Annals of Clinical and Medical Case Reports

Clinical Paper ISSN 2639-8109 Volume 10

Multicentric and Prospective Trial of Vulvovaginitis Treatment Comparing Propionibacterium Extract [Immunovag®] With Metronidazole Plus Clotrimazole [Meclon®]

Melis GB[1], Facchinetti F[2], Neri I[2], Caruso S[3], Cutello S[3], Di Carlo C[4], Siroli L[5], Neri M[6], Xholli A[7], Cagnacci A[8] and Paoletti AM[1*]

[1]University of Cagliari, Italy

[2]University of Modena-Reggio Emilia, Italy

[3]University of Catania, Italy

[4]University of Napoli Federico II, Italy

[5]Department of Agricultural and Food Sciences, Campus of Food Sciences, Cesena, Italy

[6]Azienda di Rilievo Nazionale ad Alta Specializzazione [ARNAS] G Brotzu, Cagliari, Italy

[7]IRCC-Ospedale San Martino, Clinica Ostetrica e Ginecologica, Genova, Italy

[8]IRCC-Ospedale San Martino, DINOGMI, Clinica Ostetrica e Ginecologica, University of Genova, Italy

[*]Corresponding author:

Anna Maria Paoletti,
Department of Obstetrics and Gynaecology,
University of Cagliari, Maternal Child Department,
Italy, E-mail: gineca.annapaoletti@tiscali.it

Received: 26 Jan 2023
Accepted: 21 Mar 2023
Published: 29 Mar 2023
J Short Name: ACMCR

Citation:

Paoletti AM, Melis GB, Multicentric and Prospective Trial of Vulvovaginitis Treatment Comparing Propionibacterium Extract [Immunovag®] With Metronidazole Plus Clotrimazole [Meclon®]. Ann Clin Med Case Rep. 2023; V10(16): 1-7

Keywords:

Propionibacterium acnes lysates; vulvovaginitis; Bacterial vaginosis; Candida vulvovaginitis; Metronidazole; clotrimazole

1. Abstract

Vulvo-Vaginal Infections [VVI] strongly interferes with the woman's quality of life. Antibiotic treatments, alone or combined with an antifungal compound, are commonly and successfully used. Nevertheless, they can impair genital microbiota, with long-term disorders, such as a flare-up of genital disturbances due to changes of endogenous lactobacilli. Alternatively, probiotics have been proposed. This prospective multicenter double-arm study, performed in 72 premenopausal women diagnosed with VVI, whose etiology was Gardnerella Vaginalis (n=9), Candida Albicans (n=21), or Bacterial Vaginosis (n=42), was performed to compare the clinical efficacy of a short-term treatment with a vaginal gel containing the bacteria lysate obtained by Propionibacterium acnes [Immunovag®, Depofarma, Mogliano Veneto, Italy] given for 5 days (n=40) with that of vaginal suppositories containing metronidazole and clotrimazole [Meclon®, Alfasigma SPA, Bologna, Italy] given for 6 days (n=32). The study was conducted at the gynecological Departments of the University-Hospitals of Cagliari, Catania, Catanzaro, Genova and Modena - Reggio Emilia [Italy]. At post-treatment evaluation (10 days from the screening and randomization), regardless of VVI etiology and type of treatment, a significant decrease ($p<0.05$) in symptoms (vulvovaginal itching, vulvovaginal burning, dyspareunia) and signs (erythema, leucorrhea) of VVI was observed. Immunovag® appears not less effective than Meclon® in managing signs and symptoms of VVI.

2. Introduction

Vulvo-Vaginal Infections [VVI] are very frequent pathologies of the low genital tract, that require frequent gynecological consultations and when recurrent, may impact on woman quality of life [1]. Subjective symptoms include itching and burning of vagina and vulva, frequently associated with pain at intercourse. Watery or dense vaginal discharges (i.e., leucorrhea) grey, white or yellowish-white in color, and redness of vaginal mucosa [i.e., erythema] represent typical VVI signs [2]. Because of the impact of VVI on woman quality of life, many studies have tried to understand its causes and to define mechanisms to prevent it. The vagina

has a complex ecosystem aimed to reduce growth of opportunistic or pathological bacteria [3-5]. It can be thought as an ecological niche in which different microorganisms dynamically adapt to the changes occurring in the vaginal fluid, epithelium or bacteria, to keep a balanced equilibrium [3-5]. Factors upsetting this equilibrium may predispose to different types of vaginal infections. Bacterial Vaginosis [BV], is an alteration of the vaginal microbiota in which lactobacilli are replaced by anaerobic bacteria [6-10]. Vulvo-Vaginal Candidiasis [VVC] is due to the excessive growth of Candida, a commensal of the vaginal microbiota [9-11]. Mixed flora vulvovaginitis and/or aerobic vulvovaginitis develops when several aerobic microorganisms come into play [12].

Is difficult to understand how to treat these different vaginal infections. Antibiotics alone, or in combination with antifungal agents, are considered the best treatment strategy. Metronidazole proved to be highly effective [13, 14, 15]. Yet the benefits of antibiotics come at the cost of an imbalance of the vaginal ecosystem. After the initial remission of symptoms, the induced reduction of vaginal lactobacilli may cause subsequent genital disturbance flare-ups. In addition, the development of sensitivity, allergy, and side effects, may limit the prolonged or repeated use of these therapies [15, 16]. Probiotic supplemented topically or systemically were proposed to avoid such limitations [17]. Potential therapeutic effects were obtained with bacteria lysate obtained by the mechanical fragmentation of Propionibacterium acnes [P. acnes] [18-20]. Indeed, in a large observational study, this treatment given alone improved symptoms and signs of VVI [19], with an efficacy that was like that of clyndamicine [18]. In addition, P. acnes lysate proved to potentiate the therapeutic effect of the antifungal agent fluconazole on VVC [20]. In the current study, the clinical efficacy of P. acnes lysate vaginal gel was compared to that of metronidazole, as antibiotic, associated with clotrimazole, as antifungal agent [Meclon®; Alfasigma S.p.A., Bologna, Italy].

3. Subjects and Methods

Premenopausal women [age range 18-40] diagnosed with VVI were enrolled in this prospective multicentre double-arm study. Patients were recruited from the Gynaecological Departments of the University-Hospitals of Cagliari, Catania, Catanzaro, Genova and Modena - Reggio Emilia (Italy). The study was designed and conducted following the principles outlined in the Declaration of Helsinki and the Good Clinical Practices concerning clinical investigations of medical devices for human subjects. The study was conducted at the Obstetrics and Gynaecology Departments of the University-Hospitals of Cagliari, Catania, Catanzaro, Genova and Modena - Reggio Emilia (Italy). At the screening visit, subjective symptoms of VVI (i.e., vulvovaginal burning and/or itching, and/or dyspareunia) were rated on a visual analogic scale [VAS], ranging from 0 [absence of symptoms] to 10 (intolerable symptom). Women presenting scores between 4 and 8, for one or two of the above-mentioned symptoms, were invited to participate in the study. Concurrently, a gynaecological visit was performed to grade VVI signs, such as erythema and leucorrhea, according to the 10 VAS scale. Etiology of VVI was determined by a vulvovaginal swab for the identification of Gardnerella vaginalis [GV], Candida albicans [CA], and Trichomonas vaginalis [TV], and to determine Amsel criteria for the diagnosis of BV [18,21]. Only symptomatic women meeting the inclusion criteria were included in the study [inclusion and exclusion criteria are detailed in Table 1. Subsequently, women were randomly assigned to treatment with Immunovag® or Meclon®, daily vaginally administered for 5 or 6 days, respectively. Immunovag® [Depofarma S.p.A., Mogliano Veneto, Italy] is a vaginal gel based on hyaluronic acid, polycarbophil and P. acnes lysate, available in 35 ml tube with five disposal vaginal applicators of 5 grams each. Meclon® [Alfasigma S.p.A., Bologna, Italy] is a vaginal cream based on 20% metronidazole and 4% clotrimazole, available in 30 grams' tube with six disposal vaginal applicators of 5 grams each. Post-treatment evaluation was performed after 10 days from treatment initiation. The same pre-treatment evaluations were repeated.

4. Statistical Analysis

For sample size calculation, a 50% difference in terms of symptoms and signs between the two treatment groups was defined as clinically relevant. Considering this assumption, a sample size of 30 subjects in each group of treatment yields an alpha of .05 and a beta of .76 [22]. Statistical analysis of the results was performed using the Student's t test for paired or unpaired data. Two-factor analysis of variance [ANOVA] for repeated measures was also used to analyze the data. Percentages were compared by the 2 test. Differences were considered significant for "p" less than .05. Statistical analysis was performed by the statistical software package Stat View 5.01 [SAS Institute Inc., Cary, North Carolina]. The results are expressed as mean ± standard error [SE].

5. Results

Out of 100 women, only 80 subjects were enrolled, 5 women being excluded for the presence of TV, and 15 women for not giving the consent to participate to the study. Among enrolled women 74 suffered from vulvovaginal itching, 53 from vulvovaginal burning, 61 from dyspareunia. Seventy-fivewomen had vaginal leucorrhea, and 41 vulvovaginal erythema. Out of the 80 vaginal swabs, GV was present in 10 cases, and CA in 28 cases. The remaining 42 subjects were affected by BV, diag-nosed in accordance with the Amsel criteria [21]. In women with diagnosis of GV, Amsel criteria were present [21]. Eight subjects did not attend post-treatment evaluation. Accordingly, statistical analysis was performed only in women with the complete set of data, i.e. 72 women, of which 40 treated with Immunovag® and 32 with Meclon®. Prevalence of symptoms and signs of VVI were not significantly different between women assigned to the treatment with Immunovag® or Meclon®. A significant decrease in the prevalence of the symptoms such as itching (p=0.001) and burning

(p=0.001) and the sign erythema (p=0.007) was observed in the 40 subjects treated with Immunovag®, independently from VVI etiology (Table 2). In women treated with Meclon® a significant decrease of the symptoms itching (p=0.001), and of the signs leucorrhea (p=0.006) and erythema (p=0.001) was observed (Table 2). The reduction in the prevalence of burning was more pronounced with Immunovag® (p=0.003) and that of Leucorrhea more pronounced with Meclon® (p=0.028) (Table 2). VAS values of symptoms and signs of VVI significantly decreased (p<0.001), without any significant difference between treatments (Table 3). In women with a diagnosis of VVI by CA, VAS values of the symptoms and signs significantly decreased (p<0.001) with no statistical difference between Immunovag® and Meclon® (Figure 1). Similarly, in women with a diagnosis of BV, VAS values of symptoms and signs significantly decreased (p<0.001) with no statistical difference between Immunovag® and Meclon® (Figure 2). In women with a diagnosis of VVI by GV, VAS values of symptoms and signs significantly decreased (p<0.001) with no statistical difference between Immunovag® and Meclon® (Figure 3).

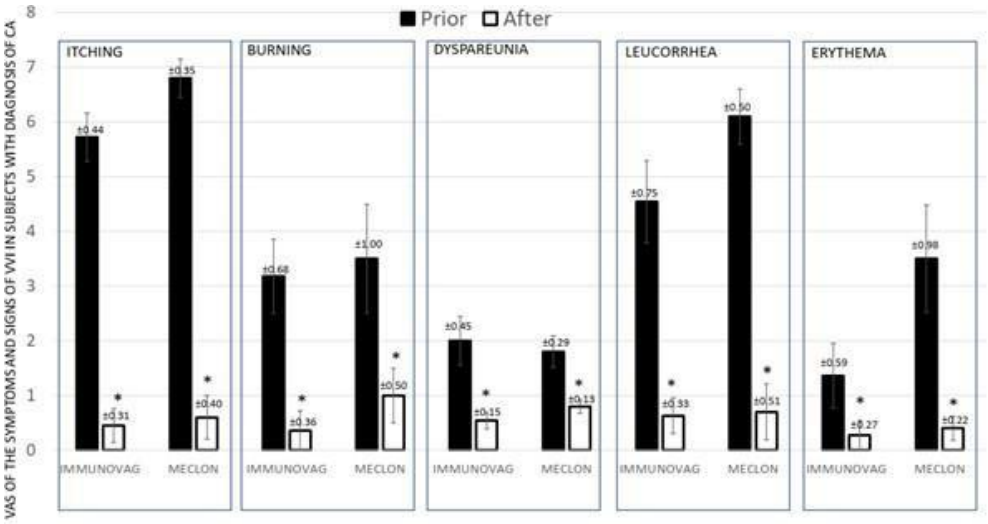

Figure 1: Mean (+SE) VAS of symptoms (Itching, Burning, Dyspareunia) and signs (Leucorrrhea and Erythema) of VVI observed in women with infection by candida (CA) prior (black bar) and after (white bar) treatment with Immunovag® or Meclon®. All symptoms and signs significantly decreased (p<0.05) without differences between treatments.

Table 1: Inclusion and Exclusion Criteria to The Study

Inclusion criteria:
1. Subjects aged between 18 and 40 years, diagnosed with VVI documented by clinical symptoms of vulvovaginal infection (vaginal itching, vulvar itching, vaginal burning, vulvar burning, dyspareunia), evaluated according to a visual analogue scale (VAS) (0 absence of the symptom, 10 if the symptom is intolerable).
2. Subjects with a negative pregnancy test.
3. Subjects who gave informed consent to the study.
4. Positivity of vaginal swab for GV or CA
5. Negative vaginal swab for TV
6. Negative vaginal swab for GV, CA, TV, but presence of Amsel criteria
The exclusion criteria were:
1. Subjects with no indication for treatment.
2. Subjects undergoing treatment with psychotropic drugs, antibiotics, antifungals, immunomodulators, cortisone drugs
3. Subjects who did not give informed consent to the study.
4. Subjects in whom the pregnancy test was positive.
5. Subjects with hypersensitivity referred to the compounds used in Immunovag® and Meclon®.
6. subjects with vaginal swab positive for TV

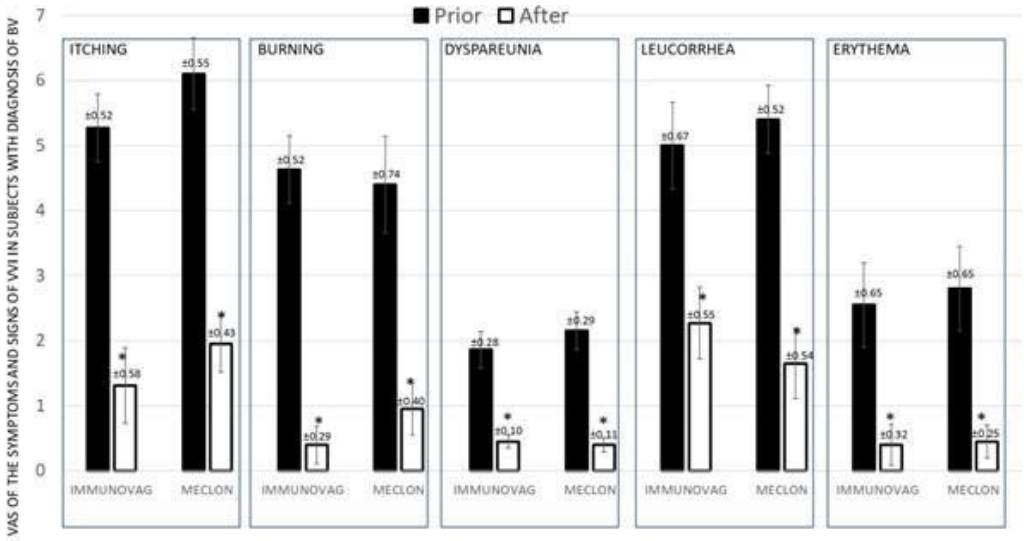

FIGURE 2

Figure 2: Mean (+SE) VAS of symptoms (Itching, Burning, Dyspareunia) and signs (Leucorrrhea and Erythema) of VVI observed in women with bacterial vaginosis (BV) prior to (black bar) and after (white bar) treatment with Immunovag® or Meclon®. All symptoms and signs significantly decreased ($p<0.05$) without differences between treatments.

Table 2: Presence of symptoms (itching, burning, dyspareunia) and signs (leucorrhea and erythema) observed in women with VVI prior and after treatment with Immunovag® (N=40) or Meclon® (N=32).

	Immunovag® (n=40)			Meclon® (n=32)			Improvements		
	Prior	After	p value	Pror	After	P value	Immunovag®	Meclon®	P value
Itching	35 (87.5%)	1 (2.5%)	0.001	27 (84.3%)	3 (9.7%)	0.001	34 (85%)	24 (75%)	0.29
Burning	31 (77.5%)	3 (7.5%)	0.001	14 (43.7%)	8 (25%)	0.111	28 (70%)	11 (34.3%)	0.003
Dyspareunia	18 (45%)	12 (30%)	0.161	17 (53.1%)	11 (34.3%)	0.132	6 (15%)	6 (18.7%)	0.677
Leucorrhea	20 (50%)	15 (37.5%)	0.262	21 (65.6%)	10 (31.2%)	0.006	5 (12.5%)	11 (34.3%)	0.028
Erythema	16 (40%)	3 (7.5%)	0.007	18 (56.2%)	3 (9.3%)	0.001	13 (32.5%)	15 (46.8%)	0.219

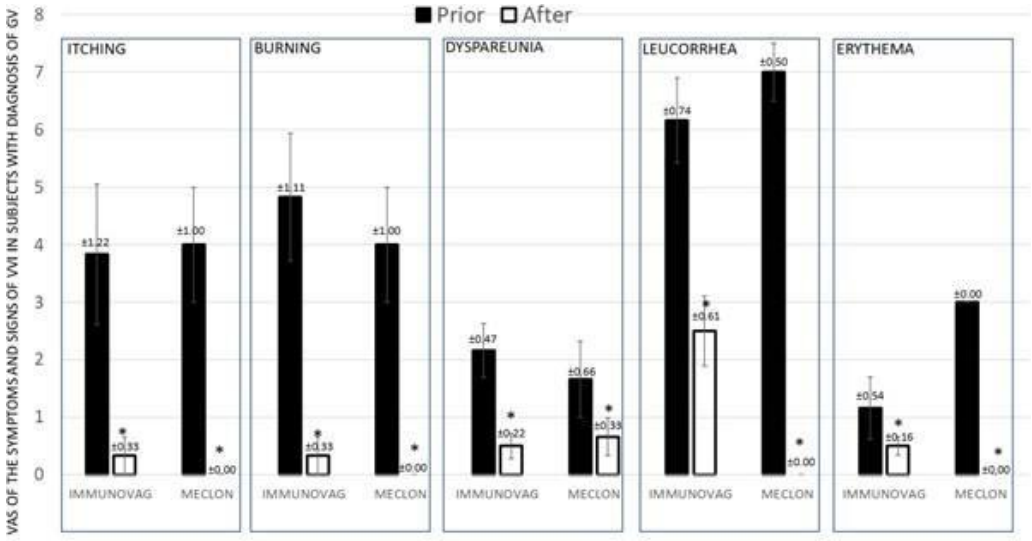

FIGURE 3

Figure 3: Mean (±SE) VAS of symptoms (Itching, Burning, Dyspareunia) and signs (Leucorrrhea and Erythema) of VVI observed in women with infection by garderenella vaginalis (GV) prior to (black bar) and after (white bar) treatment with Immunovag® or Meclon®. All symptoms and signs significantly decreased ($p<0.05$) without differences between treatments.

Table 3: Mean (±SE) of symptoms (itching, burning, dyspareunia) and signs (leucorrhea, erythema) in subjects with vulvovaginitis prior and after treatment with Immunovag® (n=40) or Meclon® (n=32).

	Prior	After	P value	Between Treaments P value
ITCHING				
Immunovag®	5.17±0.38	0.92±0.35	P<0.001	p>0.05
Meclon®	6.12±0.38	0.87±0.29	P<0.001	
BURNING				
Immunovag®	4.25±0.40	0.38±0.20	P<0.001	p>0.05
Meclon®	4.09±0.54	0.87±0.28	P<0.001	
DYSPAREUNIA				
Immunovag®	1.97±0.21	0.48±0.08	P<0.001	p>0.05
Meclon®	2.00±0.20	0.54±0.09	P<0.001	
LEUCORRHEA				
Immunovag®	5.01±0.44	1.84±0.35	P<0.001	p>0.05
Meclon®	5.75±0.36	1.30±0.38	P<0.001	
ERYTHEMA				
Immunovag®	1.97±0.42	0.33±0.20	P<0.001	p>0.05
Meclon®	3.03±0.48	0.39±0.16	P<0.001	

6. Discussion

The results of the present study indicate that in women with VVI the short-term clinical efficacy of Immunovag® and Meclon® are comparable in terms of symptoms and signs attenuation as well as in terms of VAS scores reduction of each symptom and sign. These observations are in line with our past experience related to the short-term treatment of VVI with Immunovag®. In a previous large study performed by our group on 592 women, Immunovag® administered for 5 days led to an average decrease of a 3 points [83% reduction] of VAS scores of VVI symptoms [19]. Also in this considerably smaller study [n=40] VAS scores quantifying VVI symptoms, were reduced on average of 3 points [80% reduction] by Immunovag®. Such consistency, confirms the reproducibility of previous results, and corroborates the reliability of presented findings. The ability of the P. acnes-based product to compete in terms of efficacy on VVI with standard-of-care antibiotics was already demonstrated against clyndamicine [18]. In this study Immunovag® proved to be as effective on VVI than the association of metronidazole, another antibiotic, and clotrimazole, an antifungal agent.

Metronidazole was expected to act as a potent therapeutic agent against BV and its combination with clotrimazole has certainly played a crucial role in the cases of CA positivity. Indeed, the efficacy of antibiotic and antifungal agents in treating infections is attributed to their well-establish capabilities of killing or inhibiting the growth of bacteria and fungi, respectively. Less clear is the mechanism of action of Immunovag®. The observed remission of VVI symptoms such as itching and burning sensations might be ascribed to two main factors: i] relevant antioxidant properties, eventually enabling the reduction of inflammation, have been recently associated to P. acnes fractions [23, 24]; ii] the presence of hyaluronic acid and polycarbophil, the two compounds that complement Immunovag® formulation, are thought to act as a hydrating protective barrier thanks to their moisturizing and mucoadhesive properties [25-27]. Therefore, while the two products share comparable clinical outcomes as VVI medications, the underlying mechanisms of action are completely different

Such a difference can be understood as the root cause of the presence/absence of complications associated to the use of one or the other treatment. Indeed, while no side effects have been associated with the use of Immunovag®, general disorders and administration site disturbances, are experienced after using Meclon®, including irritative symptoms such as itching, allergic contact dermatitis, rash, and hypersensitivity to the compounds present in the formulation [15]. Moreover, as antibiotics natively act against bacteria, they inevitably affect also endogenous vaginal lactobacilli – key members of the vaginal ecosystem - leading, in turn, to long-term disorders [16].

Products containing strains of lactobacilli bacteria represent, indeed, another safe and efficient probiotic-based alternative or supplementation to antibiotic treatments. According to a recent systematic review [28] summarizing the clinical data associated to BV treatment with probiotic products containing lactobacilli, both local and systemic administration of such bacteria were associated to better performances with respect to placebo in several clinical trials, some of them including populations with large sample size. Differently, controversial results were reported about whether lactobacilli can improve metronidazole effects [29-35]. A direct comparison between the efficacy of metronidazole and probiotic products containing lactobacilli in treating BV still needs to be comprehensively addressed.

Our study, by reporting a direct comparison between the perfor-

mances achieved with the sole use of a probiotic-based product [Immunovag®] and the ones obtained resorting to standard-of-care treatments [Meclon®], serves as another stepping stone towards the finding of a safe and efficient alternative strategy for VVI treatment. Nevertheless, limitations of the study such as the relatively small sample size of the population and the absence of follow-up visits need to be considered. The inclusion of VVI cases with different etiologies may have represented a confounding factor but, from another perspective, it may imply that the investigational product is indeed effective in treating a broad range of VVI. Future studies involving large sample size populations should be performed to draw solid conclusions regarding the ability of Immunovag® of matching Meclon® performances. Moreover, long follow-up times will be required to assess longer-term efficacy and safety of Immunovag® treatment.

7. Conclusion

Within the limitations of the present study, the investigational device, Immunovag®, seems to be safe and effective in managing sign and symptoms of VVI with different etiologies. Notably, its performances are comparable to those of the active control, Meclon®, but avoiding the occurrence of side effects. These preliminary results should be confirmed by studies involving a greater number of subjects, longer follow-ups and, perhaps, less heterogeneous target populations. Nevertheless, in the light of the encouraging findings reported in our study, we propose formulations such as Immunovag® as a valuable alternative to antibiotics and antifungal agents for VVI treatment.

References

1. Marrazzo JM. A Persistet(ly) Enigmatic Ecological Mystery: Bacterial Vaginosis. JID.2006; 193: 1475-8.
2. Mills BB. Vaginitis: beyond the basics. Obstet Gynecol Clin N Am. 2017; 44: 159-177.
3. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA. 2011; 108(Suppl 1): 4680-7.
4. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. Fertil Steril. 2018; 110: 327-36.
5. Younes JA, Lievens E, Hummelen R, Van der Westen R, Reid G, Petrova MI, et al. Women and their microbes: the unexpected friendship. Trends Microbiol. 2018; 26 :16–32.
6. Bagnall P, Rizzolo D. Bacterial vaginosis: A practical review. JAAPA.2017; 30: 15-21.
7. Chacra LA, Fenollar F, Diop K. Bacterial Vaginosis: What Do We Currently Know? Front Cell Infect Microbiol. 2022; 11: 672429.
8. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, et al. Hydrogen peroxide–producing lactobacilli and acquisition of vaginal infections. J Infect Dis. 1996; 174: 1058–63.
9. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med. 2005; 353: 1899-911.
10. Oakley BB, Fiedler TL, Marrazzo JM, Fredricks DN. Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. Appl Environ Microbiol. 2008; 74: 4898-909.
11. Sobel JD. Vulvovaginal candidosis. Lancet. 2007; 369: 1961-71.
12. Donders GGG, Bellen G, Grinceviciene S, Ruban K, Vieira-Baptista P. Aerobic vaginitis: no longer a stranger. Res Microbiol. 2017; 168(9-10): 845-58.
13. Donders GGG, Zodzika J, Rezeberga D. Treatment of Bacterial Vaginosis: What We Have and What We Miss. Expert Opin. Pharmacother. 2014; 15: 645-57.
14. World Health Organization. Guidelines for the Management of Symptomatic Sexually Transmitted Infections. (Geneva, Switzerzerland: World Health Organization). 2021.
15. Paladine HL, Desai UA. Vaginitis: Diagnosis and Treatment. Am Fam Physician. 2018; 97: 321-9.
16. Ferrer M, Mendez-Garcia C, Rojo D, Barbas C, Moya A. Antibiotic use and microbiome function. Biochem Pharmacol. 2017; 134: 114-26.
17. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal microbiota and reproductive health: the virtue of simplicity. Cell Host Microbe. 2018; 23: 159-68.
18. Melis GB, Marotto MF, Orrù MM, Pilloni M, Zedda P, D'Alterio M, et al. Bacterial vaginosis: efficacy of a local treatment with a gel containing a fraction derived from propinibacterium acnes. Minerva Ginecol. 2016; 68: 1-8.
19. Melis GB, Piras B, Marotto MF, Neri M, Corda V, Vallerino V, et al. The stimulation of the vaginal immune system with short-term administration of a vaginal gel containing fraction of Propionibacterium acnes, hyaluronic acid and polycarbophil is efficacious in vaginal infections dependent on disorders in the vaginal ecosystem. Gynecol Endocrinol. 2018; 34: 880-3.
20. Melis GB, Marotto MF, Orru' MM, Pilloni M, Corda V, Neri M, et al. Vulvo-vaginal candidiasis: efficacy of a local treatment with a gel containing a fraction derived from prpionibacterium acnes in addition to fluconazole. Multidisciplinary Journal of woman's Health. 2013; 2(4): 39-43.
21. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK, et al. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med. 1983; 74: 14-22.
22. Thomas L, Krebs CJ. A review of statistical power analysis software, Vol. 78. New York: Bulletin of the Ecological Society of America. 1997; Pg No: 128–39.
23. Campolo M, Gallo G, Ardizzone A, La Torre M, Filippone A, Lanza M, et al. Evaluation of a Gel Containing a Propionibacterium Extract in an In Vivo Model of Wound Healing. Int J Mol Sci. 2022; 23(9): 4708.

24. Allhorn M, Arve S, Brüggemann H, Lood R. A novel enzyme with antioxidant capacity produced by the ubiquitous skin colonizer Propionibacterium acnes. Sci. Rep. 2016; 6: 36412.

25. Grimaldi EF, Restaino S, Inglese S. Role of high molecular weight hyaluronic acid in postmenopausal vaginal discomfort. Minerva Ginecol. 2012; 64: 321-9.

26. Stute P. Is vaginal hyaluronic acid as effective as vaginal estriol for vaginal dryness relief? Arch Gynecol Obstet. 2013; 288: 1199–201.

27. 27. Fiorilli A, Molteni B, Milani M. Successful treatment of bacterial vaginosis with a policarbophil-carbopol acidic vaginal gel: results from a randomised double-blind, placebo-controlled trial. Eur J Obstet Gynecol Reprod Biol. 2005; 120: 202–5.

28. Tidbury FD, Langhart A, Weidlinger S, Stute P. Non-antibiotic treatment of bacterial vaginosis-a systematic review. Arch Gynecol Obstet. 2021; 303: 37-45.

29. Russo R, Karadja E, De Seta F. Evidence-based mixture containing Lactobacillus strains and lactoferrin to prevent recurrent bacterial vaginosis: a double blind, placebo controlled, randomised clinical trial. .Benef Microbes. 2019; 10: 19-26.

30. Laue C, Papazova E, Liesegang A, Pannenbeckers A, Arendarski P, Linnerth B, et al. Effect of a yoghurt drink containing Lactobacillus strains on bacterial vaginosis in women - a double-blind, randomised, controlled clinical pilot trial. Benef Microbes. 2018; 9: 35-50.

31. Heczko PB, Tomusiak A, Adamski P, Jakimiuk AJ, Stefański G, Mikołajczyk-Cichońska A, et al. Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. BMC Womens Health. 2015; 15: 115.

32. Hummelen R, Changalucha J, Butamanya NL, Cook A, Habbema JD, Reid G, et al. Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 to prevent or cure bacterial vaginosis among women with HIV. Int J Gynaecol Obstet. 2010; 111: 245-8.

33. Martinez RC, Franceschini SA, Patta MC, Quintana SM, Candido RC, Ferreira JC, et al. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14. Lett Appl Microbiol. 2009; 48: 269-74.

34. Anukam KC, Osazuwa E, Osemene GI, Ehigiagbe F, Bruce AW, Reid G, et al. Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes Infect. 2006; 8(12-13): 2772-6.

35. Anukam K, Osazuwa E, Ahonkhai I, Ngwu M, Osemene G, Bruce AW, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. Microbes Infect. 2006; 8: 1450-4.