
Review

Vaginal Probiotics for Reproductive Health and Related Dysbiosis: Systematic Review and Meta-Analysis

Ana López-Moreno^{1,2*} and Margarita Aguilera^{1,2,3*}

1 Department of Microbiology, Faculty of Pharmacy, University of Granada, Campus of Cartuja, 18071 Granada, Spain.

2 Instituto de Nutrición y Tecnología de los Alimentos. INYTA-Granada.

3 Instituto de Investigación Biosanitaria. Ibs-Granada.

*Correspondence: alopezm@ugr.es (A.L.-M.); maguilera@ugr.es (M.A.); Tel.: +34-9-5824-5129 (M.A.); Fax: +34 958 246235

Abstract: The use of probiotics in reproductive-related dysbiosis is an area of continuous progress due to the growing interest from clinicians and patients suffering from recurrent reproductive microbiota disorders. An imbalance in the natural colonization sites related to reproductive health—vaginal, cervicovaginal, endometrial, and pregnancy-related altered microbiota—could play a decisive role in reproductive outcomes. Oral and vaginal administrations are in continuous discussion regarding the clinical effects pursued, but the oral route is used and studied more often despite the need for further transference to the colonization site. The aim of the present review was to retrieve the standardized protocols of vaginal probiotics commonly used for investigating their microbiota modulation capacities. Most of the studies selected focused on treating bacterial vaginosis (BV) as the most common dysbiosis; a few studies focused on vulvovaginal candidiasis (VVC) and on pretreatment during in vitro fertilization (IVF). Vaginal probiotic doses administered were similar to oral probiotics protocols, ranging from $\geq 10^7$ CFU/day to 2.5×10^{10} CFU/day, but were highly variable regarding the treatment duration timing. Moderate vaginal microbiota modulation was achieved; the relative abundance of abnormal microbiota decreased and *Lactobacillus* species increased.

Keywords: vaginal probiotics; reproductive dysbiosis; bacterial vaginosis; VVC; IVF

1. Introduction

1.1. Microbiota colonization sites in women's reproductive system

The taxa composition of the microbiota appears to exert a relevant role in reproductive and hormonal health, determining states of eubiosis [1] versus dysbiosis. The effects of a microbiota imbalance seem to contribute to the triggering of reproductive [2,3], hormonal [4], and metabolic disorders [5,6]. Similarly, the reproductive-site microbiota can be affected by hormones or endocrine disruptor chemicals [7]. Conversely, if microbial dysbiosis occurs, subsequent decreased enzymes levels may diminish circulating estrogens and lead to recurrent reproductive pathologies [4,8]. Special attention has been paid to the following dysbiosis sites: vaginal, cervicovaginal, endometrial, and, indirectly, pregnancy placenta microbiota. They are described in detail below.

The vaginal microbiota shows a specific colonization pattern for each woman; in the vaginal microbiota, the *Lactobacillus* genus is dominant in the establishment of a healthy microbiome community pattern [9]. Recently, certain authors have postulated on the specific colonization of the endometrium. Specifically, a decrease in the *Lactobacillus*

population appeared to be linked with implantation failure or early miscarriage in In-Vitro-Fertilization patients [10-11]. However, there is controversy in these results and in determining the ratio of dominant microorganisms associated with health/dysbiosis. The theory of the existence of microbes in the placenta against the dogma of sterility has been experimentally approached by different authors. There is controversial research on the presence of specific microbiota in human uterine and placental sites and its effect on pregnancy and the fetus [12-13]. The hypothesis regarding the existence of microbiota in the placenta is generally considered disproven, as rigorously controlled studies found either clear pathogenic infections or no bacterial presence [12-13]. The formation and conservation of placental integrity and utility are known to be critical to fetal progress, and survival [14].

1.2. Microbial dysbiosis associated with reproductive system diseases

The reproductive tract microbiota's composition and their variable patterns seem to be associated with alterations in reproductive disorders (Figure 1). Moreover, several recent studies have demonstrated that microbial dysbiosis could be linked to long-term recurrent reproductive alterations.

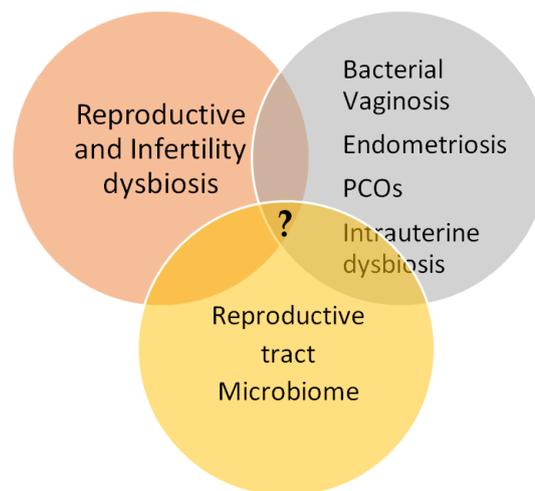


Figure 1. Intersection gap knowledge of reproductive system disorders, unexplained infertility, microbiome dysbiosis, and recurrent reproductive pathogenesis phenotypes.

Bacterial vaginosis (BV) is the most prevalent reproductive disorder and is linked with gynecological complications, like spontaneous preterm labor, abortion, and endometriosis. It can be cured by restoring the representative vaginal components of the microbiota with probiotic formula, usually species of the genus *Lactobacillus* [15-16]. Salah et al. [17] postulated that BV is strongly implicated in underestimated causes of unexplained infertility. They found that BV detection and treatment improves the pregnancy rate in women [17]. Furthermore, van Oostrum et al. [18] claim that BV is meaningfully linked with preclinical gestation loss. They claimed that infertility is generally related to BV and atypical microbiota at lower genital tract, estimating that one in every five infertile patients suffers from BV and at least one in every three has an altered vaginal taxa microbiota. Thus, they suggested that BV might be involved in the etiology and irregular pregnancies of these patients.

In addition, among women of reproductive age, there are other common dysbiosis such as endometriosis, that have been linked to an unfavorable effect on fertility; 30% to 71% of women suffering infertility showed endometriosis and 30% to 50% of women with endometriosis are infertile [19]. Moreover, polycystic ovary syndrome (PCOS), linked to multiple physiological risk factors (obesity, hypertension, dyslipidemia, and

insulin resistance), has been associated with reproductive disorders [2,20]. Furthermore, spontaneous abortions and preterm deliveries, including non-implantation of the embryo, could be highly related to episodes of dysbiosis; these could be modulated by restoring disrupted microbiota [21-24]. Therefore, one strategy to counteract these bacterial infections involves the administration of probiotics, which are safer and more natural than antibiotics [22]. However, in general, studies of probiotics in relation to pregnancy complications generally require statistically significant sample sizes or complete data with a greater number of clinical trials and the determination of microbiota at the species and strains level [24].

1.3. Probiotics for reproductive health interventions

The administration of probiotics for reproductive clinical translational investigation is continuously progressing due to the growing interest in the scientific evidence reported for demonstrating the beneficial effects related to the restoration of natural microbiome colonization sites.

Probiotics remain an important complementary intervention resource to modulate dysbiosis of the microbiota, which seems to be associated with various metabolic disorders and diseases [25-26]. Therefore, specific doses of certain probiotic strains could modulate the microbiota toward a healthier state, that is, to recover the state of eubiosis [27-28]. Conversely, the inappropriate use of probiotics might pose some risks and safety concerns in immunologically compromised individuals [29].

1.4. Administration routes of probiotics in reproductive dysbiosis

Most clinical trials on the modulation of reproductive dysbiosis have been carried out using oral probiotics [30]. However, oral administration requires transfer of the probiotic bacteria to the site of colonization to promote a specific clinical effect, which implies that the probiotics have to subsist to the low pH of the upper gastrointestinal region, which is generally demonstrated by the recovery of these specific microorganisms from fecal samples [25,31]. Specifically, in microbiota reproductive site dysbiosis, probiotics should be transferred to the dysbiotic colonization sites, such as the vagina (vaginosis), the endometrium (endometritis), and the breast (mastitis). This physical transfer can be achieved via the ascending pathway, hematogenous route and lymph node transfer [32]. Presently, there are scientific results that prove the benefits of probiotic microorganisms on reproductive health outcomes, such as the modulation of vaginosis [33], PCOS [20], mastitis [34].

Vaginal administration of *Lactobacillus* can restore the vaginal microbiota by controlling the Nugent index with the range of normal values (0–3). Furthermore, *Lactobacillus* colonization is inversely correlated with the concentration of bacteria associated with bacterial vaginosis [35]. To treat vulvovaginal infections, probiotics can be administered, preferably vaginally, to control the recolonization of *Lactobacillus* without any transfer or survival concerns at the site of action [31].

The *Lactobacillus*-dominated endometrium may also benefit embryo implantation. However, there is controversy in these results and in determining the ratio of dominant microorganisms associated with health/dysbiosis [36]. Furthermore, the same authors argued that further taxonomical analysis of the endometrial microbiota may be necessary to identify the beneficial and/or pathogenic bacteria involved in embryo implantation. This would avoid multiple interventions against the microbiota that are not dominated by *Lactobacillus*.

Additionally, in a recent research, the link between endometrial microbiota composition and pregnancy outcomes in In-Vitro-Fertilization (IVF) patients was examined. Remarkably, Moreno et al. [37] found an association between an endometrial microbial composition that was limited in *Lactobacillus* strains and adverse pregnancy outcomes. It was concluded that the negative effects of endometrial microbiota that are not dominated by *Lactobacillus* should be related with negative reproductive outcomes, such as implantation failure and pregnancy loss [38-40]. According to this, vaginal administration of probiotics could allow a direct, quicker, and targeted colonizing action to restore the altered vaginal microbiota compared to the long-term effects obtained by oral probiotics.

The main objective of the present work was to collect, scrutinize, and extract the most recent information from the high-quality and relevant scientific literature on probiotics administered vaginally and their possible qualitative and quantitative modulation capacities in reproductive-health-related dysbiosis.

2. Materials and Methods

2.1. Eligibility criteria and search strategy

All interventional studies compiling information on specific probiotic microbial strains and dosages administered for human reproductive microbiota-related dysbiosis were included. Two reviewers, ALM and MA, screened titles, abstracts, and then full-text papers independently against inclusion criteria according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [41].

These four criteria were applied for the selection of the study: (1) being published within the last fifteen years (i.e., between 2005 and 2020), specifying (2) the probiotic strain used, (3) the dose, and (4) the time/period of administration. The specific data on population, intervention, comparison, and outcome criteria for inclusion are detailed in Table 1.

Table 1. Population, intervention, comparison, and outcome (PICO) criteria for inclusion of studies.

Parameters	Inclusion Criteria
Population	Human
Intervention	Probiotics strains and doses
Comparison	Vaginal probiotics versus placebo
Outcome	Fertility parameters
Setting	Clinical trials (CTs)

Non-English-language manuscripts and documents or studies without specific data on fertility and reproductive dysbiosis biomarkers were excluded.

Each eligible article identified was reanalyzed by title and abstract, and the eligible articles were selected for full reading. The first selection was performed based on a term search through title and abstract screening, and the second selection was based on a full-text screening, and the two independent reviewers revised the publications with reference to the inclusion criteria. The study selection inter-rater agreement between the two reviewers was calculated as the proportion of positive agreement (PA) [42].

Literature search and review were carried out under the stepwise search procedure. The systematic review was developed in collaboration with University of Granada librarian support using medical subject headings (MeSH) and search keywords/terms

(described below). The following databases were used: MEDLINE/PubMed [43], Web of Science (Thomson Reuters Scientific), Scopus (Elsevier), and Cochrane Library [44]. A PRISMA flow diagram of the literature search summarizes the selection of the studies consisting of two screening phases (Figure 2).

The collective search approach was carried out using MeSH and free text search terms as follows: (probiotic* and infertility and doses); (probiotic* and microbiota and fertility); (probiotic* and microbiota and infertility); (probiotic* and “vaginal microbiota” and infertility); (probiotic* and endometriosis); (probiotic* and endometriosis and fertility); (probiotic* and endometriosis and infertility); (probiotic* and “endometrial microbiota” and infertility); (probiotic* and endometrium and infertility); (probiotic* and endometrium and fertility); (probiotic* and microbiota and “*vaginal administration”); (probiotic* and ovules); (probiotic* and reproductive and “*vaginal administration”); (probiotic* and “Polycystic Ovary Syndrome”).

2.2. Data extraction, analysis, and risk of bias (quality) assessment

The resulting data were extracted from all the selected clinical studies: publication year, study design, number of participants, characteristics of the population, including the sample size (n = number of subjects) in the intervention group, sex, and age; micro-organism probiotic strains; dose; pattern of administration; modification of the main clinical parameters, Nugent score, or alterations in fertility-related parameters. The main data results from CT were qualitatively compiled and organized into Table 2.

The risk of bias for each clinical trial selected was assessed independently by the authors using the Cochrane collaboration methodology [44]. The risk of bias was tabulated for each study (Figures 3 and 4). Each item evaluated was classified as low risk, high risk, or unclear risk according to the quality recommendations described in Chapter 8 of the Cochrane Handbook of Systematic Reviews of Interventions [44]. Analysis and corresponding figures were generated in RevMan 5.3 Review Manager (RevMan Computer program) Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2019, available at revman.cochrane.org.

2.3. Statistical analysis

To calculate the overall quantitative effect size for each relevant study regarding the modulation of vaginal microbiota capacities, the following steps were undertaken: (1) The baseline value in treatment group, baseline value in placebo group, endpoint in treatment group, and endpoint value in placebo group were extracted. If baseline values were not reported in a study, only endpoint values were used. (2) Change \pm SD from baseline was calculated for the treatment group and placebo group, separately. (3) The mean difference between changes from baseline in probiotics treatment group vs. changes from baseline in the placebo group was calculated and used as the overall effect size.

Alignment calculations and Hedges's adjusted g were used to calculate the effect size. A random-effects model was used to pool calculated effect sizes. The I^2 test was used to explore heterogeneity; an $I^2 > 75\%$ is considered high heterogeneity and an $I^2 < 25\%$ is considered low heterogeneity. Heterogeneity between subgroups was evaluated using a fixed-effects model. Sensitivity analysis was performed by omitting one study at a time to detect any significant changes in the results obtained. We used Begg's rank correlation test and Egger's regression asymmetry test to evaluate publication bias.

3. Results

Reproductive disorders are an increasing health concern. Therefore, the plausible role of the microbiota in reproductive and hormonal health has encouraged studies on vaginal probiotics. Accordingly, an initial search with the keywords “probiotics and fertility” showed a tripling of available studies over the last fifteen years. A total of 719 documents were retrieved as a result of applying the selection criteria. A total of 35 clinical studies eligible for vaginal probiotics were selected for full-text review (Figure 2). The positive agreement (PA) value was 0.85 for titles and 0.90 for abstracts. When the full texts were analyzed for the specific strain, doses, and patterns, only six articles (clinical trials ($n = 5$) and a systematic review ($n = 1$)) fulfilled the inclusion criteria. The carefully chosen and included studies were extensively analyzed and relevant qualitative outcomes are shown in Table 2.

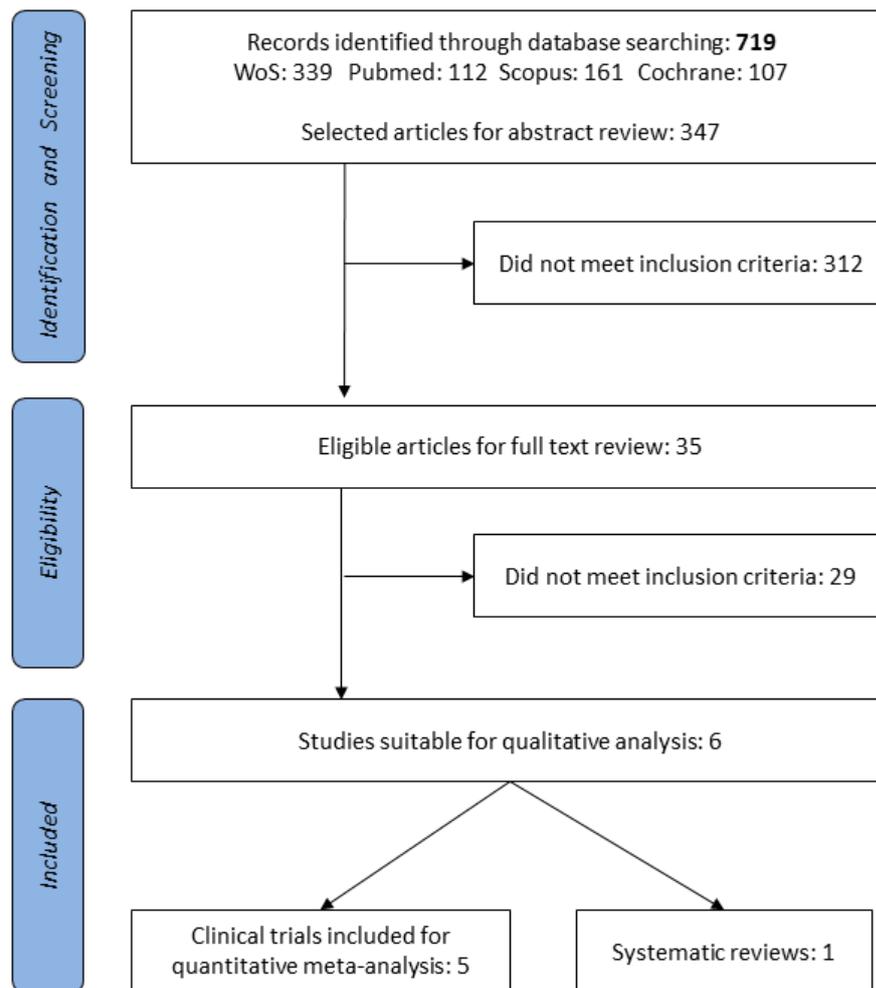


Figure 2. Vaginal probiotics Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Complementary oral probiotics PRISMA was previously performed [45].

The qualitative comparative data were extracted based on the following categories: sample number, population characteristics, probiotic strain(s), dosage and administration pattern, intervention period (weeks), disorder treated, and modulation data of clinical outcomes related with fertility disorders (Table 2).

The quality of the selected clinical studies was guaranteed based on the comprehensive method applied in the selection of the final documents and their outcomes in order to obtain comparative and useful conclusions. To evaluate the five clinical trials (CTs) in terms of their design, execution, and outcomes, the risk of bias was evaluated (Figures 3 and 4), increasing the classification of the quality standards and giving extra significance to the selected CTs, as well as allowing the validation of the revised results.

The disorders treated in the selected CTs were one of vulvovaginal candidiasis, two of BV, one of recurrent BV, and one of IVF (Table 2). The selected systematic review [46] only contained one article [47] that met the established inclusion criteria; however, the available data did not contain enough specifications to be incorporated into the quantitative analyses. The qualitative information extracted was aligned with normal microbiota restoration effects. The administration of *L. acidophilus* KS400 (Gynoflor®) to 360 women with BV did not show a significant improvement in BV symptoms, although the normal flora index (NFI) augmented meaningfully in the treated group. Further limitations beyond the restrictive design of the systematic review include the lack of studies fulfilling the strict inclusion criteria.

Furthermore, the most relevant changes and modulation capacities of vaginal probiotics on the abnormal microbiota (Figure 5) and the *Lactobacillus* genus amount (Figure 6) were revealed by quantitative examination through forest plot assessments, where the statistical impact on clinically significant parameters was verified.

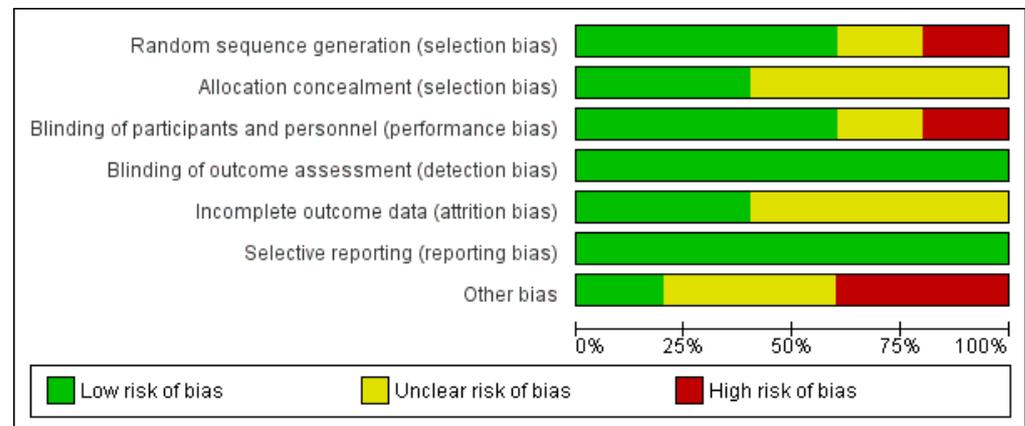


Figure 3. Risk of bias graph of clinical trial (CT): review authors' judgments about each item as percentages across all included studies.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Bisanz et al., 2014	+	?	+	+	?	+	-
Bradshaw et al., 2012	+	+	+	+	?	+	+
Gilboa et al., 2005	?	?	?	+	?	+	-
Mastromarino et al., 2008	+	+	+	+	+	+	?
Oerlemans et al., 2020	-	?	-	+	+	+	?

Figure 4. Risk of bias summary of CT: review authors' judgments about each risk of bias item for each included study low risk (+, green circle), high risk (-, red circle), or unclear risk (? , yellow circle).

Table 2. Effects of vaginal probiotic strains administered in clinical trials in reproductive and fertility-related disorders and their relevant clinical results.

Reference	Population sample (n)	Probiotic strains	Probiotic doses	Probiotic administration time (weeks)	Disorders/diseases	Clinical effects and health parameter modifications
Oerlemans et al. [48]	20 women with vulvovaginal candidiasis (VVC)	<i>Lactobacillus pentosus</i> KCA1, <i>Lactobacillus plantarum</i> WCFS1, and <i>Lactobacillus rhamnosus</i> GG	2.5×10^9 – 2.5×10^{10} CFU/day	1.5	Vulvovaginal candidiasis	Probiotic formulation restores the vaginal microbiota in 45% of women. The other 55% of women needed rescue medication (fluconazole), but, at the end of the study, these women presented a larger reduction in the amount of <i>Lactobacillus</i> sp. compared to the other group.
Mastromarino et al. [49]	39 women	Florisia®: <i>Lactobacillus brevis</i> (CD2), <i>Lactobacillus salivarius</i> subsp. <i>salicinius</i> (FV2), and <i>L. plantarum</i> (FV9)	$\geq 10^9$ CFU/day	1	Bacterial vaginosis	This probiotic product of exogenous strains of <i>Lactobacillus</i> spp. administered intravaginally restored the healthy vaginal microbiota and it can be administered to treat bacterial vaginosis (BV) disorders.
Bisanz et al. [50]	14 postmenopausal women	<i>L. rhamnosus</i> GR-1 and <i>Lactobacillus reuteri</i> RC-14	2.5×10^9 CFU/day	3 (day)	Bacterial vaginosis	Total <i>Lactobacillus</i> increased and the proportion of <i>Atopobium</i> decreased. In addition, there was a trend for <i>Gardnerella</i> and <i>Prevotella</i> reduction. No changes in Nugent score and host metabolome.
Bradshaw et al. [51]	450 healthy women	<i>Lactobacillus acidophilus</i> KS400	$>10^7$ CFU/day	12	Recurrent bacterial vaginosis	<i>Lactobacillus acidophilus</i> KS400 administered vaginally in combination of oral metronidazole during an extended course did not cure recurrent bacterial vaginosis.
Gilboa et al. [52]	117 women	Probiotic Femina®: <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i> , and <i>Bifidobacterium longum</i>	6×10^9 CFU/treatment	1 (day)	In vitro fertilization (IVF)–embryo transfer cycle	Probiotic Femina® did not affect the vaginal colonization of <i>Lactobacillus</i> during oocyte retrieval or embryo transfer and did not improve the pregnancy rate.

The quantitative outcome promoted by the diverse probiotics administered in each population studied in relation to the capacity for a reduction in abnormal microbiota and the increase in the *Lactobacillus* genus amount is indicated by black diamonds.

Interestingly, the meta-analysis showed that probiotics groups could reduce the amount of abnormal microbiota (*Gardnerella* and *Atopobium*) (Figure 5) and increase in parallel the quantity of species belonging to the *Lactobacillus* genus (Figure 6).

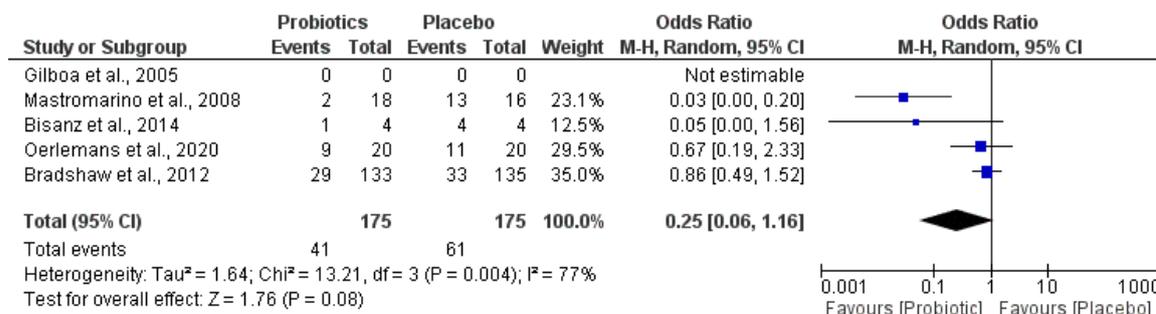


Figure 5. Effect of vaginal probiotics for modulation reduction of abnormal microbiota.

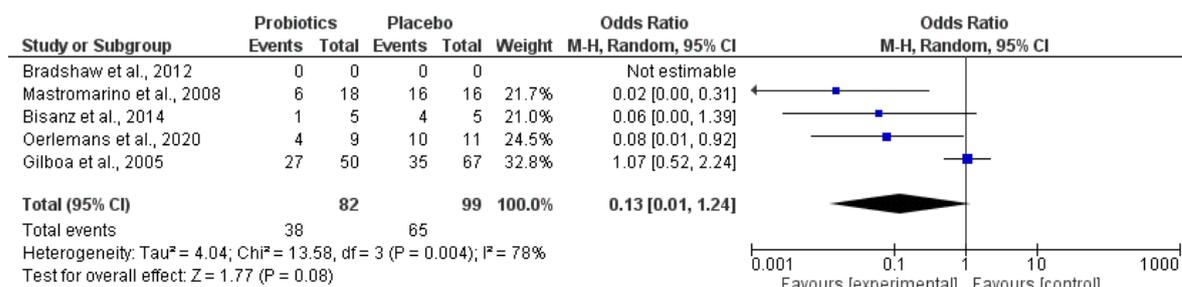


Figure 6. Effect of vaginal probiotics for modulation increase in *Lactobacillus* spp.

4. Discussion

In the last several decades, reproductive disorders and infertility cases have increased. This seems to be the result of multiple factors and hormonal imbalances triggered by different etiologies including polycystic ovary syndrome (PCOS), endometriosis, obesity or metabolic syndrome, bacterial vaginosis, infections, and even some cancers [2]. Recently, most of these metabolic disorders have been concomitantly linked to reproductive microbiota dysbiosis [2]. Consequently, many studies were conducted to establishing the healthy female reproductive microbiota, and its role in fertility dysbiosis [8-10]. Healthy microbiota at reproductive sites contains lactobacilli as the most represented bacteria, but other representative anaerobic genera may be present such as the genera *Prevotella*, *Gardnerella*, *Atopobium*, *Megasphaera*, *Sneathia*, and *Anaerococcus* [54-56]. All these bacteria seem to also be involved in diverse phases of reproduction such as gamete formation, fertilization, gestation establishment, and maintenance, and also in the bacterial transfer mother-newborn [56-57]. There are multiple factors that seem to modify the reproductive tract microbiome equilibrium, mainly triggering bacterial vaginosis as the most reported dysbiosis [58]. However, we highlight the misuse of antibiotics, together with cumulative exposure to several xenobiotics, and endocrine disruptors, which can also influence the healthy microbiome [7], especially when exposure occurs via direct contact with a high level of contaminants in hygiene products [60].

Our study highlights the probiotic modulation capacities in relation to bacterial vaginosis, which showed the restoration of a healthy microbiome. *Lactobacillus* spp. are the dominant colonizers in reproductive sites [38] and defend these sites against abnor-

mal or pathogenic microorganisms [61]. Accordingly, more probiotic interventional studies have been conducted on reproductive failures with lactobacilli imbalance, such as adverse pregnancy outcomes [16], a significant decrease in endometrial implantation [36], and altered IVF outcomes [62-63]. The *Lactobacillus* genus has optimal probiotic properties, including high hydrophobicity and self-regulation, adhesion to epithelial cells and acid production [64], and restoration of healthy urogenital microbiota [65-67]. In this systematic review, combinations of *Lactobacillus* strains were also administered in most studies, as *L. acidophilus* KS400 was present in all formulae administered as the single probiotic strain. Importantly, this strain produces bacteriocins with antimicrobial activity against relevant urogenital pathogens [68]. In addition, a combination of strains can be administered orally and vaginally, like *L. rhamnosus* GR-1 and *L. reuteri* RC-14 [50, 69-70], with the aim of reducing abnormal microbiota and recurrent dysbiosis.

Other probiotic genera administered orally, such as *Bifidobacterium* spp., are used in fertility disorders. Zhang et al. [20] managed to modulate the levels of sex hormones in patients with PCOS through the intestine-brain axis with the probiotic strain *Bifidobacterium lactis* V9. In this review, we only found one article that used *Bifidobacterium* in combination with *Lactobacillus*, the only research study on the impact of probiotic strains on embryo transfer success; the authors claimed that the supplementation of probiotics after oocyte retrieval didn't improve vaginal colonization or pregnancy rate [52]. The use of the specific probiotic strain for such dysbiosis was corroborated through modifying bacterial vaginosis parameters [46,48-50]. There was a probiotic strain in combination with oral metronidazole that was not able to reduce BV recurrence [51]. The lack of success in trials with probiotics as modulators of fertility disorders may be due to the efficacy of the probiotic being strain- and disease-dependent, as well as highly reliant on the dose, duration, administration method, and host state [71-72].

In agreement with the qualitative outcomes retrieved, we found wide variations in the administration pattern of the probiotics used in the investigations selected. The doses administered in the studies collected for the systematic review ranged from $\geq 10^7$ CFU/day [46] to 2.5×10^{10} CFU/day [48]. We found large variations in the administration time between the administration of the probiotic for 1 day [52] and its administration for 12 weeks [51]. Except for these two studies [51-52], the mean administration time found in the review was 1 week. When we compare these data with the oral administration of probiotics in reproductive disorders [45], it is observed that the range of CFU/day administered is similar to that of vaginal probiotics, from 1×10^6 CFU/day to 3×10^{10} CFU/day. However, in terms of treatment duration, the differential range is much more pronounced, varying from 3 to 24 weeks; this could be due to oral probiotics needing a longer duration to reach the natural reproductive site of colonization compared to the more localized site-direct administration for vaginal probiotics. As expected, there were more clinical trials in which probiotics were administered orally (10) than vaginally (5), which fulfilled the high-quality standards for fertility disorders. Until now, bacterial vaginosis has been the most common vaginal syndrome treated by local probiotics, but we consider that well-designed clinical studies would better support and explore the use of vaginal probiotics as therapeutic complementary solutions on reproductive site dysbiosis in relation to unexplained infertility cases.

The meta-analysis outcomes corroborated a slightly modulated vaginal probiotic capacity on the relative abundance of abnormal microbiota. This seemed to be associated with a tendency for microbiome restoration by the level of *Lactobacillus* species. Heterogeneity data were also similar for abnormal microbiota reduction (77%) and restoration of *Lactobacillus* (78%). The data analyzed were in agreement with postulates on the presence of abnormal vaginal microbiota as a factor of recurrent dysbiosis. Vaginal microbiotas of patients with BV contain more diverse and higher counts of *Gardnerella*, *Prevotella*, *AtopobiumMobiluncus*, *Peptostreptococcus*, *Sneathia*, *Leptotrichia*, and *Mycoplasma*,

whereas *Lactobacillus* are found in lower quantity and less frequently [58]. The combination of these microbial modifications can synthesize amino compounds and rise the vaginal pH, thus generating an location more inclined to several pathogenic infections and vulnerable to unhealthy disorders, including reproductive results [73]. Remarkably, reproductive site *Lactobacillus* species promotion, together with a proportionally decreasing abnormal microbiota, was also supported by in vitro studies that showed lactobacilli inhibiting the colonization of *Gardnerella vaginalis* to the vaginal epithelium tissues and producing bacteriocins, lactic acid, and/or H₂O₂, which impede the bacteria that cause BV [46].

The limitations of this review are based on the few comparative and qualitative clinical data available because of the low number of eligible studies and narrow sample size population. Furthermore, there is no standardized probiotic administration, and there are different doses and several probiotic strains. International guidelines or protocols on probiotics for reproductive-related disease prevention and treatments are required. This will allow for a more significant and unified clinical effect comparison and provide robust meta-analysis outcomes.

In future studies, all probiotics used must be safe and harmless to the target patients [74]. Next-generation probiotics (NGP) are better and have been extensively characterized regarding their physiological interaction with the host [75-76]; therefore, new clinical studies with NGP might better modulate reproductive dysbiosis. Another innovative therapeutic method for reproductive site microbiota modulation is vaginal microbiota transplantation (VMT) [77-79]. This method opens the door to a BV treatment that requires more research to advance it from conceptual analysis to clinical application.

5. Conclusions

The present study revealed that only a few clinical trials administering vaginal probiotics for fertility-related dysbiosis applied harmonized protocols for the most common reproductive disorder, bacterial vaginosis. *Lactobacillus acidophilus* remains the first probiotic species to be vaginally administered. The impact of quantitative microbiota modulation capacities in reproductive-health-related dysbiosis was similar within the selected studies, as proved by the meta-analysis outcomes in which the administration of vaginal probiotics moderately modulated the relative abundance of abnormal microbiota, coinciding with an increase in *Lactobacillus* species. The variety of fertility disorders treated with vaginal probiotics found was significantly low compared to oral administration of probiotics. Hence, vaginal intervention studies with next-generation probiotics could redirect the effort to obtain not only modulation of microbial biomarkers, but also better holistic reproductive health effects.

Author Contributions: Conceptualization, M.A.; methodology, A.L.-M. and M.A.; writing—original draft preparation, A.L.-M.; review and editing, A.L.-M. and M.A. Funding Acquisition, M.A. The Authors have read and agreed to the published version of the manuscript.

Funding: This research received no direct external funding. A.L.M. was granted with the IniciaTC programme—OTRI-UGR. Infrastructure Reference Microbiota Laboratory funding FED-ER-IE_2019-198. The APC was funded by EIN-2019-103082.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors acknowledge support from the Programs IniciaTC 2019, and INV 2019-2021 from the Plan Propio of the University of Granada. Part of results is from Ana López-Moreno doctoral thesis, Biomedicine Doctorate Program of the University of Granada.

Conflicts of Interest: The authors declare no conflict of interest.

1. García-Velasco, J. A.; Menabrito, M.; Catalán, I. B. What Fertility Specialists Should Know about the Vaginal Microbiome: A Review. *Reprod. Biomed. Online* **2017**, *35* (1), 103–112. <https://doi.org/10.1016/j.rbmo.2017.04.005>.
2. Baker, J. M.; Al-Nakkash, L.; Herbst-Kralovetz, M. M. Estrogen-Gut Microbiome Axis: Physiological and Clinical Implications. *Maturitas* **2017**, *103*, 45–53. <https://doi.org/10.1016/j.maturitas.2017.06.025>.
3. Vazquez, F.; Fernández-Blázquez, A.; García, B. Vaginosis. Vaginal Microbiota. *Enfermedades Infecc. Microbiol. Clínica Engl. Ed.* **2019**, *37* (9), 592–601. <https://doi.org/10.1016/j.eimce.2019.06.001>.
4. Torres, P. J.; Siakowska, M.; Banaszewska, B.; Pawelczyk, L.; Duleba, A. J.; Kelley, S. T.; Thackray, V. G. Gut Microbial Diversity in Women With Polycystic Ovary Syndrome Correlates With Hyperandrogenism. *J. Clin. Endocrinol. Metab.* **2018**, *103* (4), 1502–1511. <https://doi.org/10.1210/jc.2017-02153>.
5. Fontané, L.; Benaiges, D.; Goday, A.; Llauradó, G.; Pedro-Botet, J. Influence of the Microbiota and Probiotics in Obesity. *Clin. E Investig. En Arterioscler. Publicacion Of. Soc. Espanola Arterioscler.* **2018**, *30* (6), 271–279. <https://doi.org/10.1016/j.arteri.2018.03.004>.
6. Rondanelli, M.; Faliva, M. A.; Perna, S.; Giacosa, A.; Peroni, G.; Castellazzi, A. M. Using Probiotics in Clinical Practice: Where Are We Now? A Review of Existing Meta-Analyses. *Gut Microbes* **2017**, *8* (6), 521–543. <https://doi.org/10.1080/19490976.2017.1345414>.
7. Aguilera, M.; Gálvez-Ontiveros, Y.; Rivas, A. Endobolome, a New Concept for Determining the Influence of Microbiota Disrupting Chemicals (MDC) in Relation to Specific Endocrine Pathogenesis. *Front. Microbiol.* **2020**, *11*. <https://doi.org/10.3389/fmicb.2020.578007>
8. Ata, B.; Yildiz, S.; Turkgeldi, E.; Brocal, V. P.; Dinleyici, E. C.; Moya, A.; Urman, B. The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls. *Scientific Reports* **2019**, *9* (1), 2204. <https://doi.org/10.1038/s41598-019-39700-6>
9. Pramanick, R.; Mayadeo, N.; Warke, H.; Begum, S.; Aich, P.; Aranha, C. Vaginal Microbiota of Asymptomatic Bacterial Vaginosis and Vulvovaginal Candidiasis: Are They Different from Normal Microbiota? *Microb Pathog* **2019**, *134*, 103599. <https://doi.org/10.1016/j.micpath.2019.103599>
10. Garcia-Grau, I.; Perez-Villaroya, D.; Bau, D.; Gonzalez-Monfort, M.; Vilella, F.; Moreno, I.; Simon, C. Taxonomical and Functional Assessment of the Endometrial Microbiota in A Context of Recurrent Reproductive Failure: A Case Report. *Pathogens* **2019**, *8* (4). <https://doi.org/10.3390/pathogens8040205>
11. Moreno, I.; Garcia-Grau, I.; Bau, D.; Perez-Villaroya, D.; Gonzalez-Monfort, M.; Vilella, F.; Romero, R.; Simón, C. The First Glimpse of the Endometrial Microbiota in Early Pregnancy. *Am J Obstet Gynecol* **2020**, *222* (4), 296–305. <https://doi.org/10.1016/j.ajog.2020.01.031>
12. Perez-Muñoz, M. E.; Arrieta, M.-C.; Ramer-Tait, A. E.; Walter, J. A Critical Assessment of the “Sterile Womb” and “in Utero Colonization” Hypotheses: Implications for Research on the Pioneer Infant Microbiome. *Microbiome* **2017**, *5* (1), 48. <https://doi.org/10.1186/s40168-017-0268-4>
13. de Goffau, M. C.; Lager, S.; Sovio, U.; Gaccioli, F.; Cook, E.; Peacock, S. J.; Parkhill, J.; Charnock-Jones, D. S.; Smith, G. C. S. Human Placenta Has No Microbiome but Can Contain Potential Pathogens. *Nature* **2019**, *572* (7769), 329–334. <https://doi.org/10.1038/s41586-019-1451-5>
14. Sood, R.; Zehnder, J. L.; Druzin, M. L.; Brown, P. O. Gene Expression Patterns in Human Placenta. *PNAS* **2006**, *103* (14), 5478–5483. <https://doi.org/10.1073/pnas.0508035103>
15. Bohbot, J. M.; Cardot, J. M. Vaginal Impact of the Oral Administration of Total Freeze-Dried Culture of LCR 35 in Healthy Women. *Infect Dis Obstet Gynecol* **2012**, *2012*, 503648. <https://doi.org/10.1155/2012/503648>
16. Bhandari, P.; Prabha, V. Evaluation of Profertility Effect of Probiotic Lactobacillus Plantarum 2621 in a Murine Model. *Indian J Med Res* **2015**, *142* (1), 79–84. <https://doi.org/10.4103/0971-5916.162127>
17. Salah, R. M.; Allam, A. M.; Magdy, A. M.; Mohamed, A. S. Bacterial Vaginosis and Infertility: Cause or Association? *Eur J Obstet Gynecol Reprod Biol* **2013**, *167* (1), 59–63. <https://doi.org/10.1016/j.ejogrb.2012.10.031>
18. van Oostrum, N.; De Sutter, P.; Meys, J.; Verstraelen, H. Risks Associated with Bacterial Vaginosis in Infertility Patients: A Systematic Review and Meta-Analysis. *Hum Reprod* **2013**, *28* (7), 1809–1815. <https://doi.org/10.1093/humrep/det096>
19. Halis, G.; Arici, A. Endometriosis and Inflammation in Infertility. *Ann N Y Acad Sci* **2004**, *1034*, 300–315. <https://doi.org/10.1196/annals.1335.032>
20. Zhang, J.; Sun, Z.; Jiang, S.; Bai, X.; Ma, C.; Peng, Q.; Chen, K.; Chang, H.; Fang, T.; Zhang, H. Probiotic Bifidobacterium Lactis V9 Regulates the Secretion of Sex Hormones in Polycystic Ovary Syndrome Patients through the Gut-Brain Axis. *mSystems* **2019**, *4* (2). <https://doi.org/10.1128/mSystems.00017-19>
21. Romero, R.; Espinoza, J.; Chaiworapongsa, T.; Kalache, K. Infection and Prematurity and the Role of Preventive Strategies. *Semin Neonatol* **2002**, *7* (4), 259–274. [https://doi.org/10.1016/s1084-2756\(02\)90121-1](https://doi.org/10.1016/s1084-2756(02)90121-1)
22. Basavaprabhu, H. N.; Sonu, K. S.; Prabha, R. Mechanistic Insights into the Action of Probiotics against Bacterial Vaginosis and Its Mediated Preterm Birth: An Overview. *Microb Pathog* **2020**, *141*, 104029. <https://doi.org/10.1016/j.micpath.2020.104029>
23. Moreno, I.; Simon, C. Relevance of Assessing the Uterine Microbiota in Infertility. *Fertil Steril* **2018**, *110* (3), 337–343. <https://doi.org/10.1016/j.fertnstert.2018.04.041>
24. Krauss-Silva, L.; Moreira, M. E. L.; Alves, M. B.; Braga, A.; Camacho, K. G.; Batista, M. R. R.; Almada-Horta, A.; Rebello, M. R.; Guerra, F. A Randomised Controlled Trial of Probiotics for the Prevention of Spontaneous Preterm Delivery Associated with Bacterial Vaginosis: Preliminary Results. *Trials* **2011**, *12*, 239. <https://doi.org/10.1186/1745-6215-12-239>

140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199

25. Gardiner, G. E.; Heinemann, C.; Baroja, M. L.; Bruce, A. W.; Beuerman, D.; Madrenas, J.; Reid, G. Oral Administration of the Probiotic Combination Lactobacillus Rhamnosus GR-1 and L. Fermentum RC-14 for Human Intestinal Applications. *International Dairy Journal* **2002**, *12* (2), 191–196. [https://doi.org/10.1016/S0958-6946\(01\)00138-8](https://doi.org/10.1016/S0958-6946(01)00138-8) 200
201
202
26. Verna, E. C.; Lucak, S. Use of Probiotics in Gastrointestinal Disorders: What to Recommend? *Therap Adv Gastroenterol* **2010**, *3* (5), 307–319. <https://doi.org/10.1177/1756283X10373814> 203
204
27. Swanson, K. S.; Gibson, G. R.; Hutkins, R.; Reimer, R. A.; Reid, G.; Verbeke, K.; Scott, K. P.; Holscher, H. D.; Azad, M. B.; Delzenne, N. M.; Sanders, M. E. The International Scientific Association for Probiotics and Prebiotics (ISAPP) Consensus Statement on the Definition and Scope of Synbiotics. *Nat Rev Gastroenterol Hepatol* **2020**, *17* (11), 687–701. <https://doi.org/10.1038/s41575-020-0344-2> 205
206
207
208
28. Daliri, E. B.-M.; Lee, B. H. New Perspectives on Probiotics in Health and Disease. *Food Science and Human Wellness* **2015**, *4* (2), 56–65. <https://doi.org/10.1016/j.fshw.2015.06.002> 209
210
29. Doron, S.; Snyderman, D. R. Risk and Safety of Probiotics. *Clin Infect Dis* **2015**, *60* Suppl 2, S129–134. <https://doi.org/10.1093/cid/civ085> 211
212
30. de Goffau, M. C.; Lager, S.; Salter, S. J.; Wagner, J.; Kronbichler, A.; Charnock-Jones, D. S.; Peacock, S. J.; Smith, G. C. S.; Parkhill, J. Recognizing the Reagent Microbiome. *Nature Microbiology* **2018**, *3* (8), 851–853. <https://doi.org/10.1038/s41564-018-0202-y> 213
214
215
31. Mombelli, B.; Gismondo, M. R. The Use of Probiotics in Medical Practice. *Int J Antimicrob Agents* **2000**, *16* (4), 531–536. [https://doi.org/10.1016/S0924-8579\(00\)00322-8](https://doi.org/10.1016/S0924-8579(00)00322-8) 216
217
32. McDonald, B.D.; Jabri, B.; Bendelac, A. Diverse Developmental Pathways of Intestinal Intraepithelial Lymphocytes. *Nat Rev Immunol* **2018**, *18*, 514–525, doi:10.1038/s41577-018-0013-7 218
219
33. Ngugi, B. M.; Hemmerling, A.; Bukusi, E. A.; Kikuyi, G.; Gikunju, J.; Shiboski, S.; Fredricks, D. N.; Cohen, C. R. Effects of Bacterial Vaginosis-Associated Bacteria and Sexual Intercourse on Vaginal Colonization with the Probiotic Lactobacillus Crispatus CTV-05. *Sex Transm Dis* **2011**, *38* (11), 1020–1027. <https://doi.org/10.1097/OLQ.0b013e3182267ac4> 220
221
222
34. Fernández, L.; Cárdenas, N.; Arroyo, R.; Manzano, S.; Jiménez, E.; Martín, V.; Rodríguez, J.M. Prevention of Infectious Mastitis by Oral Administration of *Lactobacillus salivarius* PS2 During Late Pregnancy. *Clin Infect Dis* **2016**, *62*, 568–573, doi:10.1093/cid/civ974 223
224
225
35. Tomusiak, A.; Strus, M.; Heczko, P. B.; Adamski, P.; Stefański, G.; Mikołajczyk-Cichońska, A.; Suda-Szczurek, M. Efficacy and Safety of a Vaginal Medicinal Product Containing Three Strains of Probiotic Bacteria: A Multicenter, Randomized, Double-Blind, and Placebo-Controlled Trial. *Drug Des Devel Ther* **2015**, *9*, 5345–5354. <https://doi.org/10.2147/DDDT.S89214> 226
227
228
36. Kyono, K.; Hashimoto, T.; Kikuchi, S.; Nagai, Y.; Sakuraba, Y. A Pilot Study and Case Reports on Endometrial Microbiota and Pregnancy Outcome: An Analysis Using 16S rRNA Gene Sequencing among IVF Patients, and Trial Therapeutic Intervention for Dysbiotic Endometrium. *Reprod Med Biol* **2019**, *18* (1), 72–82. <https://doi.org/10.1002/rmb2.12250> 229
230
231
37. Moreno, I.; Codoñer, F. M.; Vilella, F.; Valbuena, D.; Martinez-Blanch, J. F.; Jimenez-Almazán, J.; Alonso, R.; Alamá, P.; Remohí, J.; Pellicer, A.; Ramon, D.; Simon, C. Evidence That the Endometrial Microbiota Has an Effect on Implantation Success or Failure. *Am J Obstet Gynecol* **2016**, *215* (6), 684–703. <https://doi.org/10.1016/j.ajog.2016.09.075> 232
233
234
38. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G. M.; Koenig, S. S. K.; McCulle, S. L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C. O.; Brotman, R. M.; Davis, C. C.; Ault, K.; Peralta, L.; Forney, L. J. Vaginal Microbiome of Reproductive-Age Women. *Proc Natl Acad Sci U S A* **2011**, *108* Suppl 1, 4680–4687. <https://doi.org/10.1073/pnas.1002611107> 235
236
237
39. Romero, R.; Hassan, S. S.; Gajer, P.; Tarca, A. L.; Fadrosch, D. W.; Bieda, J.; Chaemsathong, P.; Miranda, J.; Chaiworapongsa, T.; Ravel, J. The Vaginal Microbiota of Pregnant Women Who Subsequently Have Spontaneous Preterm Labor and Delivery and Those with a Normal Delivery at Term. *Microbiome* **2014**, *2*, 18. <https://doi.org/10.1186/2049-2618-2-18> 238
239
240
40. Romero, R.; Chaiworapongsa, T.; Kuivaniemi, H.; Tromp, G. Bacterial Vaginosis, the Inflammatory Response and the Risk of Preterm Birth: A Role for Genetic Epidemiology in the Prevention of Preterm Birth. *Am J Obstet Gynecol* **2004**, *190* (6), 1509–1519. <https://doi.org/10.1016/j.ajog.2004.01.002> 241
242
243
41. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D. G.; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* **2009**, *6* (7), e1000097. <https://doi.org/10.1371/journal.pmed.1000097> 244
245
42. Watson, P. F.; Petrie, A. Method Agreement Analysis: A Review of Correct Methodology. *Theriogenology* **2010**, *73* (9), 1167–1179. <https://doi.org/10.1016/j.theriogenology.2010.01.003> 246
247
43. Canese, K. An Updated PubMed Is on Its Way. NLM Technical Bulletin. 2019 Mar–Apr. Accessed January 20, 2021. https://www.nlm.nih.gov/pubs/techbull/ma19/ma19_pubmed_update.html 248
249
44. Higgins, J.P.T.; Green, S. (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. 250
251
45. López-Moreno, A.; Aguilera, M. Probiotics Dietary Supplementation for Modulating Endocrine and Fertility Microbiota Dysbiosis. *Nutrients* **2020**, *12* (3). <https://doi.org/10.3390/nu12030757> 252
253
46. Falagas, M. E.; Betsi, G. I.; Athanasiou, S. Probiotics for the Treatment of Women with Bacterial Vaginosis. *Clin Microbiol Infect* **2007**, *13* (7), 657–664. <https://doi.org/10.1111/j.1469-0691.2007.01688.x> 254
255
47. Ozkinay, E.; Terek, M.C.; Yayci, M.; Kaiser, R.; Grob, P.; Tuncay, G. The Effectiveness of Live Lactobacilli in Combination with Low Dose Oestriol (Gynoflor) to Restore the Vaginal Flora after Treatment of Vaginal Infections. *BJOG* **2005**, *112*, 234–240, doi:10.1111/j.1471-0528.2004.00329.x 256
257
258

48. Oerlemans, E. F. M.; Bellen, G.; Claes, I.; Henkens, T.; Allonsius, C. N.; Wittouck, S.; van den Broek, M. F. L.; Wuyts, S.; Kiekens, F.; Donders, G. G. G.; Lebeer, S. Impact of a Lactobacilli-Containing Gel on Vulvovaginal Candidosis and the Vaginal Microbiome. *Scientific Reports* **2020**, *10* (1), 7976. <https://doi.org/10.1038/s41598-020-64705-x> 259
260
49. Mastromarino, P.; Macchia, S.; Meggiorini, L.; Trinchieri, V.; Mosca, L.; Perluigi, M.; Midulla, C. Effectiveness of Lactobacillus-Containing Vaginal Tablets in the Treatment of Symptomatic Bacterial Vaginosis. *Clin Microbiol Infect* **2009**, *15* (1), 67–74. <https://doi.org/10.1111/j.1469-0691.2008.02112.x> 262
263
264
50. Bisanz, J. E.; Seney, S.; McMillan, A.; Vongsa, R.; Koenig, D.; Wong, L.; Dvoracek, B.; Gloor, G. B.; Sumarah, M.; Ford, B.; Herman, D.; Burton, J. P.; Reid, G. A Systems Biology Approach Investigating the Effect of Probiotics on the Vaginal Microbiome and Host Responses in a Double Blind, Placebo-Controlled Clinical Trial of Post-Menopausal Women. *PLoS One* **2014**, *9* (8), e104511. <https://doi.org/10.1371/journal.pone.0104511> 265
266
267
268
51. Bradshaw, C. S.; Pirodda, M.; De Guingand, D.; Hocking, J. S.; Morton, A. N.; Garland, S. M.; Fehler, G.; Morrow, A.; Walker, S.; Vodstrcil, L. A.; Fairley, C. K. Efficacy of Oral Metronidazole with Vaginal Clindamycin or Vaginal Probiotic for Bacterial Vaginosis: Randomised Placebo-Controlled Double-Blind Trial. *PLoS One* **2012**, *7* (4), e34540. <https://doi.org/10.1371/journal.pone.0034540> 270
271
272
52. Gilboa, Y.; Bar-Hava, I.; Fisch, B.; Ashkenazi, J.; Voliovitch, I.; Borkowski, T.; Orvieto, R. Does Intravaginal Probiotic Supplementation Increase the Pregnancy Rate in IVF-Embryo Transfer Cycles? *Reprod Biomed Online* **2005**, *11* (1), 71–75. [https://doi.org/10.1016/s1472-6483\(10\)61301-6](https://doi.org/10.1016/s1472-6483(10)61301-6) 273
274
275
53. Human Microbiome Project Consortium. Structure, Function and Diversity of the Healthy Human Microbiome. *Nature* **2012**, *486* (7402), 207–214. <https://doi.org/10.1038/nature11234> 276
277
54. The Human Microbiome Project Consortium. A Framework for Human Microbiome Research. *Nature* **2012**, *486* (7402), 215–221. <https://doi.org/10.1038/nature11209> 278
279
55. González, A.; Vázquez-Baeza, Y.; Knight, R. SnapShot: The Human Microbiome. *Cell* **2014**, *158* (3), 690–690.e1. <https://doi.org/10.1016/j.cell.2014.07.019> 280
281
56. Shao, X.; Cheng, H.; Zhou, J.; Zhang, J.; Zhu, Y.; Yang, C.; Di Narzo, A.; Yu, J.; Shen, Y.; Li, Y.; et al. Prenatal Exposure to Ambient Air Multi-Pollutants Significantly Impairs Intrauterine Fetal Development Trajectory. *Ecotoxicol Environ Saf* **2020**, *201*, 110726, doi:10.1016/j.ecoenv.2020.110726. 282
283
284
57. D'Argenio, V. The Prenatal Microbiome: A New Player for Human Health. *High Throughput* **2018**, *7* (4). <https://doi.org/10.3390/ht7040038> 285
286
58. Onderdonk, A. B.; Delaney, M. L.; Fichorova, R. N. The Human Microbiome during Bacterial Vaginosis. *Clin Microbiol Rev* **2016**, *29* (2), 223–238. <https://doi.org/10.1128/CMR.00075-15> 287
288
59. Gao, C.-J.; Kannan, K. Phthalates, Bisphenols, Parabens, and Triclocarban in Feminine Hygiene Products from the United States and Their Implications for Human Exposure. *Environment International* **2020**, *136*, 105465. <https://doi.org/10.1016/j.envint.2020.105465> 289
290
291
60. Cherpes, T. L.; Hillier, S. L.; Meyn, L. A.; Busch, J. L.; Krohn, M. A. A Delicate Balance: Risk Factors for Acquisition of Bacterial Vaginosis Include Sexual Activity, Absence of Hydrogen Peroxide-Producing Lactobacilli, Black Race, and Positive Herpes Simplex Virus Type 2 Serology. *Sex Transm Dis* **2008**, *35* (1), 78–83. <https://doi.org/10.1097/OLQ.0b013e318156a5d0> 292
293
294
61. Witkin, S. S.; Linhares, I. M. Why Do Lactobacilli Dominate the Human Vaginal Microbiota? *BJOG An Int J Obstet Gynaecol* **2017**, *124* (4), 606–611. <https://doi.org/10.1111/1471-0528.14390> 295
296
62. Verstraelen, H.; Senok, A. C. Vaginal Lactobacilli, Probiotics, and IVF. *Reprod Biomed Online* **2005**, *11* (6), 674–675. [https://doi.org/10.1016/s1472-6483\(10\)61683-5](https://doi.org/10.1016/s1472-6483(10)61683-5) 297
298
63. Bagga, R.; Arora, P. Genital Micro-Organisms in Pregnancy. *Front Public Health* **2020**, *8*. <https://doi.org/10.3389/fpubh.2020.00225> 299
300
64. Melgaço, A. C. C.; Blohem Pessoa, W. F.; Freire, H. P.; Evangelista de Almeida, M.; Santos Barbosa, M.; Passos Rezende, R.; Timenetsky, J.; Miranda Marques, L.; Romano, C. C. Potential of Maintaining a Healthy Vaginal Environment by Two Lactobacillus Strains Isolated from Cocoa Fermentation. *Biomed Res Int* **2018**, *2018*, 7571954. <https://doi.org/10.1155/2018/7571954> 301
302
303
65. Reid, G.; Beuerman, D.; Heinemann, C.; Bruce, A. W. Probiotic Lactobacillus Dose Required to Restore and Maintain a Normal Vaginal Flora. *FEMS Immunol Med Microbiol* **2001**, *32* (1), 37–41. <https://doi.org/10.1111/j.1574-695X.2001.tb00531.x> 304
305
66. Reid, G.; Charbonneau, D.; Erb, J.; Kochanowski, B.; Beuerman, D.; Poehner, R.; Bruce, A. W. Oral Use of Lactobacillus Rhamnosus GR-1 and L. Fermentum RC-14 Significantly Alters Vaginal Flora: Randomized, Placebo-Controlled Trial in 64 Healthy Women. *FEMS Immunol Med Microbiol* **2003**, *35* (2), 131–134. [https://doi.org/10.1016/S0928-8244\(02\)00465-0](https://doi.org/10.1016/S0928-8244(02)00465-0) 306
307
308
67. Reid, G.; Burton, J.; Hammond, J.-A.; Bruce, A. W. Nucleic Acid-Based Diagnosis of Bacterial Vaginosis and Improved Management Using Probiotic Lactobacilli. *J Med Food* **2004**, *7* (2), 223–228. <https://doi.org/10.1089/1096620041224166> 309
310
68. Gaspar, C.; Donders, G. G.; Palmeira-de-Oliveira, R.; Queiroz, J. A.; Tomaz, C.; Martinez-de-Oliveira, J.; Palmeira-de-Oliveira, A. Bacteriocin Production of the Probiotic Lactobacillus Acidophilus KS400. *AMB Express* **2018**, *8* (1), 153. <https://doi.org/10.1186/s13568-018-0679-z> 311
312
313
69. McMillan, A.; Rulisa, S.; Gloor, G. B.; Macklaim, J. M.; Sumarah, M.; Reid, G. Pilot Assessment of Probiotics for Pregnant Women in Rwanda. *PLoS One* **2018**, *13* (6). <https://doi.org/10.1371/journal.pone.0195081> 314
315
70. Anukam, K. C.; Duru, M. U.; Eze, C. C.; Egharevba, J.; Aiyebelihin, A.; Bruce, A.; Reid, G. Oral Use of Probiotics as an Adjunctive Therapy to Fluconazole in the Treatment of Yeast Vaginitis: A Study of Nigerian Women in an Outdoor Clinic. *Microbial Ecology in Health and Disease* **2009**, *21* (2), 72–77. <https://doi.org/10.1080/08910600902907475> 316
317
318

-
71. Sniffen, J. C.; McFarland, L. V.; Evans, C. T.; Goldstein, E. J. C. Choosing an Appropriate Probiotic Product for Your Patient: An Evidence-Based Practical Guide. *PLoS One* **2018**, *13* (12), e0209205. <https://doi.org/10.1371/journal.pone.0209205> 319
320
72. Sivamaruthi, B. S.; Kesika, P.; Chaiyasut, C. Effect of Probiotics Supplementations on Health Status of Athletes. *Int J Environ Res Public Health* **2019**, *16* (22). <https://doi.org/10.3390/ijerph16224469> 321
322
73. Hyman, R. W.; Herndon, C. N.; Jiang, H.; Palm, C.; Fukushima, M.; Bernstein, D.; Vo, K. C.; Zelenko, Z.; Davis, R. W.; Giudice, L. C. The Dynamics of the Vaginal Microbiome during Infertility Therapy with in Vitro Fertilization-Embryo Transfer. *J Assist Reprod Genet* **2012**, *29* (2), 105–115. <https://doi.org/10.1007/s10815-011-9694-6> 323
324
325
74. Chang, C.-J.; Lin, T.-L.; Tsai, Y.-L.; Wu, T.-R.; Lai, W.-F.; Lu, C.-C.; Lai, H.-C. Next Generation Probiotics in Disease Amelioration. *J Food Drug Anal* **2019**, *27* (3), 615–622. <https://doi.org/10.1016/j.jfda.2018.12.011> 326
327
75. Lai, K.-P.; Chung, Y.-T.; Li, R.; Wan, H.-T.; Wong, C. K.-C. Bisphenol A Alters Gut Microbiome: Comparative Metagenomics Analysis. *Environ Pollut* **2016**, *218*, 923–930. <https://doi.org/10.1016/j.envpol.2016.08.039> 328
329
76. O'Toole, P. W.; Marchesi, J. R.; Hill, C. Next-Generation Probiotics: The Spectrum from Probiotics to Live Biotherapeutics. *Nat Microbiol* **2017**, *2*, 17057. <https://doi.org/10.1038/nmicrobiol.2017.57> 330
331
77. Ma, D.; Chen, Y.; Chen, T. Vaginal Microbiota Transplantation for the Treatment of Bacterial Vaginosis: A Conceptual Analysis. *FEMS Microbiol Lett* **2019**, *366* (4). <https://doi.org/10.1093/femsle/fnz025> 332
333
78. DeLong, K.; Zulfiqar, F.; Hoffmann, D. E.; Tarzian, A. J.; Ensign, L. M. Vaginal Microbiota Transplantation: The Next Frontier. *J Law Med Ethics* **2019**, *47* (4), 555–567. <https://doi.org/10.1177/1073110519897731> 334
335
79. Lev-Sagie, A.; Goldman-Wohl, D.; Cohen, Y.; Dori-Bachash, M.; Leshem, A.; Mor, U.; Strahilevitz, J.; Moses, A. E.; Shapiro, H.; Yagel, S.; Elinav, E. Vaginal Microbiome Transplantation in Women with Intractable Bacterial Vaginosis. *Nat Med* **2019**, *25* (10), 1500–1504. <https://doi.org/10.1038/s41591-019-0600-6> 336
337
338
339
340
341