

The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections

Erdinc Ozkinay,^a Mustafa Cosan Terek,^a Murat Yayci,^b Renato Kaiser,^c
Philipp Grob,^c Gungor Tuncay^a

Objective To evaluate the effectiveness of live lactobacilli in combination with low dose oestriol for restoration of the vaginal flora after anti-infective treatment.

Design The study was designed as a single centre, randomised, placebo-controlled, double-blind clinical trial.

Setting University Hospital.

Sample Three hundred and sixty women out of 1750 were randomised.

Methods Three hundred and sixty women with the complaints of vaginal infections (bacterial vaginosis, candidiasis, trichomoniasis or fluor vaginalis) were randomly assigned two to seven days after the end of the anti-infective therapy, to therapy with live lactobacilli in combination with low dose oestriol (study group, $n = 240$) or placebo ($n = 120$). The follow up visits occurred three to seven days and four to six weeks after the end of the restoration therapy.

Main outcome measures The Normal Flora Index (NFI), which consists of numbers of lactobacilli, pathogenic microorganisms, leucocytes and vaginal pH, was used as the primary outcome of the study. Secondary outcomes included the total symptoms score, the degree of purity of the vaginal flora and the global assessment of the treatment by the investigator and the women.

Results During restoration therapy, the NFI increased significantly more in the study group than in the control group in both first and second control visits ($P = 0.002$ and $P = 0.006$, respectively). The degree of purity of the vaginal flora also increased significantly more in the study group compared with the control group ($P < 0.0001$ and $P = 0.001$, respectively). No serious adverse event was reported during restoration therapy.

Conclusion Restoration of the vaginal flora can be significantly enhanced by the administration of live lactobacilli in combination with low dose oestriol.

INTRODUCTION

Vaginal infection is the most common reason why women attend gynaecological or sexually transmitted disease clinics. Bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis are regarded as the three vaginal disorders occurring most frequently worldwide. Although anti-infective treatment is available and usually highly efficient in eradicating the pathogenic microorganisms, the long term efficiency is hampered by relapses. Furthermore, unsatisfactory results have been achieved in so-called

complicated cases including both recurrent bacterial vaginosis and recurrent candidiasis,^{1–3} as well as ‘intermediate’ bacterial vaginosis, which has recently been proposed as an independent disease called aerobic vaginitis by some authors.⁴ All these disorders do not adequately respond to standardised anti-infective therapy.^{1,2,5}

To understand the treatment failures frequently occurring vaginal infections, it is important to consider the vaginal ecosystem in health and disease. The normal microflora is dominated by lactobacilli, which produce both lactic acid to lower the vaginal pH and bactericidal compounds. Hydrogen peroxide is one antimicrobial agent generated by lactobacilli present in a healthy vagina, but interestingly mostly absent in women with bacterial vaginosis.^{6–10} Apart from that, lactobacilli compete with pathogenic microorganisms for adherence on epithelial cells.^{11,12}

Another crucial factor that influences the complex vaginal ecosystem is the local oestrogen level.¹³ A well-balanced hormonal status provides sufficient oestrogen levels to ensure the proliferation and maturation of the vaginal epithelium and an adequate supply of glycogen as nourishment for the lactobacilli.^{14,15} Vaginal infections are

^aDepartment of Obstetrics and Gynecology, Ege University Faculty of Medicine, Izmir, Turkey

^bAbdi Ibrahim Pharmaceutical Product Industry, Istanbul, Turkey

^cMedinova Ltd., Zurich, Switzerland

Correspondence: Dr M. C. Terek, Department of Obstetrics and Gynecology, Ege University Faculty of Medicine, Bornova, Izmir, 35100 Turkey.

usually accompanied with diminished number of lactobacilli, overgrowth of pathogens and a more or less damaged vaginal epithelium. Anti-infective treatment further lowers the number of lactobacilli dependent on the type and duration of the anti-infective therapy used.¹⁶ Even the antifungal agent clotrimazole has been shown to alter the vaginal microflora and was reported as bactericidal against lactobacilli and streptococci *in vitro*.^{17,18} Therefore, restoration of a protective healthy microflora and parallel restitution of a well-proliferated vaginal epithelium is necessary to avoid recurrent or re-infections.

The present study was designed to address the efficacy of exogenously applied live lactobacilli in combination with low dose oestriol in the restoration of the vaginal ecosystem after anti-infective therapy.

METHODS

Approval of the ethical committee was obtained for the Ege University Hospital in Turkey where the trial was conducted. The clinical trial was performed in accordance with the Declaration of Helsinki and the applicable cGCP guidelines. Women were informed about the objectives and possible risks of the study and gave their written informed consents.

In total, 360 women with vaginal infections were enrolled in this single centre (Department of Obstetrics and Gynecology, Ege University Faculty of Medicine, Izmir, Turkey), randomised, placebo-controlled, double-blind clinical trial which was conducted between February 1996 and October 1998. A flow diagram according to CONSORT depicting information about the numbers of participants at the different stages of the trial is shown in Fig. 1.

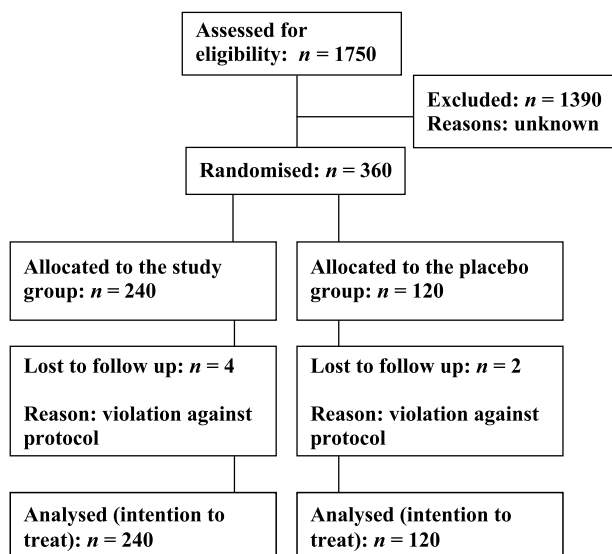


Fig. 1. Flowchart of the study.

Women, who have given their written informed consent, ranged between the age of 17 and 65 years and had the complaints of trichomoniasis, candidiasis, bacterial vaginosis or fluor vaginalis. Exclusion criteria were pregnancy, hypersensitivity to the anti-infective agent or test preparation, vaginal bleeding of unknown origin, tumours in the genital tract or breast, immunosuppression and clinically manifest sexually transmitted diseases (infections with *Neisseria gonorrhoe*, *Chlamydia trachomatis*, Herpes Simplex Virus and Human Papilloma Virus).

Diagnosis of the vaginal disorders was established by the investigator at the initial examination based on the personal history and the corresponding clinical and microscopical findings. Trichomoniasis was diagnosed based on the typical clinical symptoms and confirmed by the detection of *Trichomonas vaginalis* in the vaginal secretion at the microscopic examination. Candidiasis was diagnosed by its typical signs and symptoms (white non-watery discharge, redness of the vulva/vagina, pruritus) and confirmed by the presence of *Candida* species in the wet smears and the growth of microbial culture. Bacterial vaginosis was diagnosed by whitish-grey, homogenous discharge of fishy smell and by the detection of *Gardnerella vaginalis*. Fluor vaginalis was diagnosed when signs and symptoms of a vaginal disorder were observed but trichomoniasis, candidiasis and bacterial vaginosis could be excluded. Fluor vaginalis was confirmed by the presence of high numbers of aerobic bacteria apart from lactobacilli. The high percentage of trichomoniasis cases is explained by the social background of the patient collective and the inclusion of women usually well known to the investigators to ensure good compliance.

Initially, all women received a standardised anti-infective therapy depending on their diagnosis. For trichomoniasis, the therapy of choice was systemic metronidazole. Candidiasis was treated with oral fluconazole and serious cases additionally obtained local therapy with ketoconazole. All women with bacterial vaginosis were treated systemically with metronidazole. The only case with fluor vaginalis was treated with povidone iodine solution.

The partners of women with trichomoniasis underwent systemic metronidazole therapy and partners of women with candidiasis were treated predominantly with fluconazole. All partners of women with bacterial vaginosis were systemically treated most of them with metronidazole.

Two to three days after the end of anti-infective therapy, women were randomly allocated to the test or placebo treatment packs in five blocks with 72 women each using a random generator in balanced blocks with a block size of six (permuted blocks). The randomisation procedure was performed with the software program Random (IDV, Gauting, Germany). Of the 360 women that were enrolled and underwent anti-infective treatment, 240 were allocated to the test (study group) and 120 to the placebo preparation (control group).

At the randomisation visit, women received the study medication. Each vaginal tablet of the test preparation

(Gynoflor, Medinova, Zurich, Switzerland) contained at least 107 colony forming units (cfu) of live *Lactobacillus acidophilus*, 0.03 mg oestriol and 600 mg lactose. The placebo preparation is identical to the test preparation except that it contained neither lactobacilli nor oestriol. Test and placebo preparations were stored at 4–8°C. Premenopausal women administered one vaginal tablet (Gynoflor or placebo) daily for 6 days, and postmenopausal women for 12 days which have previously been shown to be the appropriate treatment schemes, respectively.^{19–22} Vaginal tablets were inserted deep into the vagina before going to sleep.

The first control examination (C1) took place three to seven days after the end of restoration therapy. Four to six weeks after cessation of the restoration therapy, the second control examination was performed (C2). Clinical criteria were evaluated by the investigators E. O., M. C. T. and G. T. Microscopical examinations were performed by the staff biologist of the department.

Normal Flora Index (NFI) was used as surrogate parameter to assess the status of the vaginal flora during the study, which was modified according to Petersen.²³ The number of lactobacilli, number of leucocytes, pathogenic microorganisms, and the pH of the vaginal secretion were determined in the vaginal smear. Each parameter was evaluated with a four-point scale (values from 0 to 3) consequently resulting in a range from 0 to 12 for the total NFI; the higher the total score value, the healthier the vaginal flora. The change from baseline of the NFI (NFI at C1 or C2 minus NFI at randomisation visit) was the primary outcome.

The clinical symptoms vaginal discharge, burning, itching, redness of vulva/vagina, dyspareunia were assessed using a four-point estimation scale including the categories none (0), mild (1), moderate (2) and severe (3). The total symptoms score could thus have values from 0 to 15. An improvement of the clinical picture resulted in reduced values of the total symptoms score. The vaginal pH was determined using pH stripes at the vaginal wall in the upper third of the vagina.

Vaginal secretion was examined after Gram staining using phase contrast microscopy. All samples were analysed in the same laboratory of the Microbiology Department of the Ege University Hospital. The number of lactobacilli was determined using a 1000× magnification and the average number of lactobacilli per field of view was assigned to the classes <6, 6–20, 21–50 or >50. The number of leucocytes was determined using a 400× magnification. Pathogenic microorganisms were identified using a 1000× magnification based on their morphotype and Gram category and quantified semi-quantitatively (none, few, moderate, many).²⁴

The degree of purity of the vaginal flora, also called lactobacillary grade, was assessed according to Schroeder²⁵: grade I = predominance of lactobacillary morphotypes without other bacteria; grade II = lactobacilli and other bacteria; grade III = other bacteria with few or no lactobacilli; grade IV = neither lactobacilli nor other bacteria.

The efficacy of the anti-infective therapy, the restoration phase and the combination of both therapies was assessed by the investigator and the women using a four-point estimation scale (complete, considerable, mild, poor). The tolerability was assessed at the control examinations using a four-point estimation scale (very good, good, moderate, poor) by the investigator and by the women. In addition, the type, time of onset, intensity, duration and causality of adverse events were recorded during the study.

The sample size was calculated using the program 'N', version 1.2, IDV Data Analysis and Study Planning (Munich, Germany). For a standardised difference of 0.5 with an alpha of 0.025 and a power of 90%, a sample size of 360 was chosen considering the planned weighting of 2:1 and an assumed dropout rate of 20%. The confirmatory analysis using the non-parametric Wilcoxon–Mann–Whitney *U* test was performed with the primary efficacy variable: change in NFI from baseline (NFI at C1 or C2 minus NFI at randomisation visit) on the full analysis set. The confidence intervals of the Mann–Whitney *U* test and the *P* values were calculated with the software Testimate 5.2 (IDV, Munich, Germany). Regarding the multiplicity of testing (two time points for the same variable), an adjustment of the type I error was performed using the Bonferroni equation and the significance level was set to 0.025.

Table 1. Demographic characteristics at randomisation. The values are expressed as mean [standard deviation] or median (range) or *n* (%).

	Study group (<i>n</i> = 240)	Control group (<i>n</i> = 120)
Age (years)	42 [10]	42 [10]
Diagnosis		
Trichomoniasis	182 (75)	92 (76)
Candidiasis	44 (18)	17 (14)
Bacterial vaginosis	11 (4)	8 (6)
Fluor vaginalis	0 (0)	1 (1)
Trichomoniasis and candidiasis	2 (1)	0 (0)
Trichomoniasis and bacterial vaginosis	1 (0)	2 (2)
Premenopausal women^a	160 (67)	80 (67)
Day of cycle at the entry examination	10 (0–547)	10 (2–480)
No. of births	2 (0–9)	2 (0–10)
Contraception		
Hormonal	5 (2)	3 (2)
Condom	74 (30)	41 (34)
Intrauterine device	49 (20)	21 (17)
Sterilisation	11 (4)	3 (2)
Vaginal infections during the last 12 months	1 (0)	2 (2)
NFI at randomisation	6.4 [1.6]	6.4 [1.6]
Concurrent medical disorders	2 (1)	1 (1)
Concomitant medication	0 (0)	1 (1)

^a Premenopausal women are defined as women who had a menstruation within the last two years.

Table 2. Outcomes at the first and second follow up.

	Study group ^a (<i>n</i> = 240)	Control group ^a (<i>n</i> = 120)	MD ^b /OR ^c (95% CI ^d)	<i>P</i>
First follow up				
Primary efficacy variable				
NFI change (C1–RV)	1.7 [2.0]	1.2 [1.7]	0.5 (0.1 to 1.0) ^b	0.002
Secondary efficacy variables				
NFI	8.1 [2.0]	7.6 [1.9]	0.5 (0.1 to 0.9) ^b	0.024
Total symptom score	1.9 [1.7]	2.0 [1.6]	– 0.1 (–0.4 to 0.3) ^b	0.564
Vaginal pH	4.6 [0.4]	4.6 [0.4]	–0.02 (–0.10 to 0.06) ^b	0.728
Degree of purity according to Schroeder: grade I/II	169 (70)	58 (48)	2.5 (1.6 to 4.0) ^c	<0.0001
Other microscopical outcomes				
No. of lactobacilli ^e >20	114 (48)	39 (33)	1.9 (1.2 to 3.0) ^c	0.007
No. of leucocytes ^f <20	208 (87)	103 (86)	1.1 (0.6 to 2.0) ^c	0.871
Other clinical outcomes				
Therapeutic efficacy assessed by the				
investigator: complete or clear improvement	172 (72)	69 (58)	1.9 (1.2 to 3.0) ^c	0.009
patients: complete or clear improvement	178 (74)	71 (59)	2.0 (1.2 to 3.2) ^c	0.005
Tolerability of the test preparation assessed by the				
investigator: good to very good	227 (94)	108 (90)	1.9 (0.9 to 4.4) ^c	0.125
patients: good to very good	222 (93)	108 (90)	1.4 (0.6 to 2.9) ^c	0.424
Adverse events	1 (0)	2 (2)	0.25 (0.02 to 2.75) ^c	
Second follow up				
Primary efficacy variable				
NFI change (C2–RV)	1.6 [2.3]	0.9 [2.3]	0.7 (0.2 to 1.2) ^b	0.006
Secondary efficacy variables				
NFI	8.0 [2.3]	7.4 [2.3]	0.6 (0.1 to 1.1) ^b	0.017
Total symptom score	1.8 [2.1]	2.1 [1.9]	–0.3 (–0.7 to 0.2) ^b	0.085
Vaginal pH	4.6 [0.4]	4.6 [0.4]	–0.05 (–0.13 to 0.04) ^b	0.368
Degree of purity according to Schroeder: grade I/II	155 (65)	56 (47)	2.1 (1.3 to 3.3) ^c	0.001
Other microscopical outcomes				
No. of lactobacilli ^e >20	108 (45)	33 (28)	2.2 (1.3 to 3.5) ^c	0.001
No. of leucocytes ^f <20	209 (87)	92 (77)	2.1 (1.2 to 3.6) ^c	0.015
Other clinical outcomes				
Therapeutic efficacy assessed by the				
investigator: complete or clear improvement	178 (74)	72 (60)	1.9 (1.2 to 3.0) ^c	0.008
patients: complete or clear improvement	177 (74)	73 (61)	1.8 (1.1 to 2.9) ^c	0.015
Relapses	19 (8)	15 (13)	0.6 (0.3 to 1.2) ^c	0.182

^a The values in the columns are expressed as mean [standard deviation] or *n* (%).

^b MD = mean difference, given for the continuous outcomes.

^c OR = odds ratio; given for the binary outcomes.

^d CI = confidence interval.

^e Per field of view (1000×).

^f Per field of view (400×).

Statistical analysis of the secondary efficacy variables total symptom score and the vaginal pH were performed using the Mann–Whitney *U* test. The degree of purity, the number of lactobacilli and leucocytes as well as the assessment of the therapeutic efficacy and tolerability by both the investigators and the women were analysed by group comparison using the Fisher's exact test (Statview version 5, Excel 97).

RESULTS

Among the enrolled women (*n* = 360), trichomoniasis was diagnosed in 274 cases (76.1%), candidiasis in 61 (16.9%), bacterial vaginosis in 19 (5.3%), fluor vaginalis in 1 (0.3%), and multiple diagnosis were diagnosed in 5 cases

(1.4%). As shown in Table 1, study and control group did not differ with respect to diagnosis and other demographic characteristics. Violation against the protocol had been observed for six women when the therapy had already started. During the monitoring it was observed that these six women exceeded the age limit of 65 years set in the inclusion criteria. Nevertheless, the women were included into the statistical analysis. All enrolled women underwent adequate anti-infective treatment as demonstrated by a significant increase in the NFI after anti-infective therapy. Before treatment, the NFI was 3.4 (SD 1.6) and increased to 6.4 (SD 1.6) at the randomisation visit. The subsequent restoration therapy resulted in a further NFI increase. The change in the NFI from baseline (randomisation visit) stipulated as the primary efficacy variable was significantly

higher in the study group compared with the placebo group. This was true for both control examinations C1 and C2 (Table 2).

Clinical criteria comprised the total symptoms score (vaginal discharge, burning, itching, redness of vulva/vagina, dyspareunia) and the vaginal pH. The total symptoms score decreased clearly during anti-infective therapy, from 8.5 (2.5) to 3.2 (2.0). The vaginal pH was 5.0 (0.4) and 4.8 (0.4) at the entry and the randomisation visit, respectively. The restoration therapy resulted in a further improvement of both variables irrespective of the treatment.

The degree of purity of the vaginal flora was similar in both treatment groups at the randomisation visit. However, at the end of restoration therapy at C1, the degree of purity was significantly different between the study group and the control group (Table 2). At the second control examination (C2), the difference between the treatment groups remained significant in favour of the test preparation. Significant differences in the other microscopical outcomes (i.e. numbers of lactobacilli and leucocytes) were found after restoration therapy in favour of the test preparation as shown in Table 2.

The therapeutic efficacy was assessed similarly by the investigator and by the women (Table 2). At both control examinations, the reported rates of complete or clear improvement were significantly higher for the test preparation compared with the placebo.

The tolerability of both the test and placebo preparation was rated good to very good in more than 90% of the cases by the investigator and the women.

Among the 240 women treated with the test drug, one adverse event (diarrhoea) was reported during restoration therapy, whereas two cases of adverse events (nausea, diarrhoea) were observed among 120 women treated with the placebo preparation.

DISCUSSION

The present study showed that the restoration of the vaginal ecosystem after anti-infective treatment can be significantly enhanced by exogenous applied live lactobacilli in combination with low dose oestriol when compared with placebo. The superiority of the test preparation was demonstrated by the significant increase in the NFI under restoration therapy reflecting an improvement of the vaginal milieu. The NFI, a validated surrogate parameter, is composed of four markers for the vaginal ecosystem: number of lactobacilli, pathogenic microorganisms, number of leucocytes and vaginal pH. Thus, the change in NFI is well suited as the primary efficacy variable to assess the restoration of the vaginal ecosystem.

A remarkable increase of the NFI was already expected during anti-infective therapy, and has been confirmed by our results at the status of randomisation. Following therapy with the test preparation, the restoration of the vaginal

epithelium and vaginal microflora was enhanced by its active substances, low dosed oestriol and *L. acidophilus*, leading to a significant higher improvement of the vaginal markers. Thus, Gynoflor therapy results in a significant higher increase of the NFI compared with placebo. Different authors have suggested that nutritional support of the endogenous lactobacilli already has a positive effect on the restoration of the vaginal ecosystem.^{26,27} Considering the lactose included in the placebo preparation, the efficacy of Gynoflor is probably even higher than demonstrated by our results.

The clinical symptoms vaginal discharge, burning, itching, redness of vulva/vagina and dyspareunia were combined in the total symptoms score, which was also recorded as a secondary parameter at every control examination. The total symptoms score had not been selected as the primary variable, because an adequate anti-infective therapy was expected to result in remission of clinical symptoms. However, the absence of symptoms is not a measure for the restoration of the vaginal ecosystem. In this study, the total symptoms score decreased significantly during the anti-infective treatment, whereas the decrease during the restoration therapy was negligible in both groups. These results confirmed our expectations and demonstrated that the right anti-infective treatment was used for the particular, diagnosed infection.

The degree of purity (also called lactobacillary grade) according to Schroeder is a well-known parameter to assess the status of the vaginal microflora and consequently the vaginal ecosystem.^{4,25} The significantly higher degree of purity upon test preparation compared with placebo indicated a clear shift towards a healthier vaginal flora and confirmed the results obtained with the NFI.

The present results indicate that the vaginal microflora, and consequently the vaginal ecosystem, is not restored to a healthy status immediately after cessation of anti-infective therapy. The high incidence of trichomoniasis of the study women has no importance with regard to the aim of the present study as the type of microorganism treated is not important for ensuing restoration therapy. However, restoration of a healthy microflora is important to prevent relapses, recurrences and re-infections. Although bacterial vaginosis and trichomoniasis are successfully treated with metronidazole, this therapy eliminates not only the pathogenic microorganisms but also reduces the numbers of lactobacilli leaving the patient vulnerable to relapses and re-infections. Redondo-Lopez *et al.*¹⁴ observed that post-metronidazole vaginal flora was often deficient in lactobacilli. Over 50% of women receiving metronidazole developed candidal vaginitis. Other clinical studies reported relapse rates between 14% and 39% within one month after treatment of bacterial vaginosis with metronidazole^{1,2,28}; and relapses in 30% of the cases were observed within three months.²⁹ In a further study, the relapse rate amounted to 20% with an assessment one week after treatment.¹ Recurrent vulvovaginal candidiasis is defined as four or more

episodes per year and is usually the result of relapse.^{3,30} Fifty percent of women with candidiasis will experience a recurrence of vaginitis and about 5% of adult women has recurrent, intractable infections.^{30,31}

The current attempts undertaken to reduce relapses and recurrences, such as partner treatment or prolonged maintenance therapy, have also been shown to be not the ultimate solution.^{30,32} The cessation of the maintenance regimen is accompanied by symptomatic relapse in half the women within a short time of stopping therapy.³⁰

This study was not designed to assess the efficacy of Gynoflor therapy in reducing the rate of relapses and re-infections. Nevertheless, the relapse rates in the Gynoflor and placebo groups were 7.9% and 12.5%, respectively, providing the evidence that restoration therapy with Gynoflor may reduce the rate of relapses and re-infections. However, further studies with a larger patient collective or with subpopulations where relapses and re-infections are more frequent are needed.

The use of preparations containing live lactobacilli for the treatment of urogenital infections has been recently reviewed by several authors.^{10,14,33–35} The importance of prevalent H₂O₂ producing lactobacilli to prevent bacterial vaginosis has been published in several reports and studies.^{6–8,24} Several clinical studies provided evidence that exogenously applied lactobacilli are able to reduce the re-infection rates in candidiasis and recurrent vaginitis.^{26,36,37}

The efficacy of lactobacilli in combination with oestriol has been demonstrated in clinical studies for the treatment of bacterial vaginosis, non-specific cervicovaginitis and atrophic vaginitis.^{19–21,38} In an open non-controlled study, Lauritzen *et al.*²² have shown that restoration therapy with Gynoflor results in a significant improvement of the degree of purity of the vaginal flora and the degree of proliferation of the vaginal epithelium.

The present randomised, placebo-controlled, double-blind study is the first large enough for statistical analysis of the restoration efficacy of lactobacilli preparation. We have demonstrated that therapy with Gynoflor, containing *L. acidophilus* and low dosed oestriol, enhances the restoration of the vaginal ecosystem (i.e. vaginal microflora and epithelium), and thus is justified after local and systemic anti-infective treatment.

Acknowledgements

The authors would like to thank Dr Vera Della Casa, Dr Gabriele Pohlig, Dr Susanne Gonser and Dr Valentin Rousson for their valuable support in statistical analysis and medical writing. The authors would also like to thank Medinova, Switzerland, particularly Dr Federico Graf, and Abdi Ibrahim, Turkey for sponsoring this clinical trial.

References

- Blackwell AL, Phillips I, Fox AR, Barlow D. Anaerobic vaginosis (non-specific vaginitis): clinical, microbiological and therapeutic findings. *Lancet* 1983;**17**:1379–1382.
- Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978;**298**:1429–1434.
- Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol* 1998;**178**:203–211.
- Donders GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *Br J Obstet Gynaecol* 2002;**109**:34–43.
- Hay PE, Ugwumadu A, Chowns J. Sex, thrush and bacterial vaginosis. *Int J STD AIDS* 1997;**8**:603–608.
- Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* 1989;**27**:251–256.
- Hawes SE, Hillier SL, Benedetti J, et al. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* 1996;**174**:1058–1063.
- Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H₂O₂-producing lactobacilli, and bacterial vaginosis in pregnant women. *Clin Infect Dis* 1993;**16**:S273–S281.
- Klebanoff SJ, Hillier SL, Eschenbach DA, Waltersdorff AM. Control of the microbial flora of the vagina by H₂O₂-generating lactobacilli. *J Infect Dis* 1991;**164**:94–100.
- Sobel JD. Is there a protective role for vaginal flora? *Curr Infect Dis Rep* 1999;**1**:379–383.
- Boris S, Suarez JE, Vazquez F, Barbes C. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infect Immun* 1998;**66**:1985–1989.
- Ossset J, Bartolome RM, Garcia E, Andreu A. Assessment of the capacity of *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cells. *J Infect Dis* 2001;**183**:485–491.
- Brown WJ. Microbial ecology of the normal vagina. In: Hafez ESE, Evans TN, editors. *The Human Vagina*. New York: Elsevier/North Holland Biomedical Press, 1978:407–422.
- Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis* 1990;**12**:856–872.
- Sjöberg I, Cajander S, Rylander E. Morphometric characteristics of the vaginal epithelium during the menstrual cycle. *Gynecol Obstet Invest* 1988;**26**:136–144.
- Hillier SL. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol* 1993;**169**:455–459.
- Liss RH, Letourneau RJ. Fungispecificity of fluconazole against *Candida albicans*. *Mycopathologia* 1989;**108**:173–178.
- Ross RA, Lee ML, Onderdonk AB. Effect of *Candida albicans* infection and clotrimazole treatment on vaginal microflora in vitro. *Obstet Gynecol* 1995;**86**:925–930.
- Feiks A, Grünberger W. Treatment of atrophic vaginitis: does topical application allow a reduction in the oestrogen dose? *Gynäkol Rundsch* 1991;**31**:268–271.
- Kanne B, Jenny J. Local administration of low-dosed estriol and viable *Lactobacillus acidophilus* in the post-menopausal period. *Gynäkol Rundsch* 1991;**31**:1–8.
- Parent D, Bossens M, Bayot D, et al. Therapy of bacterial vaginosis using exogenously-applied *Lactobacilli acidophili* and a low dose of estriol. *Arzneimittelforschung* 1996;**46**:68–73.
- Lauritzen C, Graf F, Mucha M. Restoration of the physiological vaginal environment with doederlein bacteria and estriol. *Frauenarzt* 1984;**4**:5–8.

23. Petersen EE. Bedeutung der Laktobazillen als Normalflora. *Gynäkologe* 1985;**18**:128–130.
24. Hillier SL, Krohn MA, Nugent RP, Gibbs RS. Characteristics of three vaginal flora patterns assessed by Gram stain among pregnant women. *Am J Obstet Gynecol* 1992;**166**:938–944.
25. Schroeder K. Zur Pathogenese und Klinik des vaginalen Fluors. *Zentralbl Gynäkol* 1921;**38**:1350.
26. Collins EB, Hardt P. Inhibition of *Candida albicans* by *Lactobacillus acidophilus*. *J Dairy Sci* 1980;**63**:830–832.
27. Reid G, Bruce AW, Taylor M. Instillation of *Lactobacillus* and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. *Microecol Ther* 1995;**23**:32–45.
28. Larsson PG. Treatment of bacterial vaginosis. *Int J STD AIDS* 1992;**3**:239–247.
29. Hillier SL, Holmes KK. Bacterial vaginosis. In: Holmes KK, Mardh PA, Sparling PF, et al., editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill, 1999:563–587.
30. Sobel JD. Treatment of vaginal *Candida* infections. *Expert Opin Pharmacother* 2002;**3**:1059–1065.
31. Ferrer J. Vaginal candidosis: epidemiological and etiological factors. *Int J Gynaecol Obstet* 2000;**71**:21–27.
32. Elsner P, Hartmann AA. *Gardnerella vaginalis* in the male upper genital tract: a possible source of reinfection of the female partner. *Sex Transm Dis* 1987;**14**:122–123.
33. Barbes C, Boris S. Potential role of lactobacilli as prophylactic agents against genital pathogens. *AIDS Patient Care STDS* 1999;**13**:747–751.
34. Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes Infect* 2000;**2**:543–546.
35. Reid G. Probiotic agents to protect the urogenital tract against infection. *Am J Clin Nutr* 2001;**73**:437S–443S.
36. Hilton E, Rindos P, Isenberg HD. *Lactobacillus GG* vaginal suppositories and vaginitis. *J Clin Microbiol* 1995;**33**:1433.
37. Shalev E, Battino S, Weiner E, Colodner R, Keness Y. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. *Arch Fam Med* 1996;**5**:593–596.
38. Bandera Gonzalez B, Conde de Vargas BI, Gonzalez Avalos GA, Toledo Medina A. Treatment of non-specific cervicovaginitis with a lyophilised preparation of lactobacilli and oestriol. *Prensa Med Mex* 1977;**XLII**(1–2):91–95.

Accepted 7 May 2004