

Use of lactobacilli and estriol combination in the treatment of disturbed vaginal ecosystem: a review

Laktobasil ve estriol kombinasyonunun bozulmuş vajina ekosisteminin tedavisinde kullanımı: Bir derleme

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Abstract

To maintain a healthy vaginal ecosystem or to restore any disturbance, sufficient estrogen levels, an intact mature vaginal epithelium, and physiological lactobacillary microflora are essential. Thus, a combination of beneficial lactobacilli and estrogen is an appealing treatment option. This article reviews the published data on the use of viable *Lactobacillus acidophilus* KS400 and a low dose of estriol (0.03 mg E3) in the form of vaginal tablets (Gynoflor®). In vitro studies demonstrated that *L. acidophilus* KS400 produces lactic acid and hydrogen peroxide (H₂O₂), inhibits the growth of relevant vaginal pathogens, and inhibits adherence of pathogens to epithelial cells. Topical administration of E3 for treatment of vaginal diseases is generally preferred, as this route of application of hormones produces a more significant local proliferative response and has no stimulating effect on the endometrium. Overall, 16 clinical studies have been published with the combination of *L. acidophilus* KS400 and 0.03 mg E3. The results of these trials have demonstrated that the combination improves the vaginal epithelium and the restoration of the lactobacillary microflora with an excellent safety profile, even during pregnancy. The combination can be used in pre- and postmenopausal women for the restoration of the vaginal flora after anti-infective therapy, for treatment of symptomatic vaginal atrophy, and for abnormal vaginal flora therapy. It can be also considered in repetitive therapy courses for the long-term prevention of recurrences of bacterial vaginosis, even though further clinical studies are needed to substantiate the benefit of this application. (J Turkish-German Gynecol Assoc 2011; 12: 239-46)

Key words: *Lactobacillus acidophilus*, estriol, abnormal vaginal flora, restoration of vaginal ecosystem, recurrence prevention, vaginal atrophy

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Özet

Sağlıklı bir vajinal ekosistemi sürdürmek veya bozulmuş olanı düzeltmek için, yeterli östrojen düzeyleri, sağlam olgun bir vajina epiteli ve fizyolojik bir laktobasiler mikroflora esastır. Bu nedenle, yararlı laktobasiller ve östrojen kombinasyonu cazip bir tedavi seçeneğidir. Bu makale vajinal tablet (Gynoflor®) formundaki düşük doz estriol (0.03 mg E3) ve canlı laktobasillus asidofilus KS400'ün kullanımı üzerine yayınlanmış verileri derlemektedir. İn vitro çalışmalar *L. asidofilus* KS400'ün laktik asit ve hidrojen peroksit (H₂O₂) ürettiğini, ilgili vajinal patojenlerin çoğalmasını baskıladığını ve patojenlerin epitel hücrelerine tutunmasını baskıladığını göstermiştir. Vajinal hastalıkların tedavisi için E3'ün genellikle topikal uygulanması tercih edilmektedir; çünkü hormonların bu şekilde uygulanması daha belirgin bir proliferatif yanıt oluşturmada ve endometriyumda uyarıcı bir etki olmamaktadır. *L. asidofilus* KS400 ve 0.03 mg E3 kombinasyonu ile ilgili toplam 16 klinik çalışma yayınlanmıştır. Bu çalışmaların sonuçları kombinasyonun vajinal epiteli ve laktobasiler mikroflora restorasyonunu iyileştirdiğini, güvenlilik profilinin ise gebelik dahil mükemmel olduğunu göstermiştir. Kombinasyon menopoz öncesi ve sonrası kadınlarda anti-enfektif tedaviden sonra vajinal floranın restorasyonu için, semptomatik vajinal atrofinin tedavisi ve anormal vajinal floranın tedavisi için kullanılabilir. Bakteriye vajinozis rekürrenslerinin uzun dönemde önlenmesi için tekrarlanan tedavi kurlerinde de düşünülebilir ancak bu uygulamanın geçerliliğini göstermek için daha fazla klinik çalışmaya gerek duyulmaktadır.

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Anahtar kelimeler: *Laktobasillus asidofilus*, estriol, anormal vajinal flora, vajinal ekosistemin restorasyonu, rekürrenslerin önlenmesi, vajinal atrofi

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Introduction

The vagina and its healthy microflora form a balanced vaginal ecosystem, with the environment controlling the microbial strains present and the flora in turn controlling the vaginal environment and engaging in the natural defence against pathogens (1, 2). Sufficient estrogen levels leading to an intact mature vaginal epithelium, as well as the physiological lactobacillary microflora are essential (3). Additionally, the vaginal immune response also influences the vaginal ecosystem and is important for overall defence (4).

During the last decade, the vaginal microflora has been intensively studied, and additionally in the framework of the human microbiome project-analysis of both the human and microbial genome (5). The microorganisms living in symbiosis with the human body can be mutualists (benefiting themselves and the host), commensals (benefiting themselves only) or pathogens (benefiting themselves by harming the host) (6). *Lactobacillus* species are the dominant vaginal mutualists (7) and reach levels of 10⁷ to 10⁸ colony forming units (cfu)/g of vaginal fluid in healthy premenopausal woman (8). The vaginal flora is in a dynamic state-the abundance and bacterial type of vaginal

microbiota can change rapidly within months or sometimes days (5, 6, 9). The predominant species of lactobacilli found in the vagina of healthy women remains controversial. Organisms previously collectively known as *Lactobacillus acidophilus* were currently shown to form a number of separate species within the *L. acidophilus* complex. Depending on the study, *L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. iners* were predominantly identified in the vagina (5, 9, 10). Beneficial lactobacilli (1) produce antibacterial compounds, (2) have the ability to colonize the vaginal epithelium, and (3) seem to influence the regulation of local vaginal immunity (4, 5, 10).

Another crucial element to be considered in the microecology of the vagina is the functional condition of the stratified, squamous nonkeratinised vaginal epithelium (1). The fluctuating sex hormones, particularly estrogens (1, 3) induce the proliferation and maturation of the vaginal epithelium (1). The breakdown of proliferated superficial vaginal epitheliocytes liberates glycogen, which is metabolised to glucose by either enzymes secreted by the vaginal or cervical cells or by lactobacilli. Hence, a mature vaginal epithelium is a prerequisite for establishing and maintaining physiological lactobacillary microflora (11). Vaginal atrophy is associated with reduced efficacy of the barrier function of the vaginal epithelial lining and, as a consequence facilitate the penetration of pathogenic micro-organisms (12, 13).

Risk factors for disturbing the vaginal ecosystem can be (1) endogenous (variations in hormone levels, contraceptives, menstruation, pregnancy and lactation, diabetes mellitus, systemic diseases) or (2) exogenous. The exogenous factors are either life-style related (smoking, stress, unprotected sex, etc.), infectious, or medical (antibacterial therapy, radiation, etc.) (1, 8, 14) It still remains unknown whether alterations of the *Lactobacillus* flora, the vaginal epithelium or the pathogens are the initial cause of an abnormal vaginal flora (15). It was found that some healthy women (7-33%) lack the *Lactobacillus* species in the vagina and have other lactic acid-producing bacteria such as *Atopobium vaginae*, *Megasphaera*, and *Leptotrichia* species instead to maintain vaginal health, i.e. remain asymptomatic (5). The studies do not address whether such 'healthy' women are in a transitional phase towards or from BV, or whether it should be called asymptomatic BV, i.e. abnormal flora but no symptoms (5).

The Gynoflor® vaginal tablet (*L. acidophilus*-0.03 mg estriol-combination) contains both lyophilised, viable *Lactobacillus acidophilus* KS400 bacteria (100 million colony forming units [cfu] per tablet) and a very low dose of estriol (0.03 mg E3). The goal of the current article is to review the published evidence in order to provide a rationale for the use of the *L. acidophilus*-0.03 mg estriol-combination vaginal tablets in daily clinical practise.

In vitro properties of *Lactobacillus acidophilus* KS400

There are numerous mechanisms by which probiotic lactobacilli ensure their ability to colonize the host and to competitively exclude the pathogens (6, 8). Firstly, vaginal lactobacilli inhibit the growth of pathogens by the production of lactic acid, or by the production of hydrogen peroxide (H_2O_2) and other antimicrobial substances, i.e. bacteriocins and bacteriocin-like substances (6, 8). Furthermore, beneficial

lactobacilli strains compete with other microorganisms, such as *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans*, *Staphylococcus aureus*, *Gardnerella vaginalis*, *Prevotella bivia*, *Mobiluncus hominis*, etc. for adherence to the vaginal epithelium and for nutrients. Additionally, other mechanisms, especially immune modulation, although yet less well understood, are at play (4, 6, 8).

Not every *Lactobacillus* strain of a single species produces a beneficial effect for the vaginal ecosystem, emphasising the importance of careful selection, study and assessment of the different properties of a specific strain in order to consider it for the prophylactic or therapeutic use as probiotic in humans. The lactobacilli strain *L. acidophilus* KS400 has been originally isolated from humans and is well characterised with regard to its in vitro properties.

The production of lactic acid by lactobacilli, via the fermentation of glycogen from desquamated vaginal epithelial cells, induces vaginal milieu acidification resulting in a normal (<4.5) vaginal pH. The acidic environment provides optimal conditions for the lactobacilli and unfavourable growth conditions for the pathogenic micro-organisms (1, 16). Lactic acid has been also shown to stimulate the immune response (17). In vitro experiments with *L. acidophilus* KS400 have demonstrated that the strain is able to produce substantial amounts of lactic acid (16). The DL-lactic acid concentration in the nutrient medium increased by about 5 mg/mL within 6-12 h and the pH of the culture decreased by ≥ 2 units within 6-12 hours of incubation. The ability of *L. acidophilus* KS400 to produce lactic acid has been confirmed also in clinical studies: a decrease and thus normalization of the vaginal pH has been observed (see below).

A further important defence mechanism of beneficial lactobacilli strains involves the production of H_2O_2 -a well-recognised mechanism of bacterial antagonism (1, 8). H_2O_2 is an oxidizing agent killing pathogens through the production of free radicals. It is thought that lactobacilli produce Fe^{3+} -activated extracellular peroxidase to protect themselves (6). The important role of H_2O_2 -producing lactobacilli is also demonstrated by clinical evidence: the vagina of 96% of healthy women was colonised by H_2O_2 -producing lactobacilli strains, compared to only 6% in women with bacterial vaginosis (7). *L. acidophilus* KS400 has been shown to produce H_2O_2 , in vitro, thus enabling it to inhibit the growth of relevant vaginal pathogens (16).

Bacteriocins are protein-like substances, usually with limited bacterial killing activity, which inhibit strains of the same or closely-related species (11). Bacteriocin-like substance is a term applied to antagonistic substances which are incompletely defined and have a broader spectrum of activity. However, the role of these substances in vivo remains to be elucidated. Bacteriocins and bacteriocin-like substances have been identified for some strains of *L. acidophilus*, *L. helveticus*, *L. fermenti* and *L. plantarum*, and shown to inhibit a wide range of both Gram-positive and -negative bacteria as well as fungi (11). Whether *L. acidophilus* KS400 produces a bacteriocin or bacteriocin-like substances has not been fully elucidated.

The overall growth inhibition activity of lactobacilli strains was also investigated with in vitro co-culture experiments. Kanne et al. (18) incubated the cultures of *E. coli*, *S. aureus* and *C.*

albicans either with or without *L. acidophilus* KS400, and have determined the growth of the respective pathogens. After 6 hours of co-incubation, the numbers of all three pathogens were significantly decreased, and *E. coli* and *S. aureus* were no longer detectable. Servin [Servin A.L., 2004, data on file] further investigated the ability of *L. acidophilus* KS400 to inhibit vaginal pathogens. Again, *S. aureus*, uropathogenic *E. coli*, *G. vaginalis*, and *P. bivia* were incubated with or without *L. acidophilus* KS400. In the presence of the lactobacilli strain, the numbers of pathogens in the culture was significantly reduced for all tested bacteria (Figure 1), confirming the earlier results and also demonstrating inhibitory activity against relevant urogenital pathogens.

Beneficial vaginal lactobacilli strongly adhere to epithelial cells and thereby prevent colonization of the vagina by pathogens by competitive exclusion. A number of studies have demonstrated the ability of lactobacilli to adhere to human epithelial cells and to diminish the pathogens (19, 20). In experiments using cervical HeLa cells and intestinal Caco-2/TC7 cells, it was shown that *L. acidophilus* KS400 adhered well to these epithelial cells. [Servin A.L., 2004, data on file] Later, *L. acidophilus* KS400 efficiently inhibited the adherence of *E. coli*, *G. vaginalis*, and *P. bivia* to epithelial cells.

Lactobacilli also have immune modulation properties. Through the production of bacterial signalling factors, lactobacilli induce human antimicrobial factors such as defensins, lysozyme and haemocidins (6, 21) and modulate the expression of 70-kDa human shock protein (hsp70) and Mannose-Binding Lectin (MBL) (4). It seems that the immune system accurately calibrates responses to pathogens and differentiates microorganisms through pattern recognition receptors, mediated by epithelial trans-membrane proteins called Toll-like receptor (TLR) signalling (4). It is thought that bacteria present in the vagina of healthy women maintain epithelial cell TLR activation at a steady level, resulting in increasing anti-inflammatory cytokines, such as IL-10, and decreasing the proinflammatory cytokines IL-1 β , TNF- α , and IL-6, thus creating a T-helper-like

response in the vaginal milieu to inhibit the proliferation of pathogens (1, 21).

More recently, additional defense mechanisms, such as the production of biosurfactants, co-aggregation and lactobacilli biofilm formation, have been discussed (6, 22).

Key characteristics of Estriol (E3)

The estrogens, estrone (E1), estradiol (E2), and estriol (E3), are female sex hormones occurring naturally in humans. E3 is specific for humans and does not occur in rodents (2). Whereas E2 and E1 can be reversibly metabolised to each other, E3 cannot be transformed back. Like all estrogens, E3 stimulates the proliferation and maturation of the vaginal epithelium. However, E3 has a lower receptor affinity (about 10 times) than E2, and thus is not able to induce estrogenic effects on the endometrium, bone and breast tissue at physiological concentrations (2). Unlike E2, after single-dose oral or vaginal applications of E3 in normal doses (oral: $\leq 8-10$ mg; vaginal: ≤ 0.5 mg), there is no, or only a weak proliferative, effect on the endometrium (23).

Estrogens administered vaginally are absorbed in a dose-dependent, bypass hepatic metabolism and are biologically active (24, 25). Vaginal estrogens are more effective in relieving urogenital symptoms than oral preparations as (1) lower doses are required due to the absence of hepatic metabolism, and (2) high local estrogen level induces direct vaginal response (2, 26). Thus, topical administration of E3 for treatment of vaginal diseases is generally preferred, as this hormone applied locally is safer than other estrogens and produces a more significant proliferation response than after oral intake (2). It is particularly important if systemic hormone replacement with estrogen is not required.

Synergy of the combination

As mentioned, both sufficient estrogen levels inducing a mature vaginal epithelium and colonisation by lactobacilli are essential to maintain or restore a healthy vaginal ecosystem. Estrogen level fluctuations, with accompanying alterations in the proliferation and maturation of the epithelium, can alter bacterial

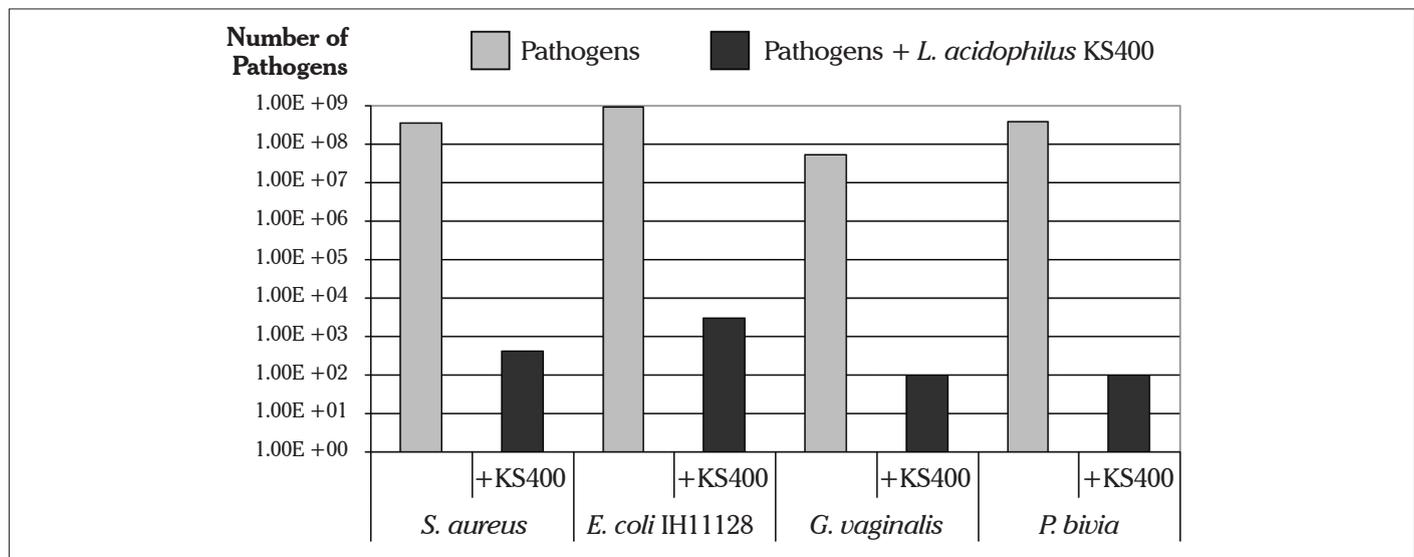


Figure 1. Inhibition of pathogens by Lactobacillus acidophilus KS400 [Servin A.L., 2004, data on file

adherence and other properties and hence affect the composition of the vaginal microflora (1, 19). It has been shown that the in vitro adherence of lactobacilli to vaginal epithelial cells is stronger on days of high concentrations of estrogens, and that administration of estrogen is able to restore vaginal colonisation in post-menopausal women (27).

A disturbed vaginal ecosystem is characterized by reduced or non-existing *Lactobacillus* flora and a more or less damaged epithelium (15). It is not always evident whether alterations of the vaginal epithelium or pathogenic micro-organisms are the primary cause of a disturbed vaginal ecosystem. Thus, treatment with a combination of beneficial lactobacilli and low-dosed estriol to support the restoration of the vaginal ecosystem on the level of the vaginal epithelium and microflora makes sense, not only in postmenopausal women, but also in women of reproductive age.

Clinical data

Pharmacokinetic studies

In a pharmacokinetic study, Kaiser et al. (28) demonstrated that the plasma level of unconjugated E3 transiently increased after the first application of *L. acidophilus*-0.03 mg estriol-combination, but was still within the normal range for untreated postmenopausal women. After the 12th daily application lactobacilli-estriol-combination however, no increase of the E3 plasma level could be observed. This was explained by the presence of a more proliferated and matured vaginal epithelium, preventing the E3 from crossing the vaginal mucosal layers. Basal plasma concentrations of unconjugated E3 during the 12 day treatment remained at the same level, indicating indirectly that no accumulation of E3 had taken place and that systemic effects were hence extremely unlikely. Thus, in contrast to the standard dose of 0.5 mg E3 (2), the *L. acidophilus*-0.03 mg estriol-combination does not result in a significant absorption and increase in systemic estriol levels

Restoration of disturbed vaginal ecosystem

For the treatment of vaginal infections or abnormal vaginal flora, as well as for the restoration therapy after use of anti-infective to prevent relapses, the same defense mechanisms induced by lactobacilli-estriol-combination are important to achieve a healthy vaginal ecosystem, i.e. the epithelial maturation, the growth inhibition of pathogens and the prevention of pathogenic vaginal colonisation. Following treatment of vaginal infections with antibiotics, a slow spontaneous recovery of the vaginal flora composition is usually seen, probiotic lactobacilli can be administered to support this restoration process.

The promising clinical data of probiotic lactobacilli treatment have been previously reviewed by other authors (29, 30). In this section, the published clinical studies of *L. acidophilus*-0.03 mg estriol-combination that have been performed to investigate the restoration of the vaginal ecosystem in different clinical indications are summarized (Table 1). The first clinical studies with lactobacilli-estriol-combination were carried out in the 1980's as open, uncontrolled trials in the treatment of vaginal infections (18, 31). Further open clinical studies demonstrating a positive

effect and benefit of *L. acidophilus*-0.03 mg estriol-combination have been published in the treatment of bacterial vaginosis (32, 33), in the perioperative treatment for gynaecological operations (34), and in the prophylaxis of infectious complications during intravaginal and intrauterine operations (35).

Parent et al. (36) performed a randomized, placebo-controlled, double-blind, multicentre study to compare *L. acidophilus*-0.03 mg estriol-combination (n=17) with placebo (n=15) in premenopausal women with bacterial vaginosis according to Amsel (37). Women were randomly assigned to a 6-day therapy protocol with verum or placebo. Follow-up took place 2 and 4 weeks after the therapy. Cure rate (defined as ≤ 1 of the 4 Amsel criteria positive) was 77% in the lactobacilli-estriol-combination group vs. 25% in the placebo group at 2 weeks follow-up visit. The corresponding numbers at 4 weeks were 88% and 22%, respectively. At both visits, the cure rate was higher for the test group than for the placebo group. In addition, a significant increase in the number of lactobacilli was observed in the vaginal smear of women treated with the test product as compared to the placebo. The therapeutic cure rate observed in this study was similar to cure rates of metronidazole and clindamycin.

Donders et al. (38) evaluated the efficacy of *L. acidophilus*-0.03 mg estriol-combination in comparison to metronidazole in the treatment of bacterial vaginal infections in a multi-centre, randomised, active-controlled pilot study. Forty six, premenopausal women between 18 to 50 years of age with a disrupted vaginal flora due to a bacterial vaginal infection (bacterial vaginosis and/or aerobic vaginitis) were included. Diagnosis was based on fresh phase contrast microscopy of vaginal fluid showing either lactobacillary grades IIb (decreased number of lactobacillary morphotypes overgrown by other bacterial morphotypes) or III (disappearance of lactobacilli due to overgrowth by other bacteria). Patients were randomized for treatment either with 12 vaginal tablets of lactobacilli-estriol-combination or with 6 vaginal suppositories containing 500 mg metronidazole (high dose). The treating physician was blinded to the medication type patients received. Eight efficacy variables were studied to assess the status of the vaginal flora at entry, at one week, one month and 4 months after the end of therapy. The combination of *L. acidophilus* KS400 and 0.03 mg E3 had equivalent efficacy to metronidazole in the short-term treatment, but was slightly less efficacious after one month. The authors suggested that continued repetitive use of the lactobacilli-estriol-combination should be considered and advised more studies to address this. In a randomised, placebo-controlled, double-blind study, Özkinay et al. (20) investigated the efficacy of *L. acidophilus*-0.03 mg estriol-combination to restore the physiological vaginal ecosystem in patients who had received specific anti-infective therapy for vaginal infections of various aetiology (bacterial vaginosis, candidiasis, trichomoniasis, and mixed infections). In total, 354 of 360 randomized pre- and postmenopausal women were included in the statistical analysis. Two to three days after the end of the anti-infective therapy, they were randomly assigned to treatment with either lactobacilli-estriol-combination or placebo. The Normal Flora Index (NFI), based on the number of lactobacilli versus the number of pathogens, vaginal pH-value, and number of leukocytes, increased signifi-

Table 1. Overview of main published clinical studies with 10⁷ cfu/g Lactobacillus acidophilus KS400 and 0.03 mg estriol (E3) combination (Gynoflor®)

Study	Design	Patients	Intervention	Outcome
Kaiser et al., 2000 (28)	Pharmacokinetic, open, monocentric, multiple-dose	8 menopausal volunteers aged 57-65	<i>L. acidophilus</i> -0.03 mg E3 1 tablet daily for 12 days	Vaginal Maturation Index (VMI) improved strongly, plasma concentration of E3 remained at the same level, no accumulation
Parent et al., 1996 (36)	Multicentric (3 centres), randomised, placebo-controlled, parallel group	32 women with bacterial vaginosis intermediate cases aged 20-52	<i>L. acidophilus</i> -0.03 mg E3 1-2 tablet daily for 6 days or placebo	Clinical cure rate (defined as ≤ 1 of 4 Amsel criteria) was significantly higher in the test group
Donders et al., 2010 (38)	Multicentric, active-controlled, randomised, single-blind study, parallel	46 premenopausal women with vaginitis or vaginosis with disrupted microflora aged 18-50	<i>L. acidophilus</i> -0.03 mg E3 1 tablet for 12 days or metronidazole 500 mg for 6 days	Short-term efficacy of <i>L. acidophilus</i> -0.03 mg E3 comparable to metronidazole, long-term efficacy was lower, repeated treatment is required
Özkinay et al., 2005 (20)	Monocentric, randomised, double-blind, placebo-controlled, parallel	360 women, restoration of flora after anti-infective therapy, aged 19-70	<i>L. acidophilus</i> -0.03 mg E3 or placebo after anti-infective therapy	Normal Flora Index (NFI) significantly improved in test group; restoration of the flora significantly enhanced
Unzeitig & Al Awad, 2006 (39)	Open, monocentric	98 women of childbearing age women with at least 4 episodes of vaginal discomfort in past year	<i>L. acidophilus</i> -0.03 mg E3; benzydamide irrigation (various schemes, 3 months)	Most of the unsuccessfully treated women reported a marked improvement and the remainder - a partial improvement
Kanne & Jenny, 1991 (45)	Double-blind, active- and placebo-controlled, randomised, parallel-group, dose-finding	15 women with atrophic vaginitis aged 51-65	<i>L. acidophilus</i> -0.03 mg E3 2 tablets daily for 6 days or E3 0.5 mg + lactobacilli or lactobacilli	No significant difference between the test medication (0.03 mg E3) and active control (0.5 mg E3)
Feiks & Grünberger, 1991 (46)	Double-blind, randomised, active-controlled, parallel groups	48 women with atrophic vaginitis aged 49-69	<i>L. acidophilus</i> -0.03 mg E3 1 tablet daily for 12 days and E3 0.5 mg 1 ovula for 12 days	No significant difference between the <i>L. acidophilus</i> -0.03 mg E3 and 0.5 mg E3
Hengst et al., 1992 (47)	Open, monocentric, perspective	Preterm prevention during pregnancy, 161 pregnant women	<i>L. acidophilus</i> -0.03 mg E3 1 tablet daily for 6-12 days	The lowest pre-term delivery rate observed in the group with <i>L. acidophilus</i> -0.03 mg E3; no adverse events
Hoyme et al., 1998 (48) Hoyme et al., 2004 (49)	Pilot multicentre (16 centres) cohort	314 pregnant women, preterm prevention during pregnancy	<i>L. acidophilus</i> -0.03 mg E3 1 tablet daily for 6 days	Reduction in preterm birth rate, delivery before week 32 and premature rupture; no adverse events
Melczar et al., 2002 (33)	Open, monocentric, before-after	100 women with bacterial vaginosis 3 of 4 Amsel criteria positive	<i>L. acidophilus</i> -0.03 mg E3 1 tablet daily for 6 days	Overall, 81% became completely free of complaints and 17 % showed substantial improvement; no adverse events

cantly more in the lactobacilli-estriol group than in the placebo group and remained at the higher level after 4 to 6 weeks. The vaginal flora in the women of the test group improved significantly more than in the placebo group, both directly after restoration therapy and at the follow-up examination, as measured by the degree of purity and number of lactobacilli. Furthermore, the number of relapses at follow-up was considerably lower in the lactobacilli-estriol-combination group (19/239, 7.9%) than in the placebo group (15/119, 12.6%).

In an open, monocentric clinical study, Unzeitig and Al Awad (39) treated 98 women of childbearing age women with at least 4 episodes of vaginal symptoms in the previous year (yeast or bacterial origin, recurrent bacterial vaginosis, etc.). The women received a single benzylamide irrigation (anti-inflammatory drug for local use) and subsequently *L. acidophilus*-0.03 mg estriol-combination for 6 consecutive days during one menstrual cycle, and during the following cycle they were again treated with the single benzylamide irrigation, but the lactobacilli-estriol-combination therapy was given in a 2-weeks interval (4 and 2 days, i.e. repetitive dosing). Two thirds of the previously unsuccessfully treated women with chronic vaginal discharge and vulvodynia reported a marked improvement, and about one fifth showed a partial improvement. Similar to the study by Donders et al., also in this study it was evident that a single 6-day therapy with lactobacilli-estriol-combination is not sufficient to achieve a long-term cure in women with recurrent disease and that a long-term maintenance therapy probably would be needed. The efficacy of repeated, probiotic lactobacilli therapy has been investigated in the long-term prevention of bacterial vaginosis recurrence, providing promising results (29, 40, 41).

Treatment of vaginal atrophy

An important quality of life issue in the management of women's menopause is an effective and safe treatment of vaginal atrophy and atrophic vaginitis. In recent years, the use of locally applied vaginal low-dose estrogen has been advocated in preference to systemic treatment and is considered to be the best therapy for vaginal atrophy (3, 12, 13). Another broadly discussed question is the possible use of low-dosed vaginal estrogens for the treatment of the symptomatic vaginal atrophy (vaginal dryness, dyspareunia, etc.) in breast cancer survivors taking aromatase inhibitors. Due to lack of proper safety data, the available clinical experience remains controversial and requires further investigation (42, 43).

The clinical studies for E3 have been reviewed by Head (2). She concluded that intravaginal E3 appears to be effective in controlling urogenital symptoms of menopause and found no incidence of endometrial hyperplasia for a conventional vaginal E3 dose of 0.5 mg. From a meta-analysis of clinical studies, Vooijs and Geurts (23) concluded that once daily treatment with intravaginal 0.5 mg E3 in post-menopausal women is safe and has no increased risk of endometrial proliferation or hyperplasia.

The first evidence of efficacy of *L. acidophilus*-0.03 mg estriol-combination in the treatment of atrophic vaginitis has been reported in an open, uncontrolled study (44) Kanne and Jenny (45) used vaginal *L. acidophilus*-0.03 mg E3-combination

in a randomised, double-blind, controlled, dose finding study, including 14 post-menopausal women with atrophic vaginitis. Women received twice daily vaginal therapy during 6 days with *L. acidophilus* supplemented with either a 0.03 mg or 0.5 mg E3 dose. Both therapies led to a significant improvement in proliferation and maturation of the vaginal epithelium, but without relevant differences between both groups.

Feiks and Grünberger (46) treated 48 post-menopausal women (49-83 years) with clinical findings of atrophic vaginitis in a randomized, blinded and controlled study. The patients were assigned to daily vaginal therapy for 12 days with either *L. acidophilus*-0.03 mg estriol-combination or with conventional dose of 0.5 mg E3. The degree of proliferation significantly improved in both treatment groups; from 1.44 to 2.19 ($p < 0.0001$) and 1.35 to 2.62 ($p < 0.0001$), in the first and second treatment group respectively. Interestingly, despite a 16-fold higher dose in the conventional dosed product, efficacy between the groups did not differ ($p = 0.094$), i.e. results were equivalent.

In a recent, unpublished controlled study Jaisamrarn et al. have demonstrated that the low-dose 0.03 mg E3 combined with *L. acidophilus* is sufficient to treat symptomatic atrophic vaginitis adequately and that a twice weekly maintenance therapy is on average sufficient to prevent relapse of vaginal atrophy.

Safety and tolerability

In a total of 16 published clinical studies, 1,715 patients have been treated with *L. acidophilus*-0.03 mg estriol-combination, and only 46 adverse drug reactions were observed (2.7%), none of which were serious. Most of the adverse events were local reactions (burning, irritation, pruritus, local allergic reactions, and reddening), which were mostly mild and usually occurred temporarily at the start of therapy. The typical estrogen side effects have not been reported.

The use of *L. acidophilus*-0.03 mg estriol-combination during pregnancy can also be considered as safe, because these substances are present physiologically in the human vagina, and systemic absorption of E3 is negligible. Furthermore, the blood level of E3 increases during pregnancy up to 1000 times compared to non-pregnant women. A total of 151 pregnant women have been intentionally treated with *L. acidophilus*-0.03 mg E3-combination in clinical studies, and no adverse effects on pregnancy, the foetus or new-born have been observed.

Hengst et al. (47) screened 443 pregnant women for an increase in vaginal pH (> 4.5) within a pre-term birth prevention programme. In the prospective patient group, 161 pregnant women were included. Forty-nine of the 102 women with an increased vaginal pH received lactobacilli-estriol-combination once daily for 12 days to normalise the vaginal pH. Pre-term delivery rates were compared, with results indicating that the lowest one was observed in lactobacilli-estriol combination group in all studies. No adverse events were reported for the lactobacilli-estriol-combination.

Within the pre-term prevention program in Erfurt, Germany by Hoyme et al., (48) 314 pregnant women checked their vaginal pH periodically. Fifty-nine of these women were identified as having a higher risk of pre-term delivery. Subsequently, 52 of these cases were treated with lactobacilli-estriol-combination, and in 19 cases clindamycin cream was also used for therapy.

No adverse events were reported, indicating the safe use of lactobacilli-estriol-combination during pregnancy.

Melczer et al. (33) treated 50 pregnant (12 in first, 25 in second, and 13 in third trimester of pregnancy) and 50 non-pregnant women with bacterial vaginosis with *L. acidophilus*-0.03 mg estriol-combination once daily for 6 days. The pregnant women tolerated the lactobacilli-estriol vaginal tablets well and no adverse effects on pregnancy, the foetus or new-born have been observed.

Conclusions

The available data on *L. acidophilus* KS400 demonstrate that this human lactobacilli strain possesses beneficial properties to inhibit the growth of vaginal pathogens by means of production of lactic acid, hydrogen peroxide and possibly yet unidentified antimicrobial substances. Furthermore, *L. acidophilus* KS400 adheres to epithelial cells and prevents the adherence of pathogens. E3 has about a 10-times lower receptor affinity than E2, and thus is regarded as a short-acting estrogen. Vaginal E3 doses of up to 0.5 mg daily have a full vaginotrophic activity resulting in the proliferation and maturation of the epithelium, but show a negligible uterotrophic and systemic effect. The published clinical studies with *L. acidophilus*-0.03 mg estriol-combination have demonstrated its efficacy in improving the vaginal epithelium and the restoration of the lactobacillary microflora. The matured vaginal epithelium is a prerequisite for the lactobacilli colonization and depends on the estrogen level, which fluctuates during the menstrual cycle and with age. Thus, a combination of beneficial lactobacilli and a low-dose of estriol is beneficial for women of any age.

The unique combination of viable *L. acidophilus* KS400 and low dose 0.03 mg E3 is efficient in establishing and maintaining a healthy vaginal ecosystem, with an excellent safety profile, including also during pregnancy. The combination can be used for restoring the vaginal flora after local and/or systemic treatment with anti-infective agents, for treatment of symptomatic vaginal atrophy due to estrogen deficiency, and for women with a vaginal discharge of unknown origin, when use of antibiotic therapy is not necessary. It can be also considered in repetitive therapy courses for the prevention of long-term recurrences of bacterial vaginosis, even though further clinical studies are needed to support this application.

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Conflict of interest

CU&GD are members of Global Advisory Board of Medinova AG, Switzerland.

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