

Multi-Gyn Gel in the Prevention and Treatment of Bacterial Vaginosis; an evidence-based cytological evaluation.

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Summary:

Objectives; To investigate the effects of the application of Multi-Gyn Gel on BV related coccoid flora by means of the evaluation of cervical and vaginal smears. *Study methods;* The Leiden Cytology and Pathology Laboratory (LCPL) evaluates some 60,000 smears per year and is covering 17% of the routine cervical smears (PAP smears) in the Netherlands for screening. Because the LCPL has developed a special line of interest for the vaginal ecology and its relationship to pathogenic conditions it routinely notes the flora in these smears and reports this to the GP. Multi-Gyn Gel combines a low pH of 4.1 with the therapeutic properties of a bio-active polysaccharide complex derived from Aloe Barbadensis gel, directly blocking adhesion of pathogens. *in vitro* tests and numerous case reports have shown the clinical efficacy of Multi-Gyn Gel in BV. In this evidence-based study the GP was asked to advise the patient in which a BV flora was detected in the routine cervical smear to apply Multi-Gyn Gel during 5 days and to return for a control smear. *Results;* The smears of a total of 128 patients with cytological evidence of BV could be evaluated. 69 of the patients had reported complaints of BV. These were itching, burning, excessive discharge and fishy odour of the discharge. 59 told the GP that they had no vaginal discomfort at the time of the routine PAP smear. In 74 % of the control smears after treatment with Multi-Gyn Gel a lactobacilli flora was observed and in 26% smears a mixed flora consisting of lactobacilli and cocci. No coccoid overgrowth was found. The control smears of the patients with Trichomonas in the original smear, 4 showed exclusively lactobacilli and 3 a mixed flora after treatment with Multi-Gyn Gel. In none Trichomonas was found back. Of the 69 patients that reported complaints of BV, 78% had experienced relief of itch and burning by the application of Multi-Gyn gel. The other 22% still had complaints of excessive discharge. They all had a mixed flora in the control smear. No side effects were reported of the use of Multi-Gyn gel. *Conclusion;* As coccoid overgrowth is a frequent vaginal ecological disbalance, this clinical evidence based study on the efficacy of the application of Multi-Gyn gel shows that application of Multi-Gyn gel seems to be a suitable tool for the treatment and prevention of bacterial vaginosis.

Key words: vaginal ecosystem, bacterial vaginosis, Multi-Gyn gel

Introduction

1. Cervical cytology and vaginal microbiology.

The Leiden Cytology and Pathology Laboratory (LCPL) services 900 General Practitioners (GPs). With the evaluation of some 60,000 smears per year it is covering 17% of the routine GP cervical smears in the Netherlands for screening.

Microbiologic changes have long been associated with alterations in vaginal health (1,2,3,4). Therefore the vaginal flora in these smears is evaluated as well and the GP is informed of any abnormal observation. The LCPL has developed a special line of interest for the vaginal ecology and its relationship to pathogenic conditions, resulting in several research programs and publications.

The advantage of studying the vaginal flora in smears as opposed to cultivation of these microorganisms is that the microorganisms can be observed in their natural habitat.

The vaginal environment can be divided in three ecosystems (1):

- a. The Döderlein flora that is represented by lactobacilli and a low pH < 4.5 established by the lactate production of the lactobacilli. The low pH of the lactobacilli flora inhibits the growth of most (possible) pathogenic organisms. Candidiasis is observed in association with this flora. Trichomonas is seldomly seen in this flora. Recently it has become evident that an overgrowth of lactobacilli is in itself a possible cause of complaints. This new diagnosis is called lactobacillosis. Actinomyces is seldomly observed.
- b. The mixed flora that is represented by lactobacilli and cocci and a pH 4.5 - 5. In this flora lactobacilli and cocci are in equilibrium. It may show the presence of candida and trichomonas. Actinomyces is most often observed. With this flora complaints are however rarely reported.
- c. The coccoid flora that is associated with the clinical presentation of bacterial vaginosis (BV) and a pH > 5. In this flora there are few or no lactobacilli. This flora is often associated with trichomoniasis. Actinomyces is also observed but in this flora candida is never seen.

In the context of the vaginal ecosystem, it is of interest to investigate the association of vaginal microbiology with abnormal cytology; CIN I-II (= PAP I-II) versus CIN III (= PAP III).

The vaginal microbiology of 666 CIN I-II cases and 293 CIN III cases is shown in Table 1.

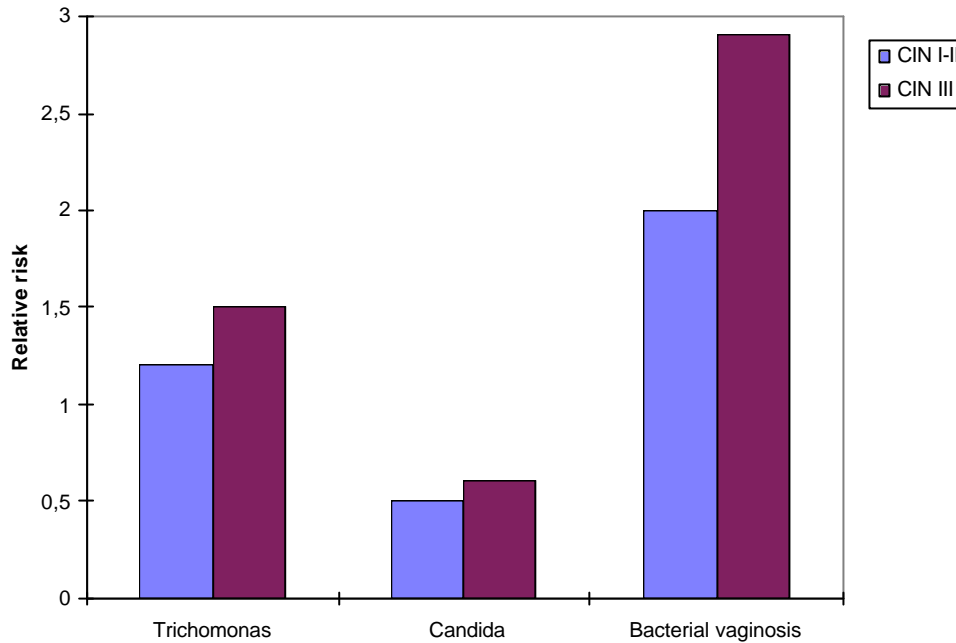
It is clear that bacterial vaginosis is quite prominent in CIN I-II and the CIN III group.

Table 1. Vaginal microbiology of 666 CIN I-II cases and 293 CIN III cases.

	CIN I-II	CIN III
	%	%
Trichomonas	0.75	1.02
Candida	1.25	1.2
Bacterial vaginosis	13.21	19.11

The relative risk for CIN (RR) in the three types of vaginal microbiology (trichomonas, Candida, bacterial vaginosis) is shown in Figure 1. The relative risk is calculated as the incidence of the type of vaginal microbiology in CIN smears versus its incidence in negative smears. A RR of 1 implies that there is no association between CIN and the vaginal microbiology values < = 1 mean that there is a negative relationship and values > 1 mean a positive relationship. It is clear that Candida women have less CIN and women with bacterial vaginosis have more CIN. Trichomonas (also associated with an alkaline pH of the vagina) has also a higher RR.

Fig. 1. Relative risk for CIN I-II and CIN III related to three types of vaginal microbiology



2. Bacterial vaginosis and the vaginal ecosystem.

In view of the pathogenicity of microorganisms the preferable ecosystem is that with a pH of < 5. The relationship between the vaginal pH and bacterial vaginosis (BV) has been well established and can be considered as a first observation of the existence of a vaginal ecosystem. Bacterial vaginosis is a very common disorder affecting many women. BV affects approximately 15 to 20% of pregnant women and it has been associated with preterm labor. It is caused by several anaerobic bacteria such as *Gardnerella vaginalis*, *Bacteroides*, beta-Streptococci and *Mobiluncus/Falcivibrio* sp. *Trichomonas vaginalis* is a STD that is closely related to BV (5). In smears no determination can be made of the species. They are observed as roundish coccoid organisms as opposed to the rod shaped lactobacilli. A typical overgrowth of cocci shows "clue cells", an abundance of coccoid bacteria glued to epithelial cells. This condition is most often associated with the complaints of BV such as excessive discharge, pain, itch and fishy odor.

Several types of antibiotics are used to treat this condition. Metronidazole, an anti-microbial agent with activity against protozoa and anaerobic bacteria, was found to be the most effective but none was completely successful either in cure or prevention (6, 7). Medication with Metronidazole also has reported side effects of stomatitis, headaches, vertigo and gastrointestinal problems and alcohol consumption is prohibited.

It is obvious that medication by which microorganism are erased for instance for (non gynecological) sore throat, will influence the ecosystem of the vagina. Antibiotics for non gynecological complaints that kill lactobacilli often give rise to symptoms of bacterial vaginosis. Local general disinfectants such as chlorhexidine and povidone iodine are also detrimental for lactobacilli and the vaginal pH. Other disruptive influences on the vaginal pH are coitus and hygiene (5). Semen is alkaline (pH 7) and many women have postcoital complaints due to the elevated vaginal pH that creates a condition for the overgrowth of cocci. These complaints usually start with a fishy odor. Alkaline soap is also of influence on the local vaginal pH.

Because attachment is the first step in infection and because clue cells are such a prominent feature of BV the adherence characteristics of the anaerobic bacteria have been studied. *G. vaginalis* adheres

better to vaginal epithelial cells at between pH 5 and 6 and all other bacteria involved in BV at a pH of >5.5! (6,7).

The corrective manipulation of a pH > 5 seems to be a tool for the treatment of complaints that are caused by the overgrowth of the coccoid flora (BV) and for the prevention of the growth of these organisms. Alternative treatments have indeed been proven to be useful and efficacious. There are several clinical situations such as pregnancy or in patients with recurrent BV in which they are the treatment of choice. Some authors claim that lactate-gel is the most promising of all alternative substances. The only effects of this gel are to re-establish a normal acid environment in the vagina and to facilitate recolonization with lactobacilli (8, 9,10,11).

3. Multi-Gyn Gel.

Multi-Gyn Gel combines a low pH of 4.1 with the therapeutic properties of bio-active polysaccharides - the bio-active polysaccharide complex Galactoarabinan Polyglucuronic Acid Crosspolymer (proposed) derived from Aloe barbadensis Gel - of directly blocking adhesion of pathogens to host tissue. It also provides relief of pain and itch. The gel adheres well to mucous.

The LCPL has participated with the evaluation of vaginal smears in a previous study of women using Multi-Gyn for the treatment of atypical vulvitis and vaginitis. The results of that study have been the reason to pursue a follow-up study with Multi-Gyn on the monitoring of BV by the principle of the correction of the vaginal pH and the blocking of the adhesion of pathogens in combination with the relief of the complaints of pain and itch.

Multi-Gyn gel is commercially available in drugstore and pharmacy. It comes in a 50 ml. aluminium tube with a 5 cm. nozzle for internal application in the vagina. Application is described on the information leaflet and advised twice per day for the treatment of vaginal problems and once per three days for the prevention of vaginal infection. In cases of vaginal discomforts such as itch or burning, application can be as often as relief is needed, because the product is non toxic, safe and harmless. Multi-Gyn gel is based on bio-active polysaccharides (polysaccharide complex; Galactoarabinan Polyglucuronic Acid Crosspolymer (proposed)) which is derived from Aloe barbadensis gel (83%) with an analysed high amount of acetylated polymannose.

bio-active polysaccharides is responsible for the inhibition of adhesion of a number of pathogenic bacteria. It also improves phagocytosis of macrophages and has proven to be immunomodulatory and effective in cell processes that are involved in inflammation (13).

(14, 15) Aloe gel components appear to have pain killing and itch stopping properties. (13, 16) The thickener of Multi-Gyn Gel is Xanthan gum that promotes adhesion of the product to mucous.

In vitro tests have shown that Multi-Gyn Gel does not affect growth of lactobacilli; a detrimental effect related to antibiotics and antiseptics. In these tests it was shown that Multi-Gyn Gel increases growth of lactobacilli but kills coccoid fecal bacteria as well as *Candida albicans*. In vitro tests with a pH neutral Multi-Gyn also showed increased growth of lactobacilli and killing of *Candida*. The log reduction of the coccoid flora was less than with the Multi-Gyn pH 4.1. It can therefore be assumed that the bio-active polysaccharides are the main responsible factor for the observed effects and that the pH value is synergistic.

Study design.

Patients come to the GP's office for a regular PAP smear for the early detection of cervical dysplasia. The materials (slide, brush, tempex slide-box and expedition envelope) are provided by the LCPL to the GP's of its client group and the smear is sent to the LCPL. In these routine PAP smears the LCPL also evaluates the vaginal flora. Any special observation so also the status of the flora, is reported to the GP. Coccoid overgrowth is observed in approximately 7% of the smears.

In this study the smears of patients of a group of 40 participating GP's were selected for coccoid overgrowth with and without clue cells. When the LCPL informed the GP on the coccoid overgrowth of a patient, the GP would contact the patient and advise application of Multi-Gyn gel. The patient was asked to come back to the GP's office after one week treatment with Multi-Gyn for a control smear of

the vagina. Small questionnaires were provided to the GP to collect the relevant information on the vaginal discomforts of the patient with coccoid overgrowth and the comments on the application of Multi-Gyn gel.

During the control visit the questionnaire was completed and afterwards returned to the LCPL together with the control smear.

The evaluation of the flora of control smear was the parameter of the efficacy of the application of Multi-Gyn gel in the elimination of the BV related coccoid flora and the restoration of the Lactobacilli (Doderlein) flora.

Results

At the LCPL a total of some 2100 (7%) smears with coccoid overgrowth are observed in a total of 30,000 routine PAP smears over a period of 6 months. Of the group of 40 GPs who were to cooperate in this study 159 smears were diagnosed for significant coccoid overgrowth; cocci +++. In seven cases *Trichomonas* was also present. A response of 80% control smears was received after treatment with Multi-Gyn gel together with the comments of the GP and the patient in the questionnaire. A total of 128 patients could be evaluated in this study. Patients had used the Multi-Gyn gel 5-10 days before their follow-up appointment with the GP.

69 = 54% of the patients reported complaints of BV. These were itching, burning, excessive discharge and fishy odor of the discharge (Figure 2). 59 = 46% told the GP that they had not experienced any vaginal discomfort at the time of the routine PAP smear (Figure 2).

In 74% = 95 of the Multi-Gyn treatment control smears a lactobacilli flora was observed and in 26% = 33 smears a mixed flora consisting of lactobacilli and cocci. No coccoid overgrowth was found (Figure 3). The Multi-Gyn treatment control smears of the patients with *Trichomonas* in the original smear, 4 showed exclusively lactobacilli and 3 a mixed flora. In none *Trichomonas* was found back.

Of the 69 patients that reported complaints of BV, 78% = 54 had experienced relief of itch and burning by the application of Multi-Gyn gel (Figure 4). They had applied the gel externally and internally with an average of 5 times per day for 3 days. After the disappearance of the complaints they continued with the application of Multi-Gyn gel two times per day. The other 15 patients still had complaints of excessive discharge. They all had a mixed flora in the control smear. Because no indication of BV could be detected in their smears, it is possible that this discharge is physiological for these individual women. None reported malodorous discharge (Figure 4).

No side effects were reported of the use of Multi-Gyn gel.

Because of the acidity of the gel some women complained about a transient stinging effect directly after application during the first days of use.

Fig. 2. Complaints in women with coccoid overgrowth

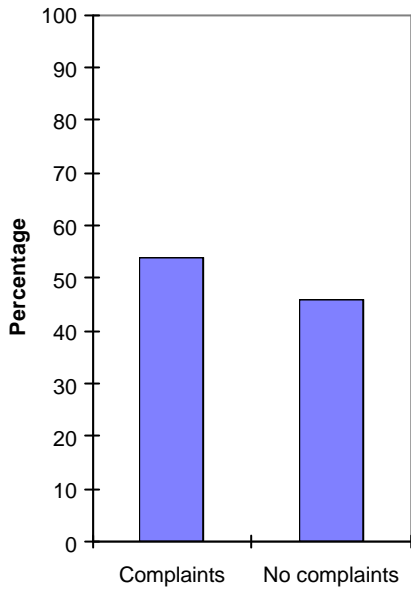


Fig. 3. Flora of control smears after use of Multi-Gyn gel

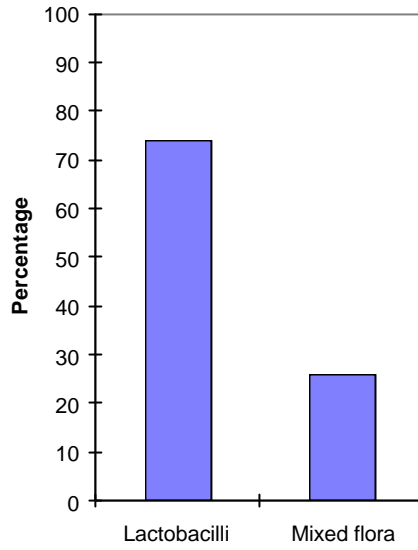
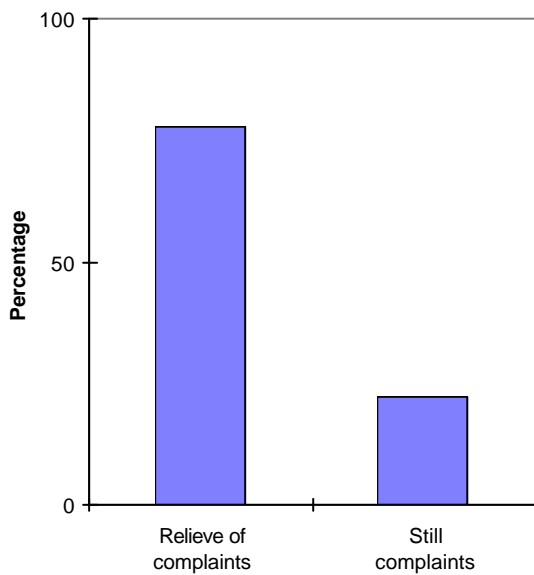


Fig. 4. Relieve of complaints after use of Multi-Gyn gel



Discussion

This study was performed with the microscopic observation of the vaginal flora in Papanicolaou-stained smears. Rod-shaped lactobacilli, and roundish coccoid micro-organisms can be observed in their natural habitat. No effort was made to isolate and determine the anaerobes. In previous studies it has been established that the occurrence of exclusively lactobacilli is associated with a pH < 4.5 as a result of the lactate production of these lactobacilli, a combination of lactobacilli and cocci is associated with a pH 4.5 to 5 and a coccoid overgrowth without presence of lactobacilli is associated with a pH > 5. We have found that both extremes of this scale are related to clinical symptoms. A pH of 3.8 and an overgrowth of lactobacilli may show candida-mimicking complaints. This condition has only recently been recognized as Lactobacillosis (12). On the other side of the scale a pH of > 5.5 and a coccoid overgrowth often shows the clinical signs of BV. Contrary to what is frequently said about the presence of coccoid micro-organisms we find that a mixed flora is seldomly associated with clinical symptoms. Only disbalances of the vaginal flora may be associated with clinical symptoms. In this study the microscopic observation of the coccoid overgrowth was matched only after the receipt of the control smears with the clinical signs of BV as reported by the patients in the questionnaires. 69 reported clinical signs as expressed in irritation of the vaginal area (itch, burning) , excessive discharge and a foul smell of the discharge. Patients had not visited the GP for these complaints in the first place. 59 patients did not report any complaints. This shows that a vaginal ecological disbalance of coccoid overgrowth is not necessarily related to clinical symptoms of BV.

The results of this study show that regular application of Multi-Gyn gel is an effective tool in the prevention and treatment of coccoid overgrowth and the recurrence of BV.

The manipulation of the vaginal pH into an acidity that is unfavorable for the habitat of anaerobic coccoid vaginal flora appears to be both clinically and microbiologically effective. Previous results of a study on the treatment of BV with an acid lactate cream are in agreement with the effects of the acid Multi-Gyn gel. As coccoid overgrowth is a frequent vaginal ecological disbalance, application of Multi-Gyn gel seems to be a suitable tool for the treatment and prevention of bacterial vaginosis.

Moreover, in Multi-Gyn gel the combination of an optimal vaginal pH and bio-active polysaccharides seems to be effective also for the relief of the vaginal discomforts (such as itch and burning) in clinical BV.

The effect of bio-active polysaccharides as an antiadhesive for pathogenic microorganisms as super-imposed to the effect of the correction of the vaginal pH could not be evaluated in this study. This would need a placebo controlled study design.

However in vitro tests have shown that the coccoid flora as well as Candida albicans were killed by Multi-Gyn Gel at a neutral pH while the growth of Lactobacilli was increased. This indicates that the polysaccharides can be hold responsible for the clinical efficacy of Multi-Gyn Gel in the treatment and prevention of BV and that the acidification of the vaginal environment has a synergistic effect.

As coccoid overgrowth is a frequent vaginal ecological disbalance, this clinical evidence based study on the efficacy of the application of Multi-Gyn gel shows that application of Multi-Gyn gel seems to be a suitable tool for the treatment and prevention of bacterial vaginosis.

References

1. Boon ME, Suurmeijer AJH. The Pap Smear. Third Edition. Harwood Academic Publishers, Amsterdam - Tokyo, 1996. The PAP Smear.
2. Boon ME, Marres EM, Hoogeveen MM, Goedbloed AF, Milios J. Visualization of vaginal flora in cervical smears using a modified microwave silver-staining method. *Histochem J* 1998; 30: 75-80.
3. Detection of bacterial vaginosis in Papanicolaou smears. Platz-Christensen J.P., Larson P., Sundström E., Bondeson L. *Am. J. Obstet. Gynecol.* 1989, 160; 19
4. The cytologist and bacterial vaginosis of the vaginal-ectocervical area. Schnadig V.J., Davie K.D., Shafer S.K., Yandell R.B., Islam M.Z., Hannigan E.V. *Acta Cytol.* 1989, 3; 287
5. Bacterial vaginosis. Spiegel C.A. *Clin. Microbiol. Rev.* 1991, 4; 485
6. Bacterial vaginosis is not a simple ecological disorder. Fredericsson B., Englund K., Weintraub L., Ölund A., Nord C-E. *Gynecol. Obstet. Invest.* 1989, 28; 156
7. Adhesion of *Gardnerella vaginalis* to epithelial cells; variables affecting adhesion and inhibition by metronidazole. Peeters M., Piot P. *Genitourin. Med.* 1985, 61; 391
8. Treatment of bacterial vaginosis with an acid cream; A comparison between the effect of lactate gel and metronidazole. Andersch B., Forssman L., Lincoln K., Torstensson P. *Gynecol. Obstet. Invest.* 1986, 21; 19
9. Treatment of bacterial vaginosis in pregnancy with a lactate gel Holst E., Brandenberg A. *Scand. J. Infect. Dis.* 1990, 22; 625
10. Bacterial vaginosis and the effect of intermittent prophylactic treatment with an acid lactate gel. Andersch B., Lindell D., Dahlen I., Brandeberg A. *Gynecol. Obstet. Invest.* 1990, 30; 114
11. The vagina as an ecologic system Overman B.A J. *Nurse-Midw.* 1993, 38, 3; 146
12. Vaginal lactobacillosis Horowitz B.J., Mardh P.A., Nagy E., Rank E.L. *Am. J. Obstet. Gynecol.* 1994; 857
13. Aloe vera; a scientific approach Davis R.H. Vantage press. New york. 1997
14. Upregulation of phagocytosis and candidicidal activity of macrophages exposed to the immunostimulant acemannan. Stuart R.W., Lefkowitz D.L., Lincoln J.A., Howard K., Gelderman M.P., Lefkowitz S.S. *Int. J. Immunol.* 1997, 19,2; 75
15. A beta-linked mannan inhibits adherence of *pseudomonas aeruginosa* to human lung epithelial cells. Azghani A.O., Williams I., Holiday D.B., Johnson A.R. *Glycobiology* 1995, 5, 1; 39
16. Management of psoriasis with Aloe vera extract in a hydrophilic cream; a placebo-controlled double blind study. Syed T.A., Ahmad S.A., Holt A.H., Ahmad S.A., Azfal M. *Trop. Med. Int. Health* 1996, 1, 4; 505

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