the therapeutic is a hormone. In an embodiment, the therapeutic is female sex hormone.

In an embodiment, the therapeutic is a contraceptive. In an embodiment, the contraceptive is a hormone (e.g. etonogestral or estradiol). In an embodiment, the
35 contraceptive is a non-hormonal contraceptive (e.g. HC4-N as described in Thurman et al., 2023 or an anti-CD52g antibody Baldeon-Vaca et al., 2021).

In an embodiment, the therapeutic is a biotherapeutic. In an embodiment, the biotherapeutic is live biotherapeutic (a microorganism) or a component thereof. As used herein, “microorganism” or “microorganisms” refers to microscopic organisms including bacterial, viral, fungal or eukaryotic organisms.

5 In an embodiment, the biotherapeutic is probiotic. As used herein, “probiotic” refers to live microorganism which when administered in an adequate amount confers a health benefit to a subject as described herein.

In an embodiment, the biotherapeutic is a postbiotic. As used herein, a “postbiotic” refers to a preparation of inanimate microorganisms and/or a component of
10 the microorganism that confers a health benefit on the host directly or indirectly (e.g. as described in Vinderola et al., 2022). In an embodiment, the inanimate microorganism confers the health benefit to the host directly or indirectly. In an embodiment, the inanimate microorganism confers the health benefit to the host directly. In an embodiment, the inanimate microorganism confers the health benefit to the host
15 indirectly. In an embodiment, one or more components of the microorganism confers the health benefit to the host. In an embodiment, the postbiotic comprises a microorganism that has been killed (e.g. by heat or chemical treatment).

As used herein, “a component”, “component of the microorganism” and “postbiotic component” refers to a part (fragment) of the cell or a product secreted from
20 a cell. Examples of postbiotic components are described for example in Zólkiewicz et al. (2020) and Vinderola et al. (2022). In an embodiment, the component is selected from one or more of: a cell wall fragment, cell supernatant, cell lysate, intracellular metabolite, secreted metabolite, enzyme, protein, short chain fatty acid, vitamin, phenol, amino acid, bacteriocin, extracellular vesicle, and exopolysaccharide.

25 As used herein, “prebiotic” refers to a substrate that is selectively utilized by host microorganisms conferring a health benefit.

In an embodiment, a prebiotic may additionally be administered.

In an embodiment, wherein the biotherapeutic is a strain of bacteria.

In an embodiment, the bacteria are bacteria as described in Happel et al (2017).

30 In an embodiment, the bacteria are selected from one or more of: *Lactobacillus*, *Leuconostoc*, *Bifidobacterium*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*.

In an embodiment, the bacteria are *Lactobacillus*. In an embodiment, the bacteria
35 are *Leuconostoc*. In an embodiment, the bacteria are *Bifidobacterium*. In an embodiment, the bacteria are *Pediococcus*. In an embodiment, the bacteria are *Lactococcus*. In an

embodiment, the bacteria are *Streptococcus*. In an embodiment, the bacteria are *Aerococcus*. In an embodiment, the bacteria are *Carnobacterium*. In an embodiment, the bacteria are *Enterococcus*. In an embodiment, the bacteria are *Oenococcus*. In an embodiment, the bacteria are *Sporolactobacillus*. In an embodiment, the bacteria are *Tetragenococcus*. In an embodiment, the bacteria are *Vagococcus*. In an embodiment, the bacteria are *Weissella*. In an embodiment, the bacteria are *B. longum* ssp. *Longum*. In an embodiment, the bacteria are *Lactobacillus rhamnosus*.

In an embodiment, the *Lactobacillus* is selected from one or more of: *Lactobacillus crispatus*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus gasseri*.

In an embodiment, the *Lactobacillus* is selected from one or more of: *Lactobacillus crispatus*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus gasseri*.

In an embodiment, the *Lactobacillus* is a *Lactobacillus crispatus*. In an embodiment the *Lactobacillus crispatus* is CTV-05. In an embodiment, the *Lactobacillus* is *Lactobacillus acidophilus*.

In an embodiment, the *Lactobacillus* is *Lactobacillus rhamnosus*. In an embodiment, the *Lactobacillus rhamnosus* is GR-1TM.

In an embodiment, the *Lactobacillus* is a *Lactobacillus plantarum*. In an embodiment, the *Lactobacillus plantarum* is P 17630. In an embodiment, the *Lactobacillus plantarum* is *Lactobacillus crispatus* IP174178.

In an embodiment, the *Lactobacillus* is a *Lactobacillus reuteri*. In an embodiment, the *Lactobacillus reuteri* is RC-14TM.

In an embodiment, the *Lactobacillus* is *Lactobacillus rhamnosus* is GR-1TM and *Lactobacillus reuteri* is RC-14TM.

In an embodiment, the strain of bacteria is isolated from the vagina.

In an embodiment, the biotherapeutic is selected from: Lactin-V, Canesflor®, PhysioFlor®, V-spot, Bioflora Vagiforte® Plus, ReuterinaTM Femme, UltraFlora® Women's and Provacare® Probiotic Vaginal Care. In an embodiment, the biotherapeutic is Lactin-V (a live probiotic). In an embodiment, the biotherapeutic is Canesflor®. In an embodiment, the biotherapeutic is PhysioFlor®. In an embodiment, the biotherapeutic is V-spot. In an embodiment, the biotherapeutic is Bioflora Vagiforte® Plus. In an embodiment, the biotherapeutic is ReuterinaTM Femme. In an embodiment, the biotherapeutic is UltraFlora® Women's. In an embodiment, the biotherapeutic is Provacare® Probiotic Vaginal Care.

Administration

A person skilled in the art will appreciate that the compositions, methods, uses as described herein are administered topically to the genitals of a subject and/or a sexual partner of the subject.

5 In an embodiment, the composition is for daily administration. In an embodiment, the composition is for twice daily administration. In an embodiment, the composition is administered three times per day. In an embodiment, the composition is for continuous or semi-continuous administration (occurring at regular or irregular intervals). In an embodiment, the composition is for periodic presumptive dosing e.g. once a week, after
10 menses to help with optimal vaginal microbiome, or in proximity to sex (before or after).

In an embodiment, administration is for about 7 days. In an embodiment, administration is for about 7 days to about 70 days. In an embodiment, administration is for about 20 days to about 65 days. In an embodiment, administration is for about 25 days to about 65 days. In an embodiment, administration is for about 30 days to about 60
15 days. In an embodiment, administration is for at least about 30 days. In an embodiment, administration is for at least about 45 days. In an embodiment, administration is for at least about 60 days.

In an embodiment, the composition is in the form selected from a: gel, cream, capsule, ovule, foam or lubricant. In an embodiment, the composition is a gel. In an embodiment, the composition is a cream. In an embodiment, the composition is a capsule. In an embodiment, the composition is an ovule. In an embodiment, the composition is a foam. In an embodiment, the composition is on a wipe. In an embodiment, the composition is a lubricant. In an embodiment, the composition is a lubricant gel. In an embodiment, the composition is not a lubricant gel.
20

25 In an embodiment, the composition is administered using a device selected from a: pessary, intravaginal ring, vaginal film, tampon, suppository, wipe or sponge. In an embodiment, the device is a pessary. In an embodiment, the device is an intravaginal ring. In an embodiment, the device is a vaginal film. In an embodiment, the device is a tampon. In an embodiment, the device is a suppository. In an embodiment, the device is
30 a wipe. In an embodiment, the device is a sponge.

In an embodiment, the device is a multipurpose (comprises one or more other therapeutic as described herein). In an embodiment, the device is a multipurpose intravaginal ring (comprising e.g. a contraceptive, a hormone, antiretroviral for example as described in Moss et al., 2013).

In an embodiment, the composition is thermosensitive. In an embodiment, the composition comprises a thermosetting polymer. In an embodiment, the polymer is a hydrogel as described for example in (Fan et al., 2022).

5 In an embodiment, the composition is administered before, after or at the same time as one or more other therapeutics as described herein. In an embodiment, the composition is administered before the one or more other therapeutics as described herein. In an embodiment, the composition is administered after the one or more other therapeutics as described herein. In an embodiment, the composition is administered at the same time as one or more other therapeutics as described herein.

10 In an embodiment, the other therapeutic is an orally administered therapeutic.

In an embodiment, the other therapeutic is a topically administered therapeutic.

In an embodiment, the other therapeutic is a vaginally administered therapeutic.

In an embodiment, the composition is administered in an applicator or device.

15 In an aspect, the present invention also provides an applicator or device having the composition as described herein. In an embodiment, the device is a vaginal delivery device. In an embodiment, the device is a multipurpose delivery device.

In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

20 In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40 % w/v to about 2.5% w/v, wherein the composition is iso-osmolal with cervicovaginal fluid.

In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40% w/w to about 4.5% w/w, wherein the composition has an osmolality of 300 to 800 mOsm/Kg.

25 In an aspect, the present invention provides a vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40% w/v to about 4.5% w/v, wherein the composition has an osmolality of 300 to 800 mOsm/Kg.

30 **EXAMPLES**

Example 1 – Methods and materials

Gels for evaluation in the EpiVaginal tissue model (VEC-100) were manufactured under Good Manufacturing Practice (GMP) in a GMP suite. Gels were manufactured using the following materials: water, sodium hydroxide, methyl paraben sodium, propyl paraben sodium, sodium chloride, hydrochloric acid, hydroxyethyl cellulose (HEC) 2000
35 cP, L-lactic acid, racemic lactic acid, and D-lactic acid. Materials were NF and USP

Grade and satisfy regulations by regulatory bodies such as the US Food and Drug Administration.

The method to prepare the lactic acid gels is as follows:

- (i) components were added to water in the following order, and mixed until the solution was clear after the addition of each component: methyl paraben (0.18% w/w final concentration), propyl paraben (0.02% w/w final concentration), NaCl (adjusted based on lactic acid concentration – range 0 – 0.46% w/w), lactic acid (from 1.40 – 2.5% w/w);
- (ii) pH was measured to confirm it was within the target range (3.7 ± 0.2), if necessary adjusted with either 1N HCl or 5M NaOH solution;
- (iii) HEC (2.4% w/w final) added under mixing at 600 RPM;
- (iv) after mixing, pH measured again to confirm it was within the target range (3.7 – 3.8) and adjusted if necessary, with either 1N HCl or 5M NaOH solution.

Example batch records for gels prepared using this method are summarized below:

Table 1: SYN Batch records

Product name	Batch number	Density	Ave. Concentration w/w	Concentration w/v
Placebo lactic acid gel	SYN.23.0251	1.0104	N/A	N/A
Lactic acid gel 1.64%	SYN.23.0260	1.0128	1.61	1.59
Lactic acid gel 2.25%	SYN.23.0189	1.0157	2.41	2.37
Lactic acid gel 4.5%	SYN.23.0176	1.0209	4.61	4.52

Alternately, the lactic acid gels can be prepared using the following protocol:

- (iii) Material used for formulation of the gels included water, sodium hydroxide, methyl paraben sodium NF, propyl paraben sodium NF, sodium chloride, hydrochloric acid (12 M), hydroxyethylcellulose (HEC) 2000 cP NF, L-lactic acid, racemic lactic acid and D-lactic acid. Gels with 1.9 % w/w, 2.3% w/w, and 4.5% w/w lactic acid comprising L-lactic acid or racemic lactic acid or lactic acid at a ratio of 1.5 :1 D-lactic acid: L-lactic acid were prepared in a GMP suite.

(i) Components were added to water in the following order: methyl paraben from a 1.8% w/w stock solution in water (0.18% w/w final concentration), propyl paraben

from a 2% w/w stock solution in water (0.02% w/w final concentration), a volume of L-lactic acid to achieve final concentration (i.e. 1.9%, 2.3% or 4.5% w/w), a volume from a 5 M Sodium hydroxide stock, a volume of 10% sodium chloride stock, pH of solution measured to pH target of 3.50 (range 3.45 – 3.55) and adjusted with 5M NaOH, mass of HEC added to achieve 2.4% w/w final concentration, solution transferred to a roller mixer and mixed for at least 7 hours.

(ii) The 1.5: 1 ratio of D-lactic acid: L-lactic acid gel was similar as above except that D-Lactic acid (solid) was prepared as a 30% w/w stock and L-lactic acid as a 20% w/w stock in water. After greater than or equal to 7 hours mixing, the gel was assessed for clarity (free of particles) and the pH measured and adjusted to pH 3.75 (range 3.70 – 3.80) with 5 M NaOH stock if necessary.

(iii) If pH was adjusted the solution was mixed for at least another 7 hours and the pH then measured to determine if at target pH 3.75 (range 3.70 – 3.80).

While lactic acid is a known preservative, other preservatives such as methyl paraben and propyl paraben were included in the formulations to prevent microbial growth and in the placebo gel that lacks lactic acid. Preservatives were included in the lactic acid gels since many of them were formulated with levels that were considerably lower [$<4.5\%$ (w/w) lactic acid] than over the counter (OTC) gels that were packaged in single use plastic applicators and where the only identifiable preservative was lactic acid (e.g. balance active). However, it is plausible that the present gels may be re-formulated with concentrations of lactic acid on the higher end of the range to reduce the need to rely on additional preservatives, i.e., generation of preservative-free gels.

To assess that the gel formulation specifications were within allowable thresholds following manufacture, a number of assays were performed. pH was measured and recorded during the formulation for Product # FP-023-0002, # FP-023-0003 and # FP-023-0004 using a TPS Aqua pH meter. The viscosity of the gels was determined at 37°C using a Rheology Test on Rheology instrumentation Anton Paar MCR302 Rheometer as per manufactures instructions. Osmolality was determined using the Osmolality test for assessment of feminine hygiene gels and liquids using a cryoscopic osmometer (Gonotec Osmomat 030-D, Gemini BV, Apeldoorn, Netherlands) as described in Tyssen et al. (2022). And a commercial enzyme assay was used to quantify D- and L-lactic acid levels in gels and stock solutions using the D-lactic acid/L-lactic acid kit (R-Biopharm, Darmstadt, Germany) as described in Tyssen et al. (2022).

Osmolality and lactic acid content in the OTC gels were determined using the same methods outlined above for the gels, while pH was determined using a pH electrode connected to a pH meter (TPS-Aqua meter and IJ sensor, Vintessential Laboratories)

which allows accurate pH measurement of viscous fluids as described in Tyssen et al. (2022).

The nine LA-containing gels, one placebo gel and 11 OTC vaginal products were evaluated in an EpiVaginal human tissue model (VEC-100) developed by MatTek Corporation (Ayehunie et al., 2006). Safety Assessment of Vaginal Test Formulations in the EpiVaginal *in vitro* Human Vaginal Model was conducted as described in Tyssen et al. (2022).

Briefly, neat gel (100 μ L) was added apically to the EpiVaginal model and incubated at 37°C 5% CO₂. Cell viability was determined using the MTT assay, a colorimetric assay that measures cellular metabolic activity based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals. Epithelial barrier integrity was assessed by measuring transepithelial electrical resistance (TEER) at 4 h and 24 h post addition as described in Tyssen et al. (2022). Gels were considered acceptable in this model if they demonstrated $\geq 50\%$ viability and TEER at 24 h post treatment compared to untreated vaginal tissue. Qualitative histological effects were also recorded to examine tissue architecture as part of the assessment.

An analysis of the production of soluble cytokines and chemokines (including inflammatory markers) following the gel treatment of the EpiVaginal human tissue model (VEC-100) was conducted as described in Tyssen et al. (2022) using the human magnetic Luminex assay following manufacturer's protocol (R&D systems) for Macrophage inflammatory protein-3 α (MIP3 α), interleukin (IL)-1 β , IL-6, IL-1 α and IL-1 receptor antagonist (IL-1RA). IL-8 was measured in samples by ELISA (R&D systems) following the manufacturers protocol. For this analysis, basolateral supernatants were collected and analysed at the 24 h time point.

LA concentrations in gels are in w/w while LA concentrations in solution/media are in w/v. LA concentrations in w/w and w/v are similar see (Table 1).

Example 2 – Physicochemical properties of gels

Nine new lactic acid-containing gels containing two ratios of the lactic acid isomers (L- and D- LA) and intended concentrations of 1.9% (w/w), 2.3% (w/w) and 4.5% (w/w) of lactic acid were prepared. Details of formulations are described in Table 2. The actual lactic acid concentrations of the new gel formulations, as quantified by a lactic acid enzyme assay, ranged from 1.4% to 4.5% (w/w) (Table 2). Ratios of D- to L-lactic acid in the nine formulations averaged 1.5 to 1. Some of the formulations comprise racemic lactic acid (i.e. 1:1 D:L-LA) and some comprise L-lactic acid alone. The ratio of

- D- to L- lactic acid for the racemic lactic acid gels was close to 1 (range 0.92-0.95) while for the 1.5:1 ratio of D- to L-lactic acid the actual ratio ranged from 1.34 to 1.38 (**Table 2**). The intended pH was 3.75 ± 0.05 for lactic acid containing gels with all gels within the pH 3.7 – 3.8 range. The pH of the placebo gel was within 0.5 of the pH 5.0 target
- 5 (**Table 2**). The pH of the placebo was deliberately set at pH 5.5 to be able distinguish lactic acid and low pH effects from the placebo gel. The majority of the nine new lactic acid gels were in the iso-osmolal range (iso-osmolal with the cervicovaginal fluid) except for gels with actual total lactic acid concentrations that were greater than 3.62% (w/w). The viscosity of the new gels ranged from 64.2 to 149.5 Pa.s (**Table 2**).
- 10 The relative physicochemical characteristics of each over the counter (OTC) gels were diverse (**Table 3**). Six of the 11 OTC gels contained only the L-isomer of lactic acid while the remaining 3 lactic acid containing gels contained racemic lactic acid (1:1 D:L lactic acid). The majority of OTC lactic acid containing gels had supraphysiological concentrations of lactic acid between 4.8 and 7.2% (w/w) lactic acid, except for Gynofit
- 15 (1.43%) and Restore (1.06%), which were within the physiological levels found in women with *Lactobacillus*-dominated vaginal microbiota. Almost all the OTC gels were hyperosmolal (>400 mOsmol/kg) ranging from 454 to >3000 mOsmol/kg, with the exception of Restore, which was iso-osmolal at 293 mOsmol/kg.

20 **Table 2: Physicochemical properties of lactic acid gels.**

Gel #	Gel product [^]	pH	Viscosity (Pa.s)	Osmolality* (mOsmol/kg)	SEM	Total LA (%) ^{&}	SEM	Proportion of D:L
1	1.9% L-LA	3.78	64.2	390	0.0	1.64	0.07	N/A
2	2.3% L-LA	3.74	108.1	385	0.9	2.25	0.09	N/A
3	4.5% L-LA	3.71	103.9	660	4.7	4.5	0.22	N/A
4	1.9% DL-LA racemic	3.80	82.9	335	1.5	1.64	0.05	0.95
5	2.3% DL-LA racemic	3.72	119.6	387	0.3	2.38	0.12	0.93
6	4.5% DL-LA racemic	3.72	128.2	631	4.1	3.95	0.23	0.92
7	1.9% DL-LA 1.5:1 (D:L)	3.74	128.8	382	0.7	1.44	0.08	1.34
8	2.3% DL-LA 1.5:1 (D:L)	3.77	148.6	329	0.3	1.64	0.08	1.38
9	4.5% DL-LA 1.5:1 (D:L)	3.70	149.5	563	1.5	3.62	0.12	1.38
10	0% LA (placebo)	5.10	72.1	294	0.6	0	N/A	N/A

^ LA-gels (with target LA concentrations) are made in 2.4% HEC gel.

pH measurement n=1

Viscosity data from n=2

Osmolality data from n=3

5 Lactic acid data from n=5

* Osmolality of healthy cervicovaginal fluids is 370 +/- 40 mOsmol/kg. Bold indicates hyperosmolar gels.

&Determined via lactic acid enzyme assay - Yellow Line- Roche D-lactic acid/L-lactic acid kit (R-Biopharm, Darmstadt, Germany). Catalogue number 111 2821 035.

10

Table 3: Descriptive summary of VEC-100 tissue histology following single topical application of test articles and incubation for 4 h and 24 h.

	Run#1	Run#2
Test Article	Effect on histology	Effect on histology
Untreated Control	Healthy tissue	Healthy tissue
Gynol II (3% N-9)	Loss of glycogen filled layers at 4 h. At exposure time 24 h the tissue was completely damaged.	Loss of glycogen filled layers at 4 h. At exposure time 24 h the tissue was completely damaged.
TA1 1.6% L-LA	Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 4 h. At 24 h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal at and suprabasal layer.	Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 4 h. At 24 h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal layer.
TA2 2.3% L-LA	Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 4hr. Separation from the underlying membrane at exposure time 24 h.	Loss of tissue at 4 h exposure time, Thinning and perturbation of apical and glycogen filled layer at 24 h exposure time.
TA3 4.5% L-LA	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.
TA4 1.6% DL-LA	Tissue damage at some spots on suprabasal layer and minor perturbation of the apical layer at exposure time 4 hr. Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 24 h.	Slight perturbation of apical and glycogen filled layer at exposure time 4 hr. reorganization of basal and suprabasal cell layer at exposure time 24 h.
TA5 2.4% DL-LA	Perturbation of apical and glycogen filled layer at exposure time 4 h. Thinning of epithelium at exposure time 24 h.	Perturbation of apical and glycogen filled layer at exposure time 4 h. Thinning of epithelium at exposure time 24 h.

TA6 4% DL-LA	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.	Perturbation of apical and glycogen filled layer at exposure times 4 h and 24 h.
TA7 1.4% DL-LA 1.5:1	Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 4 h. At 24h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal and suprabasal layers.	Separation of apical and glycogen filled layer from the basal and suprabasal layers at exposure time 4h. At 24 h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal and suprabasal layers.
TA8 1.6% DL-LA 1.5:1	Slight separation of apical and glycogen filled layer and intact basal and suprabasal layers at exposure time 4 h. At 24 h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal and suprabasal layer	Slight separation of apical and glycogen filled layer and intact basal and suprabasal layers at exposure time 4 h. At 24 h exposure time, apical and glycogen filled layer was sloughed off and reorganization of basal layer.
TA9 3.6% DL-LA 1.5:1	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 hr	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.
TA10 Placebo gel	Healthy tissue	Healthy tissue
TA11 Lactigel	Loss of apical and glycogen filled layer and thinning of epithelium at 4 h exposure. Perturbation and thinning of epithelium at 24 h exposure time.	Epithelial thinning and separation from the underlying membrane at exposure time 4 h. Thinning of epithelium at 24 h exposure time.
TA12 Relactagel	Perturbation of apical and glycogen filled layer at exposure time 4 h. Perturbation of apical and glycogen filled layer, epithelial thinning, and separation from the underlying membrane 24 h exposure.	Perturbation of apical and glycogen filled layer at exposure time 4 h. Perturbation of apical and glycogen filled layer, epithelial thinning, and separation from the underlying membrane 24 h exposure.
TA13 Balance Active	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.	Perturbation of apical and glycogen filled layer at exposure time 4 h. Epithelial thinning and separation from the underlying membrane at exposure time 24 h.
TA14 Gynofit	Separation from the underlying membrane and perturbation at exposure time 4 hr and 24 h.	Perturbation of apical and glycogen filled layers at exposure time 4 h. Loss of tissue at exposure time 24 h,

TA15 Canesbalance	Perturbation of apical and glycogen filled layers and separation from the underlying insert membrane at exposure times 4 hr and 24 hr. Thinning of epithelium at 24hr exposure	Perturbation of apical and glycogen filled layers and separation from the underlying insert membrane at exposure times 4 hr and 24 hr. Thinning of epithelium at 24hr exposure
TA16 Geliofil Classic	Separation from the underlying membrane and perturbation at exposure time 4 h and loss of tissue at exposure time 24 h.	Perturbation of apical and glycogen filled layers and separation from the underlying insert membrane at exposure times 4 h and 24 h. Thinning of epithelium at 24 h exposure
TA17 Caya Gel	Separation from the underlying membrane and perturbation at exposure time 4 h and 24 h.	Thinning of tissue at exposure time 4 h. Slight perturbation of apical layer at exposure time 24 h.
TA19 FleurStat	Damage to glycogen filled layer at exposure time 4 h. Intact basal cell layer and loss/disorganization of suprabasal, apical and glycogen filled layers at exposure time 24 h.	Disorganization of suprabasal, apical and glycogen filled layers at exposure times 4 hr and 24 hr.
TA20 Restore	Slight perturbation of apical layer and intact basal layer at exposure time 4 h. Perturbation of apical and glycogen filled layer and reorganization of the basal and suprabasal layers at exposure time 24h.	Slight separation of apical layer and intact basal layer at exposure time 4 h. Perturbation or loss of apical and glycogen filled layer and reorganization of the basal and suprabasal layers at exposure time 24 h.
TA21 Aci-Gel	Separation from the underlying membrane and perturbation of apical and glycogen filled layer and thinning of epithelium at exposure times 4 h and 24 h.	Separation from the underlying membrane and perturbation of apical and glycogen filled layer and thinning of epithelium at exposure times 4 h and 24 h.
TA22 Contragel	Separation from the underlying membrane and perturbation of apical and glycogen filled layer and thinning of epithelium at exposure times 4 h and 24 h.	Separation from the underlying membrane and perturbation of apical and glycogen filled layer and thinning of epithelium at exposure times 4 h and 24 h.

Example 3 – Effects of gels on cytotoxicity and epithelial barrier integrity in the EpiVaginal model

An evaluation of the over the counter (OTC) gels was performed using the organotypic EpiVaginal model (VEC-100; MatTek Corp.), which is composed of primary human cervicovaginal epithelial cells that form highly differentiated, stratified tissues closely resembling native human vaginal and ectocervical epithelium (Aychunie et al., 2006). Using this model, cytotoxicity, impact on epithelial barrier integrity and immunomodulatory effects of the gels were evaluated. The positive control Gynol-II was included, a spermicide containing 3% nonoxynol-9, which has been shown to damage

the vaginal epithelial barrier and increase HIV transmission in women (Van Damme et al., 2002). The OTC gels included Lactigel, Relactagel, Balance Active, Gynofit, Canesbalance, Geliofil, Restore, two gels used as a contraceptive, Contragel and Caya Gel, as well as the vaginal gels, Aci-Jel and Fleurstat, where the active ingredients are acetic acid and astrodimer, respectively instead of lactic acid.

Cytotoxicity of the new lactic acid gels was compared in parallel with 11 OTC products designed for intravaginal use. In this vaginal tissue model, MatTek defines MTT and TEER measurements that are less than 50% relative to the untreated controls as significantly cytotoxic or a disruptor of barrier integrity, respectively (Ayehunie et al., 2016). The data indicate that the gel formulations containing up to 1.6% (w/w) lactic acid have no significant effects on the viability of vaginal epithelial tissues (**Figure 1A**). This is in dramatic contrast to the majority of OTC LA-containing gels which were highly cytotoxic in this system (**Figure 1B**), likely due to their high lactic acid content and hyperosmolal properties (**Table 3**). The exceptions were Fleurstat and Restore, with the latter containing the lowest lactic acid content of all OTC lactic acid gels and both with osmolality values that were close to normal (**Table 3**). A similar effect was observed on the epithelial barrier integrity, where gels with up to 1.6% lactic acid demonstrated no significant effect on barrier integrity (**Figure 1C**), while the majority of OTC gels exhibited significant barrier disruption (**Figure 1D**). The only two non-cytotoxic OTC gels, Fleurstat and Restore (1.06% lactic acid), did not significantly decrease TEER (**Figure 1D**). These data indicate that the majority of OTC gels were cytotoxic, likely due to their hyperosmolality, with these gels also demonstrating decreased barrier integrity.

L-lactic acid but not D-lactic acid is metabolised by human cells. However, comparison of the same concentration (1.6%) of L-lactic acid vs racemic lactic acid vs 1.5:1 ratio of D- to L- lactic acid did not show any dramatic differences in cytotoxicity or TEER (**Figure 1A** and **1C**). These data suggest that under the conditions of this assay, gel formulations with D-lactic acid do not appear to have greater toxicity in the EpiVaginal model compared to gels containing L-lactic acid alone.

Example 4 – Immunomodulatory effects of gels in the EpiVaginal model

Genital inflammation is associated with an increased risk for adverse sexual health outcomes including HIV acquisition in women (Masson et al., 2015). The production of pro-inflammatory cytokines and chemokines can be elicited from cervicovaginal epithelial cells in response to microbial associated molecular patterns (MAMPs), including those that mimic BV-associated bacteria and HIV envelope,

seminal plasma (Hearps et al., 2017), and chemical agents that cause vaginal irritation (Ayehunie et al., 2016). For this study the pro-inflammatory cytokines IL-1 α , IL-1 β , IL-6 and the pro-inflammatory chemokines MIP3 α and IL-8 were measured. All these immune mediators are produced by cervicovaginal epithelial tissue and are elevated in women who are at risk of acquiring HIV (Masson et al., 2015; Anahtar et al., 2015; Arnold et al., 2016; Masson et al., 2019). IL-1 α and IL-1 β are considered valuable markers of vaginal irritation and are elevated in vaginal lavages following dosing with the irritant, benzalkonium chloride in the rabbit vaginal irritation model and the EpiVaginal tissue model (Ayehunie et al., 2016). The anti-inflammatory cytokine IL-1RA which is elicited by lactic acid and is a powerful blocker of pro-inflammatory cytokines IL-1 α and IL-1 β was also measured (Hearps et al., 2017).

To determine if the gels promote vaginal inflammation, soluble immune mediators released into the basolateral medium of EpiVaginal tissues were quantified following apical treatment. Comparison of untreated tissue with the gels containing lactic acid at concentrations of up to 1.6% (w/w) revealed that these elicited no or very low production of the pro-inflammatory cytokines IL-1 α and IL-1 β , and the pro-inflammatory chemokine MIP3 α (**Figure 2A, 2B, and 2G**). In contrast, the positive control, Gynol-II, elicited a significant increase in IL-1 α and IL-1 β (**Figure 2B and 2G**). All new gels containing lactic acid elicited production of high levels of the anti-inflammatory factor IL-1RA (**Figure 2C**). These data are consistent with *in vitro* observations that lactic acid alone elicits the production of IL-1RA from cervicovaginal epithelial cells as well as vaginal tissue (Hearps et al., 2017). In contrast, gels with lactic acid levels greater than 1.6 % (w/w) demonstrated a dose-dependent increase in IL-1 α , IL-1 β , and MIP3 α , while IL-6 and IL-8 levels were similar to untreated tissue (**Figure 2A, 2B, 2G, 2H and 2I**). Given that untreated vaginal tissues produce basal levels of IL-6 and IL-8 (**Figures 2H and 2I**), the decrease in these immune mediators at lactic acid concentrations greater than 1.6% is likely due to cytotoxicity (**Figure 1A**).

All of the OTC gels tested, except Fleurstat, elicited production of the powerful pro-inflammatory cytokine IL-1 β compared to untreated cells (**Figure 2E**). In contrast, Fleurstat elicited the greatest increase in the pro-inflammatory chemokine, MIP3 α , and the second highest increase in IL-8 compared to the other OTC gels (**Figure 2D**). In contrast, MIP3 α and IL-8 levels for all lactic acid gels up to 4.5% lactic acid were significantly lower than Fleurstat (**Figure 1A and 1I**). While Restore, which contains 1% DL-lactic acid and is iso-osmolal (**Table 3**), demonstrated modest increases in IL-1 α and IL-1 β compared to untreated tissue (**Figure 2J and 2E**), it elicited the greatest increase in IL-6 and IL-8 compared to all of the OTC gels tested (**Figures 2K and 2L**) including

all gels tested up to 4.5% lactic acid (**Figure 2H** and **2I**). Increases in immune mediators elicited by Restore was observed in the absence of significant cytotoxicity or decrease in barrier integrity of the vaginal tissue (**Figure 1B** and **1D**). These data suggest that Restore contains ingredients distinct from the new lactic acid gel formulations that are eliciting a dramatic increase in IL-6 and IL-8. Restore contains additional additives compared to the new lactic acid gels which include organic aloe barbadensis leave juice, xanthan gum, natural flavour, potassium sorbate and sodium benzoate, the latter two representing preservatives. A previous study has reported that the preservative, sorbic acid, elicits the production of IL-8 from HEC-1A epithelial cells (Gali et al., 2010) suggesting that potassium sorbate in Restore may be responsible for the increase in IL-8 observed in the EpiVaginal model.

Taken together, these data demonstrate that lactic acid gels with concentrations up to 1.6% (w/w) lactic acid do not elicit the production of pro-inflammatory mediators in the VEC-100 vaginal tissue model and have a superior safety profile compared to all of the OTC gels tested including Restore, an iso-osmolal OTC lactic acid-containing gel. Additionally, various lactic acid gels with concentrations up to 4.5% (w/w) lactic acid had favourable properties in relation to the reduction in various pro-inflammatory mediators as compared to particular OTC gels, specifically Fleurstat and Restore. This suggests that the present lactic acid gels have utility in the treatment of vaginal inflammatory disorders.

Example 5 – Histological effects of gels in the EpiVaginal model

As a qualitative measure to assess the effect of the various gels on the VEC-100 EpiVaginal tissue model, one tissue sample at 4 h and 24 h were processed and stained using Haematoxylin and Eosin (H&E staining) following the epithelial barrier integrity (TEER) measurement. A histopathology-experienced technician assessed and graded each tissue section (Tyssen et al. 2022) according to a number of parameters that were a) loss of glycogen filled layers, b) tissue thinning, c) separation or perturbation of apical and glycogen filled layers, and d) reorganization/disorganization of suprabasal, apical and glycogen layers. These four parameters, each carrying different weightings, were then used to determine an overall tissue damage score (**Table 4**).

Consistent with previous assays, the positive control (PC) exhibited only mild effects (+) on the tissue at 4 h and severe tissue damage (+++) at 24h (private communication S. Ayehunie, MatTek) compared to the untreated control (NC1). The placebo gel (**Table 4**, TA10) demonstrated no effect on the tissue (-) compared to the untreated control (NC1) following 4 h or 24 h of treatment. Four hours treatment with

the lactic gels showed little effect on tissue structure (- or +) (**Table 4**). After 24 h treatment, low (+), mild (++) or severe (+++) tissue damage was observed for all lactic acid-containing gels, although this effect was not dose-dependent.

At 4 h following treatment, seven of the 11 OTC gels demonstrated low (+) to moderate (++) tissue damage while four of the gels exhibited no effect on the tissue (**Table 2**). As observed for the gels, at 24 h the majority of the OTC gels showed moderate (++) tissue damage, with the exception of Gynofit (**Table 4**, TA14) where little morphological effects were observed in 1 of the 2 runs. The 24 h tissue for the second run was lost and so was not available for analysis and the result is based on only one tissue at 24 h.

One of the OTC gels, Good Clean Love – Restore (**Table 4**, TA2), was previously tested by MatTek in their VEC-100 model and showed no tissue damage (Ayehunie et al 2018). This is in contrast to the moderate tissue damage, predominately glycogen layer separation and disorganization, that was observed following 24 h treatment in the present study. This is supported by the lack of a dose-dependent effect of the new lactic acid gels on tissue damage as observed by histology (**Table 4**). In addition, there was little correlation between the histology data (**Table 4**) and the cytotoxicity and epithelial barrier integrity data (**Figure 1**) which showed a dose-dependent effect where higher concentrations of lactic acid were toxic. To confirm the results studies will be performed in the VEC-100 full thickness model (VEC-100FT) EpiVaginal model, which additionally includes a basal layer of ectocervical fibroblasts. Both the VEC-100 and VEC100FT provide comparable responses in regard to toxicity (MTT) and epithelial barrier integrity (TEER) when compared to the rabbit vaginal irritation model.

Table 4: Summary of histological effects of gels in the EpiVaginal model tissue model (VEC-100).

Sample ID	Test Material	Loss of Glycogen Filled Layers				Thinning				Separation or Perturbation of Apical and Glycogen Filled Layers				Reorganization/ Disorganization of Suprabasal, Apical and Glycogen Layers				Tissue Damage (Mean of RUN-1 & Run-2)	
		4hr		24hr		4hr		24hr		4hr		24hr		4hr		24hr		4hr	24hr
		Run-1	Run-2	Run-1	Run-2	Run-1	Run-2	Run-1	Run-2	Run-1	Run-2	Run-1	Run-2	Run-1	Run-2	Run-1	Run-2		
NC1	Untreated	-	-	-	-	-	-	-	-	+++	+++	+++	+++	-	-	-	-	-	-
PC	Gyrol H (containing 0% Nanosyringol-3)	++	+++	++	+++	-	-	-	-	+++	+++	+++	+++	-	-	-	-	+	+++
TA1	1.6% L-LA in 2.4% HEC	-	-	++	+++	-	-	++	+++	+++	+++	+++	+++	-	-	+++	-	-	++
TA2	2.3% L-LA in 2.4% HEC	-	N/A	+	+	+	N/A	+	+	++	N/A	-	-	-	N/A	-	-	-	++
TA3	4.5% L-LA in 2.4% HEC	-	-	-	+	-	-	+	++	-	-	-	-	-	-	-	-	+	++
TA4	1.6% DL-LA in 2.4% HEC (racemic)	-	+	+++	+++	-	-	++	+++	-	-	+++	-	-	-	+++	-	-	++
TA5	2.4% DL-LA in 2.4% HEC (racemic)	-	-	++	-	-	-	+	-	-	-	-	-	-	-	-	-	-	++
TA6	4.0% DL-LA in 2.4% HEC (racemic)	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+
TA7	1.4% DL-LA in 2.4% HEC (1.5D : 1.0L)	-	-	-	++	+	+	-	+	+++	+++	+++	+++	+	+	++	+++	+	+
TA8	1.6% DL-LA in 2.4% HEC (1.5D : 1.0L)	-	-	++	+++	-	-	-	+++	++	++	++	+++	+	+	+++	-	+	+++
TA9	3.6% DL-LA in 2.4% HEC (1.5D : 1.0L)	-	-	++	++	-	-	++	++	-	-	-	++	-	-	+++	+++	-	++
TA10	0% LA (allanol) in 2.4% HEC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TA11	Lachgel	++	+	-	+	++	-	+	+	++	-	++	++	-	+	-	-	++	++
TA12	ReoDagel	+++	-	+++	++	++	-	++	+	++	-	++	+	++	-	+++	++	++	++
TA13	Balanea Active	++	-	+	+	++	-	++	++	+	-	++	++	++	-	++	++	++	++
TA14	Gynast	-	-	-	N/A	-	-	-	N/A	-	-	-	-	-	+	-	N/A	-	-
TA15	Canasolance	++	++	++	++	+	+	++	++	++	++	++	++	++	++	++	++	+	++
TA16	Galich Classic	-	-	N/A	++	-	-	N/A	+	+	+	N/A	-	-	-	N/A	+	+	++
TA17	Cayre Gel (diaphragm gel)	-	-	++	-	++	++	++	-	-	-	++	-	-	-	++	-	-	+
TA18	ReoStat (1% Asproline)	-	+++	+++	+++	-	-	++	++	-	-	++	+++	-	++	+++	+++	+	++
TA20	Resine	+	-	++	++	-	-	++	+++	-	-	++	++	+++	+++	++	++	-	++
TA21	Acu-Jel	-	-	+	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+
TA22	Contragel (light/contraphase)	+	++	+	++	+	++	+	++	+	++	+	++	-	-	+	+	+	++

It was found that the new lactic acid-containing gel formulations exhibit a superior safety profile in the EpiVaginal model (VEC-100) compared to vaginal gels currently on the market.

Most lactic acid containing OTC gels tested were hyperosmolal, with values in excess of the WHO interim limit of 1200 mOsm/kg (Ayehunie et al., 2018). These gels mostly contained supraphysiological concentrations of lactic acid [$>4\%$] that likely contributed to the increased osmolality. One exception was Gynofit which contained physiological concentrations of lactic acid (1.43%); however, the osmolality was high at 2026 mOsm/kg suggesting other gel components contributing to the cytotoxicity observed in the EpiVaginal model. For OTC gels and the lactic acid gels, cytotoxicity in the EpiVaginal model was associated with a significant decrease in epithelial barrier integrity and significant increases in the pro-inflammatory cytokines IL-1 α and IL-1 β .

Restore, an iso-osmolal OTC gel that contains 1% DL lactic acid, demonstrated a lack of cytotoxicity and no significant decrease in the epithelial barrier. However, it elicited a modest increase in IL-1 β and greater levels of IL-6 and IL-8 compared to the other OTC gels and the best performing lactic acid gels. Of note, most of the OTC lactic acid gels only contained the L-isomer of lactic acid, while Lactigel, Relactogel and Restore contained racemic D:L lactic acid.

The results suggest that the maximum concentration of lactic acid that is not toxic in the VEC-100 EpiVaginal Model is about 2.5% (w/w). This concentration (or lower) did not elicit cytotoxicity, epithelial barrier disruption, minimal effects on the production of the pro-inflammatory cytokines IL-1 α and IL-1 β as well as tissue morphology. Obvious differences in cytotoxicity between gels containing L-lactic acid alone or different ratios of L- and D- lactic acid when comparing gels with the same total lactic acid content in the EpiVaginal model was not observed. Since both D- and L-lactic acid isomers are produced by beneficial *Lactobacillus spp.* (i.e. *L. crispatus*) (Tachedjian et al., 2017) it is desirable to advance a gel formulation that contains both lactic acid isomers to better recapitulate the effects of an optimal vaginal microbiota compared to L-lactic acid alone.

Example 6 – Evaluation of lactic acid bactericidal activity on anaerobic bacteria

The inhibitory activity of lactic acid (LA) and/or LA containing gels on the growth of various anaerobic bacteria was assed using a bactericidal assay. Bactericidal activity was determined counting bacterial colonies (representing viable bacteria) following a short exposure (1-2 h at 37°C) of anaerobic bacteria to lactic acid LA, LA-containing gel, and control gel (placebo gel).

Briefly, *Lactobacillus crispatus*, *Lactobacillus iners* and *Gardnerella vaginalis* were grown in De Man, Rogosa and Sharpe (MRS), NYC-III and Brain Heart Infusion (BHI), respectively in pre-reduced broth incubated in anaerobic conditions for 24 h at 37°C.

5 Depending on the experiment, treatments contained D-LA, L-LA and DL-LA in solution or diluted gel containing DL-LA (or placebo gel). The different treatments were adjusted to the required pH using either NaOH or HCl. Of each treatment (190 µl) was incubated with 10 µl of bacterial culture containing 1×10^6 colony forming units per ml (CFU/ml) of the relevant bacteria. The treatment-bacteria mix was then incubated for 1
10 h at 37°C under anaerobic conditions. After incubation, the treated bacteria were serially diluted in medium and 20 µl of each dilution was plated into the appropriate agar plates and further incubated for 3 days at 37°C under anaerobic conditions. After this incubation, the number of visible colonies on the plates were counted to calculate CFU present the original inoculum.

15 The results shows DL-lactic acid gel (1%) at pH 3.8 had potent bactericidal activity against *L. iners* and *G. vaginalis*, but not *L. crispatus* or *L. gasseri* after 1 hour incubation at 37°C under anerobic conditions compared to untreated bacteria incubated in medium without pH adjustment (untreated not pH adjusted) (**Figure 3**). The decrease in *G. vaginalis* and *L. iners* viability mediated by the lactic acid containing gel was
20 greater than placebo gel that was pH adjusted to 3.8 using HCl (Placebo 3.8). These data indicate that the bactericidal activity of lactic acid against *G. vaginalis* and *L. iners* is greater than gel acidified with HCL alone. No bactericidal activity was observed with the placebo gel at pH 5.5 (placebo 5.5) (*L. gasseri* was not tested). Bacteria treated with media acidified to pH 3.8 with HCl (water 3.8) was not as potent at killing *L. iners*
25 compared to lactic acid (1% DL-LA gel final). Error bars represent standard error of the mean from n=4 independent assays. Statistical analysis was performed using the Mann-Whitney test where * p<0.05 and ** p<0.01

The results show L-lactic acid, D-lactic acid and racemic lactic acid (1% w/v) in solution are bactericidal against *G. vaginalis* and *L. iners* but not *L. crispatus* at pH <
30 5.5. Bacteria were incubated with lactic acid in medium adjusted to different pH values for 1 hour at 37°C under anaerobic conditions (**Figure 4**). Following incubation bacteria were plated to determine viability as determined by counting colonies. The bactericidal activity of lactic acid against *G. vaginalis* and *L. iners* increases in potency as the pH values decrease around and lower than the pKa of lactic acid (3.86). These data indicate
35 that it is uncharged lactic acid mediating bactericidal activity against *L. iners* and *G. vaginalis* and not the lactate anion. In contrast *L. crispatus* retained viability under all

conditions tested indicating that this bacterial species is resistant to the bactericidal effects of lactic acid. Error bars represent standard error of the mean from at least n=2 independent assays.

5 **Example 7 – Randomised, double-blind placebo-controlled mechanistic study to determine antimicrobial and immunobiological activities of the new lactic acid formulations in women with BV**

Study design: Randomised, double-blind placebo-controlled mechanistic study to determine antimicrobial and immunobiological activities of the new lactic acid
10 formulations in women with BV. Eligible women presenting to the clinic with BV will be invited to participate and informed consent obtained. Non-pregnant women (n=50) randomized randomized 1:1 to placebo or lactic acid gel. Block randomization performed to ensure balance across groups with permuted block sequences generated using statistical software. Women instructed to self-administer 4-5 ml of gel intravaginally
15 nightly on 7 consecutive days (Days 1–7), a dosing regimen that is effective for BV treatment. Based on a previous study ~17% end-point attrition is expected due to lost-to-follow-up and STI positivity.

Lactic acid gel preparation: Clinical batches of placebo and lactic acid formulations prepared under Good Manufacturing Practice (GMP) and packaged with
20 sufficient gel for the entire course packaged in metal tubes for use with single use vaginal applicators (blinded as gel A or B). Gels will be subjected to quality control and undergo physicochemical, microbiological and stability testing by prior to use in this project.

Recruitment: Women with BV will be recruited.

Inclusion/exclusion criteria: Inclusion criteria: (i) women aged ≥ 18 years and pre-menopausal, (ii) BV according to standard clinical practice, (iii) willing to abstain from
25 vaginal sex for study duration, (iv) able to provide informed consent and complete all study procedures. Exclusion criteria: pre-existing STI, current/past cervicovaginal disease, pregnancy/breastfeeding, recent antibiotic use, current/recent use of a copper intrauterine device, which promotes BV, and current/imminent menstruation.

Sample collection: On Day 1 (pretreatment), women will complete a demographic
30 and behavioral questionnaire and provide urine for pregnancy and STI testing. Contraceptive use and stage in the menstrual cycle will be recorded. A sexual health research nurse or clinician, experienced in examining women and undertaking BV clinical trials, will visually inspect the vaginal mucosa, collect four lateral wall swabs
35 and instruct women to collect neat vaginal fluid using a menstrual cup. Participants will be instructed on how to administer the vaginal gel and self-collect swabs at home;

instructional information sheets, gels in single use applicators, and swabs/transport medium will be provided. Women will self-collect two vaginal swabs immediately prior to administering the gel daily on Days 2-7. Daily swabs will be used for microbiome analyses (taxon qPCR) and to measure cytokines and prostate specific antigen (PSA) to evaluate abstinence from unprotected sex (which can alter the vaginal microbiome). On Day 8 (post-treatment), women will return to the clinic with their self-collected swabs and the research nurse will repeat the clinical examination and sample collection as on Day 1. On Days 1 (pre-dosing) and 8 at the clinic, swabs will be collected to determine markers of epithelial barrier integrity (proteomics), and menstrual cup samples will be collected for metagenomics, bacterial cultivation and metabolite (lactic acid and short chain fatty acid (SCFA)) quantitation. If a BV diagnosis is made on Day 8, women will be offered standard recommended treatment (oral metronidazole for 7 days). To explore the durability of any observed effect after lactic acid treatment cessation, an extra vaginal swab will be self-collected 21 to 30 days post treatment initiation and posted back to the clinic. All swabs and samples will be stored in buffers and at conditions validated to preserve sample integrity prior to processing.

Vaginal sample analyses: Sample analyses will be blinded. Swabs will be processed to diagnose BV. Nugent score will be assessed by gram stain by an experienced microbiologist in real time at the clinic Amsel clinical criteria will be used to diagnose symptomatic BV at the clinic. Vulvovaginal candidiasis will be diagnosed by standard clinical parameters. Urine will be tested for STIs (Chlamydia, gonorrhea, trichomoniasis and Mycoplasma genitalium) by qPCR at enrolment. Samples from participants with an STI will be subsequently excluded from analyses. PSA will be measured using the Human kallikrein 3/PSA Quantikine ELISA kit. Levels of lactic acid (both L- and D-lactic acid isomers, which are differentially produced by distinct *Lactobacillus spp.*) in vaginal samples will be quantified by enzymatic assay. SCFAs, that are elevated in vaginal fluid of women with BV, will be quantified in Day 1 and Day 8 samples using their SCFA targeted panel analysis (8 analytes); they have proven expertise in the analysis of vaginal samples. To prevent gel carry-over, samples will be collected ~24 h after dosing when the gels will have largely dissipated due to dilution by vaginal secretions (6 ml/24 h), diffusion and leakage.

Taxon qPCR: Eight daily swabs will be processed to purify DNA for taxon specific qPCR using a validated TaqMan-based assay targeting the 16S rRNA gene. *G. vaginalis* other key BV-associated bacteria (*Fannyhessea vaginae* [previously *Atopobium*], *Prevotella spp.*, and *Megasphaera spp.*) and 4 common vaginal *Lactobacillus spp.* (incl. *L. crispatus* and *L. iners*) will be quantified in 8 daily swabs,

using primers and probes as published. Total bacterial load in each sample will be quantified as previously.

Metagenomics: Shotgun metagenomic sequence libraries will be constructed from DNA and sequenced on the Illumina NovaSeq system (>90 million reads/sample, which provides sufficient depth to analyse the bacterial metagenome among an abundance of host DNA). The taxonomic composition of each metagenome will be established by mapping reads to VIRGO, a vaginal non-redundant gene catalogue and omics data analytical platform, enabling the identification of subspecies in each sample. To identify the presence and abundance of bacteria at the strain-level, metagenome assembled genomes will be generated as published and analysed with the InStrain tool (using a strict percent identity threshold). These studies will reveal if lactic acid vs. placebo gel changes bacterial load, decreases levels of key BV-bacteria and *L. iners*, and changes the overall vaginal microbiome composition to optimal.

Cytokines and chemokine analysis: Cytokines and chemokines will be quantified from daily swabs using a multiplex Luminex assay (a measure of total cytokine and chemokines). In addition to TNF α , inflammatory mediators associated with increased HIV risk *in vivo* and inhibited by lactic acid *in vitro* (e.g. IL-1 α /b, IL-6, IL-8, IP-10, MIP-1 α /b, RANTES) will be measured. Women with genital inflammation will be identified based on their inflammatory cytokine and chemokine profiles by K-means (Masson et al., 2020). The anti-inflammatory cytokine IL-1RA will also be quantified in vaginal fluids as well as other anti-inflammatory mediators (e.g. IL-10) that are robustly detected in vaginal fluids.

Metaproteomics: Day 1 (pre) and Day 8 (post-treatment) vaginal samples will be subjected to metaproteomics analysis using shotgun liquid chromatography tandem mass spectrometry, as described in Delgado-Diaz et al. 2022 and Alisoltani et al. 2020. This innovative application of metaproteomics will enable unbiased measurement of host protein abundance to reveal mechanistic insights of lactic acids effect on the epithelial barrier and other host functions, such as innate immune and inflammatory responses through biological process gene ontology analysis (Alisoltani et al., 2020). Protein identification will be conducted in MaxQuant using a custom cohort-specific vaginal database (Ma et al., 2020), and key differentially abundant epithelial barrier proteins and gene ontologies identified using the limma R package (Alisoltani et al. 2020).

Example 8 – Bacterial cultivation and experimental setup

Bacteria used in the examples 8 to 11 were plated from frozen stocks onto solid media and incubated for three days. Cultivation was performed at 37°C under anaerobic

conditions using an anaerobic chamber. All media were pre-reduced by being placed in the anaerobic chamber overnight before cultivation. *Lactobacillus crispatus* ATCC 33820, *Lactobacillus iners* ATCC 55195, and *Gardnerella vaginalis* ATCC 14018 were grown from single colonies for 18 hours in de Man, Rogosa and Sharpe (MRS), New York City (NYC-III), and Brain Heart Infusion (BHI) media, respectively.

Example 9– Bactericidal and bacteriostatic activity of lactic acid

Overnight cultures were adjusted to inoculate 1 ml of pre-reduced S-broth, so that the final bacteria concentration was 0.03 OD600, at pH 6.3, media adjusted to pH 4.5 (using HCl), and 1% DL-lactic acid pH4.5.

S-broth consisted of 37 g/L BHI broth, 10 g/L yeast extract, and 1 g/L dextrose, which was autoclaved, cooled and then supplemented to a final concentration of 5% fetal bovine serum (FBS), 5% Vitamin K1-Hemin, and 2% IsoVitaleX Enrichment. This nutrient-rich, serum-containing S-broth supported the growth of the three bacterial species from the female genital tract. Cultures were incubated for 48 hours under anaerobic conditions at 37°C, with 50 µl samples taken at 0, 24, and 48 hours to quantify bacterial viability. Viability (colony forming units, CFU/ml) was determined by plating serially diluted samples onto MRS, NYC-III, and BHI solid media for *L. crispatus*, *L. iners*, and *G. vaginalis*, respectively.

Results show that lactic acid treatment has bactericidal activity against *G. vaginalis*, with a sharp decrease in viable bacterial numbers after 24 hours (**Figure 5**). Lactic acid (pH 4.5) exhibited bacteriostatic activity against *L. iners* after 24 hours incubation at 37°C under anaerobic conditions. In contrast, media adjusted to the same pH 4.5 with HCl had little effect on *L. iners* growth compared to bacteria grown in untreated medium (pH 6.3). Neither lactic acid at pH 4.5 or media adjusted to the same pH mediated no observed effects on the growth of *L. crispatus* compared to untreated medium (pH 6.3).

Example 10 – Lack of activity of lactic acid against probiotic Lactobacillus strains

Canesflor (*Lactobacillus plantarum* P17630) and Physioflor (*L. crispatus*) probiotics were plated onto solid MRS media and incubated at 37°C under anaerobic conditions for 48 hours. Once single colonies were visible, a colony was inoculated into pre-reduced MRS media and further incubated for 18 hours.

To assess lactic acid bactericidal activity, bacteria was inoculated in MRS media at pH 5.7, MRS adjusted to pH 3.8 with HCl and MRS containing 1% DL-LA at pH 3.8. On the day of the experiment, bacterial cultures were adjusted to an OD600 of 1, and 10

µl were inoculated into 190 µl of pre-reduced treatments. This mixture was then incubated for 1 hour at 37°C under anaerobic conditions. After incubation, cultures were serially diluted and plated onto MRS agar, followed by further incubation for 48 hours. Finally, colonies were counted to calculate the CFU present in the original culture after treatment.

The result show that 1% DL-LA treatment at pH 3.8 for 1 hour under anaerobic conditions did not significantly decrease the viability of either Canesflor or PhysioFlor compared to bacteria grown in untreated medium (pH 5.7) (**Figure 6**).

Example 11 – Bactericidal activity of lactic acid against *L. iners* and *G. vaginalis* but not *L. crispatus* in a mixture of female genital tract bacteria

All media and treatments were pre-reduced by being placed in the anaerobic chamber overnight before cultivation. Frozen stocks of *G. vaginalis*, *L. iners*, and *L. crispatus* were plated onto appropriate solid media and incubated for three days under anaerobic conditions at 37°C. Single colonies were picked to prepare liquid cultures in BHI, NYC-III, and MRS media, respectively, and further incubated for 18 hours at 37°C. On the day of the experiment, bacterial monocultures were adjusted to OD600 of 0.03 for *G. vaginalis* and *L. iners* and 0.001 for *L. crispatus*. Aliquots of monocultures (20 µl of *G. vaginalis*, 20 µl of *L. iners*, and 2 µl of *L. crispatus*) were added to 1 ml of each treatment, either as single cultures or combined to form a mixed culture of all three bacteria (mix culture). Treatment conditions were S-broth unadjusted pH 6.3, S-Broth adjusted to pH 4.5 and S-broth 1% DL-LA pH 4.5.

The viability of the input monoculture (T0) was calculated by plating a sample at the beginning of the incubation period to determine the starting ratio of bacteria. Mono- and mix-cultures were then incubated for 24 hours at 37°C under anaerobic conditions. After this period, aliquots of monocultures were assessed for viability by serially diluting and calculating CFU/ml after 24 hours.

Quantitation of bacteria was performed using taxon-specific PMA-qPCR. Bacteria were harvested by centrifugation at 2500 x g and labeled with 200 µl of 50 µM PMAXx. PMAXx (propidium monoazide) is a membrane-impermeant photo-reactive dye that binds to DNA. In a population of live and dead cells, only dead cells are susceptible to DNA modification due to compromised cell membranes. DNA from dead cells cannot be amplified by PCR. After staining, bacterial pellets were stored at -20°C until DNA extraction.

DNA from bacterial cultures was purified using the MagAttract PowerMicrobiome kit (Qiagen). Relative growth within mixed bacteria was assessed by

quantitative PCR of PMAxx treated cultures. Briefly, 2 µl of DNA extracted from either monoculture or the mixture of bacteria subjected to the different experimental treatments were used for the specific detection of either *L. crispatus*, *L. iners*, or *G. vaginalis* target sequences, employing the following primers and probes:

- 5 • For detection of *G. vaginalis*:
 - o Gv Fw 5'-ACCTGGGCTTGACATGTGCCT-3' (SEQ ID NO:1)
 - o Gv Rev 5'-CATGCACCACCTGTGAACCTG-3' (SEQ ID NO:2)
 - o Probe 5'-FAM-CTGCAGAGATGTGGTTTCCTTTTCG-3IABkFQ-3' (SEQ ID NO:3).
- 10 • For detection of *L. iners*:
 - o Li Fw 5'-GATGCTAATACCGGATAACAACAGAT-3' (SEQ ID NO:4)
 - o Li Rev 5'-CACCGCAGGTCCATCCAAGA-3' (SEQ ID NO:5)
 - o Probe 5'-TGCCTATCAACTGTTTAAAAGATGGTTCT-3IABkFQ-3' (SEQ ID NO:6).
- 15 • For detection of *L. crispatus*:
 - o Lc Fw 5'-TCTTGACATCTAGTGCCATTTGT-3' (SEQ ID NO:7)
 - o Lc Rev 5'-TGCACCACCTGTCTTAGC-3' (SEQ ID NO:8)
 - o Probe 5'-FAM-CCGAAGGGAACCTTGTATCTCTAC-3IABkFQ-3' (SEQ ID NO:9).

20 The PCR reaction volume was 20 µl and contained 1X Taqman Gene Expression Master Mix, primers at a concentration of 800 nM, and dual-labeled probes at a concentration of 250 nM (FAM and 3' Iowa Black® FQ (3IABkFQ)). Assays were performed at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, then 60°C for 1 minute. Data analysis was performed using QuantStudio Real-Time PCR
 25 software. Copy numbers were quantified against a standard curve prepared by 10-fold serially diluted plasmids containing the target sequences. All samples were amplified in duplicate.

Taxon-specific PMA-qPCR results for quantifying viable bacterial showed that 1% lactic acid treatment for 24 hours / 37°C at pH 4.5 resulted in a >100-fold decrease
 30 in viable *G. vaginalis* compared to bacteria grown in media at pH 6.3. A similar decrease in viable *G. vaginalis* was observed in both in mono-cultures and when *G. vaginalis* was grown in a mixture with *L. crispatus* and *L. iners*. Taxon-specific PMA-qPCR data for mono-cultures were consistent with the gold standard viability assessment by CFU/ml. *L. iners* viability was decreased in the presence of lactic acid at pH 4.5 by >31 fold
 35 compared to untreated controls in both mono-cultures and mixtures. In contrast media acidified to the same pH 4.5 with HCL had little effect on *L. iners* viability. The viability

of *L. crispatus* propagated in monocultures or in a mixed cultures with *L. iners* and *G. vaginalis* in the presence of lactic acid at pH 4.5 or in media acidified to pH 4.5 with HCl was not affected compared to the untreated control. Results are presented in **Figure 7**, **Figure 8** and **Figure 9**.

5

Example 12 – Lactic acid alone or in combination with metronidazole decreases concentrations of viable *G. vaginalis* and *L. iners* but not *L. crispatus*

To assess lactic acid activity in the presence of metronidazole (MTZ) overnight cultures were used to inoculate 1 ml of pre-reduced S-broth at a final cell concentration of OD600 of 0.03. The treatments were: unadjusted media at pH 6.3, media adjusted to pH 4.5 (using HCl), MTZ 50 µg/ml pH 4.5, 1% DL-lactic acid pH 4.5 and combination of 50 µg/ml MTZ together with 1% DL-lactic acid at pH 4.5. Bacterial cultures were incubated for 24 h at 37°C in anaerobic conditions. Viability was quantified by calculating CFU/ml.

lactic acid at pH 4.5 dramatically decreased the levels of viable *G. vaginalis* and *L. iners* compared to untreated bacteria and was more potent than media acidified to pH 4.5 with HCl. Metronidazole led to a decrease viable *G. vaginalis* with greater inhibition observed with both metronidazole and lactic acid (MTZ + LA pH 4.5). In contrast to metronidazole, where there was no observable decrease in the levels of viable *L. iners*, media containing lactic acid at pH 4.5 and the combination of MTZ and lactic acid (pH 4.5) led to a >1000-fold decrease in viable bacteria. Neither lactic acid or metronidazole alone or in combination decreased levels of viable *L. crispatus*. Results are presented in **Figure 10**.

25 **Example 13 – Discussion**

To overcome the observed negative effects of OTC lactic gels (gels which are hyperosmolal and contain additional components that promote genital inflammation), new vaginal lactic acid compositions were formulated iso-osmolal with the human cervicovaginal fluid. Matching placebo gel were also formulated without harmful excipients and the compositions were tested in an EpiVaginal model. The new lactic acid gel compositions were not cytotoxic, did not substantially disrupt epithelial barrier integrity and did not elicit proinflammatory cytokines compared to placebo. Lactic acid was stable in the formulations for 18 months at room temperature and the compositions had a buffering capacity to maintain a pH<4.5 (when added to an equal volume of seminal fluid, pH 8).

35

The prevailing view is lactic acid kills BV-associated bacteria (e.g. *Gardnerella vaginalis*) but not vaginal *Lactobacillus* spp. *In vitro*. An *L. crispatus*-dominated vaginal microbiota is protective against adverse sexual and reproductive health outcomes and is considered stable and less likely to shift to BV while the less protective and typically
5 unstable *L. iners*, predominates following antibiotics used to treat BV, readily transitions back to BV. Using American Type Culture Collection strains (verified by sequencing), it was discovered that the new lactic acid compositions described herein are bactericidal against *G. vaginalis* but not *L. crispatus* yet unexpectedly kills *L. iners in vitro*, challenging current assumptions. The data show that the selective bactericidal activity
10 against *L. iners* versus *L. crispatus* is mediated by lactic acid and is not due to other components in the gel. The bactericidal activity of lactic acid against *L. iners* is greater (around 10,000 fold greater in the assay) than treating *L. iners* with media at the same low pH (placebo gel at pH 3.8 vs 1% DL LA gel pH 3.8) indicating that the observation is not simply a low pH effect. The data suggests that the new lactic acid compositions as
15 described herein are suitable for shifting the vaginal microbiome to an optimal *L. crispatus*-dominated microbiome, promoting a non-inflammatory vaginal environment and increasing expression of epithelial barrier integrity markers compared. The data also suggests that the new lactic acid compositions could be used as an adjunct to help select for *L. crispatus* following BV treatment with antibiotics and/or to promote colonization
20 of *L. crispatus* based live biotherapeutics.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The
25 present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

This application claims priority from Australian Provisional Application No. 2023903187 entitled "Vaginal compositions and methods and uses thereof" filed on 4 October 2023, the entire contents of which are hereby incorporated by reference.

30 All publications discussed and/or referenced herein are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of
35 these matters form part of the prior art base or were common general knowledge in the

field relevant to the present invention as it existed before the priority date of each claim of this application.

REFERENCES

- Alisoltani et al (2020) *Microbiome* 21;8(1):165.
- Anahtar et al (2015) *Immunity* 42:965-76.
- Arnold et al (2016) *Mucosal Immunol* 9:194-205.
- 5 Ayehunie et al (2006) *Toxicol In Vitro* 20:689-98.
- Ayehunie et al (2016) Pre-validation of in vitro-in vivo assays for vaginal irritation abstract Society for Toxicology 2016 Annual Meeting New Orleans, Louisiana.
- Ayehunie et al (2018) *Toxicol Rep* 5:134-140.
- Baldeon-Vaca et al (2021) *EbioMedicine* 69:103478.
- 10 Delgado-Diaz et al (2022) *Microbiome* 10:141.
- Division of Aids Adverse Event Grading Tables (2007) Additional Grading Table Resources. Addendum 1 Female Genital Grading Table for Use in Microbicide Studies. <https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables>
- Fan et al (2022) *Polymers (Basel)* 14(12): 2379.
- 15 Gali et al (2010) *Antimicrob Agents Chemother* 54:5105-14.
- Gupta et al (1998) *J Infect Dis* 178:446–50.
- Happel et al (2017) *BMC Women's Health* 17,7.
- Hearps et al (2017) *Mucosal Immunol* 10:1480-1490.
- Ma et al (2020) *Nat Commun* 11:940.
- 20 Masson et al (2015) *Clin Infect Dis* 61:260-9.
- Masson et al (2019) *Sex Transm Infect* 95:5-12.
- McKinnon et al (2019) *AIDS Res Hum Retroviruses* 35(3): 219–228.
- Moss et al (2013) *Antimicrob Agents Chemother* 57(8): 3994–3997.
- O'Hanlon et al (2011) *BMC Infect Dis* 11:200.
- 25 Tachedjian et al (2017) *Res Microbiol* 168:782-792.
- Thurman et al (2023) *Am J Obstet Gynecol* 228(6):716.e1-716.e12.
- Tyssen et al (2022) *Front Rep Health*. 2022;4:915948.
- Van Damme et al (2002) *Lancet* 360:971-7.
- Vinderola et al (2022) *Foods* 11:1077.
- 30 Żółkiewicz et al (2020) *Nutrients* 12(8): 2189.

CLAIMS

1. A vaginal pharmaceutical composition comprising:
 - a) lactic acid at about 1.40 % w/w to about 2.5% w/w;
 - 5 b) a mucoadhesive agent;
 - c) a viscosity of about 50 to about 150 Pa.s;wherein the composition is iso-osmolal with cervicovaginal fluid.
2. The composition of claim 1, wherein the composition comprises lactic acid at
10 about 1.64% w/w to about 2.25% w/w.
3. The composition of claim 1 or claim 2, wherein the composition comprises lactic acid at about 1.64% w/w.
- 15 4. The composition of any one of claims 1 to 3, wherein about 18% to about 82% of the lactic acid is protonated.
5. The composition of any one of claims 1 to 4, wherein the composition comprises L-lactic acid.
20
6. The composition of any one of claims 1 to 5, wherein the composition comprises D-lactic acid.
7. The composition of any one of claims 1 to 6, wherein the composition comprises
25 D-lactic acid and L-lactic acid.
8. The composition of claim 7, wherein the ratio of D-lactic acid to L-lactic acid is selected from:
 - a) about 0.8 to 1 to about 1.5 to 1;
 - 30 c) about 1 to 1; and
 - d) about 1.5 to 1.
9. The composition of any one of claims 1 to 8, wherein the composition comprises an osmolality of about 300 to about 410 mOsm/Kg.
35

10. The composition of claim 9, wherein the composition comprises an osmolality selected from:
- a) about 330 to about 390 mOsm/Kg;
 - b) about 330 to about 380 mOsm/Kg;
 - 5 c) about 380 to about 390 mOsm/Kg; and
 - d) about 370 mOsm/Kg.
11. The composition of any one of claims 1 to 10, wherein the mucoadhesive agent is selected from one or more of: hydroxyethylcellulose (HEC) or a derivative thereof, a
- 10 carbomer/a Carbopol®, methylhydroxypropyl cellulose, a hydrogel, and hydroxypropyl methylcellulose.
12. The composition of any one of claims 1 to 11, wherein the composition comprises the mucoadhesive agent at a concentration of about 1% to about 3% w/w.
- 15 13. The composition of any one of claims 1 to 12, wherein the composition comprises the mucoadhesive agent at a concentration of about 1.32% w/w.
14. The composition of any one of claims 1 to 13, wherein the composition has a pH
- 20 of about 3.2 to about 4.2.
15. The composition of any one of claims 1 to 14, wherein the composition has a pH of about 3.7 to 3.8.
- 25 16. The composition of any one of claims 1 to 15, wherein the composition additionally comprises one or more of: a paraben, sodium lactate and sodium chloride.
17. The composition of claim 16, wherein the paraben is methylparaben and/or propylparaben.
- 30 18. The composition of claim 16 or claim 17, wherein the paraben is present at a concentration of about 0.01 to about 0.2%, or about 0.02% to about 0.18%, or about 0.18%, or about 0.02%.
- 35 19. The composition of any one of claims 16 to 18, wherein the paraben is methylparaben and is present at about 0.18% w/w.

20. The composition of any one of claims 16 to 19, wherein the paraben in propylparaben and is present at about 0.02% w/w.
- 5 21. The composition of any one of claims 16 to 19, wherein the composition is not cytotoxic to cervicovaginal epithelial cells.
22. The composition of any one of claims 1 to 21, wherein the composition does not disrupt the integrity of the cervicovaginal epithelial barrier and/or increases the integrity
10 of the cervicovaginal epithelial barrier.
23. The composition of any one of claims 1 to 22, wherein the composition does not substantially increase the level of pro-inflammatory cytokines and/or pro-inflammatory chemokines.
- 15 24. The composition of any one of claims 1 to 23, wherein the composition reduces the level of pro-inflammatory cytokines and/or pro-inflammatory chemokines.
25. The composition of any one of claims 1 to 24, wherein the composition does not
20 substantially increase the expression of one or more pro-inflammatory cytokines selected from: IL-1 α (interleukin 1 alpha), IL-1 β (interleukin 1 beta) and IL-6 (interleukin 6).
26. The composition of any one of claims 1 to 25, wherein the composition reduces the expression of one or more pro-inflammatory cytokines selected from: IL-1 α
25 (interleukin 1 alpha), IL-1 β (interleukin 1 beta) and IL-6 (interleukin 6).
27. The composition of any one of claims 1 to 26, wherein the composition does not substantially increase the expression of a pro-inflammatory chemokines selected from MIP3 α (macrophage inflammatory protein-3 alpha), IL-8 (interleukin 8), IP-10 (IFN—
30 gamma-inducible protein 10), and RANTES (regulated upon activation, normal T-cell expressed and presumably secreted).
28. The composition of any one of claims 1 to 27, wherein the composition does not substantially reduces the expression of a pro-inflammatory chemokines selected from
35 MIP3 α (macrophage inflammatory protein-3 alpha), IL-8 (interleukin 8), IP-10 (IFN—

gamma-inducible protein 10), and RANTES (regulated upon activation, normal T-cell expressed and presumably secreted).

29. The composition of any one of claims 1 to 28, wherein the composition increases
5 the expression of the anti-inflammatory factor interleukin-1 receptor antagonist (IL-1RA).

30. The composition of any one of claims 1 to 29, wherein the composition does not
10 comprise one or more or all of: ethylenediaminetetraacetic acid (EDTA),
glycerine/glycerol, polyethylene glycol (PEG), citric acid, organic aloe barbadensis leave
juice, xanthan gum, natural flavour, potassium sorbate, potassium bitartrate, acetic acid,
butyric acid, succinic acid, boric acid, propylene glycol, caprylyl glycol, levulinic acid,
P-anisic acid, xylose, clary sage oil, phenethyl alcohol, thymus mastichina herb oil,
15 triethanolamine, hydrogenated castor oil ethoxylated, ethylparaben, propionic acid,
sodium benzoate, chlorhexidine gluconate and sorbic acid.

31. The composition of any one of claims 1 to 30, wherein the composition is for
daily administration.

20 32. The composition of any one of claims 1 to 31, wherein the composition is for
continuous, semi-continuous administration or periodic presumptive dosing.

33. The composition of any one of claims 1 to 32, wherein the composition is in the
25 form selected from a: gel, cream, capsule, ovule, lubricant or foam.

34. The composition of any one of claims 1 to 33, wherein the composition is
administered using a device selected from a: pessary, intravaginal ring, vaginal film,
tampon, suppository, wipe or sponge.

30 35. The composition of any one of claims 1 to 34, wherein the composition comprises
a thermosensitive polymer.

36. The composition of any one of claims 1 to 35, wherein the pH of the composition
was adjusted with sodium hydroxide or hydrochloric acid.

37. The composition of any one of claims 1 to 36, wherein the composition is administered before, after or at the same time as one or more other therapeutic/s.
38. The composition of any one of claims 1 to 37, wherein the therapeutic is a small molecule or a biotherapeutic.
39. The composition of any one of claims 1 to 38, wherein the therapeutic is an antibiotic, hormone, contraceptive, a probiotic or a component thereof or a postbiotic.
40. The composition of any one of claims 1 to 39, wherein the biotherapeutic is a bacteria or a part thereof selected from one or more or: *Lactobacillus*, *Leuconostoc*, *Bifidobacterium*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*.
41. The composition of claim 40, wherein the bacteria are *Lactobacillus crispatus*, preferably *Lactobacillus crispatus* is CTV-05.
42. The composition of any one of claims 1 to 41 for treating or preventing genital inflammation in a subject.
43. The composition of any one of claims 1 to 42, wherein the subject is female.
44. The composition of any one of claims 1 to 43, wherein the subject is male.
45. The composition of claim 42 or claim 43, wherein the inflammation is cervicovaginal inflammation.
46. The composition of claim 45 wherein the cervicovaginal inflammation is the cause or result of a condition selected from one or more of: bacterial vaginosis, *Chlamydia trachomatis*, gonorrhoea, human papillomavirus human immunodeficiency virus, trichomonas infection, vaginitis, pelvic inflammatory disease, allergy, ureaplasma, mycoplasma, campylobacter, syphilis, improper hygiene, douching/vaginal cleaning practices, and herpes simplex virus.

47. The composition of any one of claims 1 to 43 or 45 or 46 for use in maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome.
- 5 48. The composition of any one of claims 1 to 43 or 45 to 47 for use in maintaining or increasing the relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome.
49. The composition of any one of claims 1 to 43 or 45 to 48 for use in maintaining
10 or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome.
50. The composition of any one of claims 1 to 43 or 45 to 49 for use in decreasing the relative abundance of *Lactobacillus iners* or *Gardnerella vaginalis* in the
15 cervicovaginal microbiome.
51. The composition of any one of claims 1 to 43 or 45 to 50 for use in increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners*, the ratio of *Lactobacillus gasseri* to *Lactobacillus iners*, *Lactobacillus crispatus* to *Gardnerella vaginalis*, or *Lactobacillus jensenii* to *Lactobacillus iners* in the cervicovaginal microbiome.
20
52. The composition of any one of claims 1 to 43 or 45 to 46 for use in maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.
- 25 53. The composition of any one of claims 1 to 52, wherein the composition is an over the counter (OTC) composition.
54. A method of treating or preventing cervicovaginal inflammation in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to
30 53 to the subject.
55. A method of maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the cervicovaginal microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
35

56. A method of maintaining or increasing the relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
- 5 57. A method of maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
58. A method of decreasing the relative abundance of *Lactobacillus iners* or
10 *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
59. A method of increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners*
15 in the cervicovaginal microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
60. A method of increasing the integrity of the cervicovaginal epithelial barrier in a subject, the method comprising administering the composition of any one of claims 1 to
20 43 or 45 to 53 to the subject.
61. A method of preventing urogenital tract inflammation in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the
25 subject.
62. A method of maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the urogenital tract microbiome in a subject, the method comprising administering the composition of any one of claims 1 to 43 or 45 to 53 to the subject.
- 30 63. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for treating or preventing cervicovaginal inflammation in a subject.
64. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus*
35 *crispatus* in the cervicovaginal microbiome in a subject.

65. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus gasseri* in the cervicovaginal microbiome in a subject.
- 5 66. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of a medicament for maintaining or increasing the relative abundance of *Lactobacillus jensenii* in the cervicovaginal microbiome in a subject.
67. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of
10 a medicament for decreasing the relative abundance of *Lactobacillus iners* or *Gardnerella vaginalis* in the cervicovaginal microbiome in a subject.
68. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of
15 a medicament for increasing the ratio of *Lactobacillus crispatus* to *Lactobacillus iners* in the cervicovaginal microbiome in a subject.
69. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of
20 a medicament for maintaining or increasing the integrity of the cervicovaginal epithelial barrier in a subject.
70. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of
a medicament for preventing a urogenital tract inflammation in a subject.
71. Use of lactic acid at about 1.40 % w/w to about 2.5% w/w in the manufacture of
25 a medicament for maintaining or increasing the relative abundance of *Lactobacillus crispatus* in the urogenital tract microbiome in a subject.
72. The use of any one of claims 62 to 71, wherein the medicament additionally
30 comprises a mucoadhesive agent, a viscosity of about 50 to about 150 Pa.s and wherein the composition is iso-osmolal with cervicovaginal fluid.
73. An applicator or device having the composition of any one of claims 1 to 53.
74. A vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w
35 to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.

75. A vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 2.5% w/w, wherein the composition is iso-osmolal with cervicovaginal fluid.
- 5 76. A vaginal pharmaceutical composition comprising lactic acid at about 1.40 % w/w to about 4.5% w/w, wherein the composition has an osmolality of 300 to 800 mOsm/Kg.
77. A vaginal delivery device comprising a pharmaceutical composition comprising lactic acid at about 1.40% w/w to about 4.5% w/w, wherein the composition has an
10 osmolality of 300 to 800 mOsm/Kg.
78. The vaginal pharmaceutical composition or the vaginal delivery device of claim 76 or claim 77, wherein the composition has an osmolality of 500 to 800 mOsm/Kg.