Review Article

The Impact of Female Genital Microbiota on Fertility and Assisted Reproductive Treatments

Pedro Brandão; M.D.[1,2], Manuel Gonçalves-Henriques; M.D.[3]

1 Department of Reproductive Medicine, Infertility Institute of Valencia, Valenica, Spain
2 Faculty of Medicine, University of Porto, Porto, Portugal
3 Department of Obstetrics and Gynecology, Prof. Doutor Fernando da Fonseca - Amadora Hospital, Lisbon, Portugal

Received June 2020; Revised and accepted September 2020

Abstract

Objective: To review publish data about human microbiome. It is known to modulate many body functions. In the field of Reproductive Medicine, the main question is in what extent may female genital tract microbiome influence fertility, both by spontaneous conception or after Assisted Reproductive Treatments (ART). The aim of this work is to review publish data about this matter.

Materials and methods: This is a systematic review on the effect of the microbiota of the female genital tract on human fertility and on the outcomes of ART.

Results: Fourteen articles were retrieved, concerning female lower genital tract and endometrium microbiota, including 5 case-controls studies about its impact on fertility, 8 cohort studies regarding ART outcomes and 1 mixed study. The main variables considered were richness and diversity of species, *Lactobacillus* dominance and the role of other bacteria. Results and conclusions of the various studies were quite diverse and incoherent. Despite the inconsistency of the studies, it seems that vaginal, cervical and endometrial microbiome may eventually play a role. Whether high richness and diversity of species, low amounts of *Lactobacillus spp.* or the presence of other bacteria, such as *Gardnerella spp.,* may adversely affect reproductive outcomes is not clear.

Conclusion: The influence of female genital microbiota on the ability to conceive is still unclear, due to the paucity and inconsistency of published data.

Keywords: Assisted Reproductive Techniques; Endometrium; Infertility; Microbiota; Next Generation Sequencing; Vagina

Introduction

It is estimated that bacteria constitute 1-3% of human body. The indigenous microbial communities that colonize the human body are known as microbiota, together with the environment they inhabit and their genetic profile form the microbiome (1,2). Human microbiome is highly variable between individuals and it's still unclear what extent may its interaction with eukaryotic cells have and its repercussion in health and well being. Furthermore, some parts of the human body have for long time been thought to be sterile, such as the uterus or the placenta, yet recent evidence has shown that most of them have their own low-abundance microbiome (3). Since the advent of Next Generation Sequencing (NGS) techniques, a hidden ocean of microbial diversity has been found, including some genital organs such as the uterus or the testicles, once thought to be devoid of bacteria (4).

Correspondence:
Dr. Pedro Brandão
Email: pedro.brandao@ivirma.com

Culture and microscopic based methods are not expensive, but they are highly operator dependent, time-consuming, require specific media for bacteria to grow and have a limited discriminatory power, based on morphology or biochemical reactions. Also, many bacteria are uncultivable and high abundant and fast growing bacteria may prevail resulting in unreliable conclusions (5).

Quantitative polymerase chain reaction (qPCR) is a well-established method for the detection, quantification, and typing of different microbial agents, monitoring deoxyribonucleic acid (DNA) amplification in real time through fluorescence. It's a fast, affordable and well established method, but like other sequencing techniques, it does not discriminate between viable and dead organisms. It may identify microorganisms otherwise not detectable by microscopic and/or culture methods, but when compared to NGS, it has a more limited range (6).

The 16s rRNA (ribosomal ribonucleic acid) gene has been used to identify bacteria and study bacterial phylogeny and taxonomy at a level that was not possible with culture, microscopy or qPCR. This gene is present in virtually all bacteria, remains conserved over time and it has regions of sequence conservation which can be used as target for PCR, as well as regions of variable sequencing which can be used to differentiate bacteria. Nine hypervariable variable regions (V1 to V9) are commonly used as target. The detected 16s rRNA gene is used to identify taxa defined as operational taxonomic unit (OTU). It has, though, a relatively low taxonomic resolution – usually genus-level, at the species level it may be limited (7). There are a few international databases that can be used as reference to classify bacteria based on the results of 16s rRNA targeting. (8) Alternatively to 16s rRNA, it is possible to target interspacer regions (ITS), such as 16S–23S rRNA ITS (9,10).

Whole genome sequencing (WGS) is a more advanced technique which has an unmatched ability to reliably discriminate highly related lineages of bacteria, not only at the species level, but also strains. It's based on massive genome sequencing. However, it has higher costs and requires more complex analyses. It can be useful when new lineages with no known close relatives are present, as it doesn't require a previously defined database to match results (11,12).

These techniques allow not only the identification of genera, species or even strains, but they can also measure the richness and diversity of species, within and between samples. These measures are of a great value to understand not only the number of different species – richness of species - but also the evenness of distribution of those species - the diversity of species. The most frequently used indexes are the Chao1 index for richness of species and Shannon (SDI) or Simpson's indexes for diversity of species. (13,14) The higher these indexes, the higher the richness or diversity of species. Diversity can be measured within the same site/sample - alpha diversity, or between habitats/samples – beta diversity (15).

Some parts of human microbiome remain unknown, despite all research conducted so far. The female lower genital tract, especially the vagina, is highly colonized by different species of bacteria, dominated mainly by *Lactobacillus spp*. These species produce large amounts of hydrogen peroxide and lactic acid which keep pH low, and other substances such as bacteriocins which prevent colonization by harmful bacteria (16). There is a considerable inter and intra individual variance of the vaginal microbiota (modulated by many factors such as sexual intercourse, hormonal status, stress, vaginal douching, tampons and vaginal infections), reason why researchers have defined 5 Community State Types (CST), according to the dominant species: type I is dominated by *L. crispatus*, type II *L. gasseri*, type III *L. iners*, type V *L. jensenii* and type IV is not dominated by *Lactobacillus spp.*, but by different anaerobic bacteria (such as *Gardnerella spp., Prevotella spp., Megasphera spp. or Sneathia spp.*) (17–20). The balance of different species is thought to be of upmost importance to vaginal health (21). Knowledge about cervical microbiome is a little bit more limited but it seems to be quite similar to the vagina (22).

The upper genital tract, in particular the uterus, on the other hand, has for long been considered sterile, but with the advent of NGS, recent research has focused on endometrial microbiota (EM) (4,23,24). Most of the studies acknowledge *Lactobacillus spp.* to be the dominant genus in most of the women, but many other entities have been identified, such as *Bacteroides spp., Streptococcus spp., Staphylococcus spp., Enterobacteriaceae, Pseudomonas spp., Atopobium spp., Corynebacterium spp., Bifidobacterium spp., Prevotella spp.* and others (25–27). Whether EM has any relation with VM, and similar to what happens to the latter, whether if it is modulated by external factors such as hormones, sexual intercourse or uterine diseases remain unclear (28,29). The main disadvantage of studying the

endometrial microbiota is that it requires more invasive methods. Apart from endometrial biopsy, some researchers use the embryo catheter tip or directly collect the endometrial fluid at the time of transfer with an intrauterine insemination (IUI) catheter. It seems safe to do it right before embryo transfer (ET), but some authors question if it reliably reflects the endometrial flora (30).

Microbiome is a subject of even more complexity than simple description of microorganisms based on metagenomics, it also involves the understanding of the interaction between bacteria, their three-dimensional biofilms and their interaction with human cells (31).

In spite of being a matter of debate in Reproductive Medicine field nowadays, yet not many studies have been published so far about the impact of microbiome on assisted reproductive treatments (ART) (32).

The aim of this work is to review all published data on the impact of the microbiota of the female genital tract (based only on sequencing techniques) on human fertility and the outcomes of assisted reproductive treatments.

Materials and methods

Data sources and study selection: A systematic review of all articles listed in Pubmed, SCOPUS and Cochrane Library was conducted in March 2020 using the query: (microbiome or microbiota or biofilm or 16s) and (infertility or "assisted reproductive" or "assisted reproduction" or "IVF" or "in vitro fertilization" or "intrauterine insemination"). Only original, finished research addressing human fertility or outcomes of ART were included. Reviews, case reports, case series, editorials, letters to the editor, comments, corrigenda, replies, articles of opinion, book chapters, study protocols and works on animals were excluded. Articles written in any language other than English, Portuguese, Spanish or French were included only if researchers, after being contacted, provided information in one of these languages, or a reliable translation was obtained. No limit of date was set. References of the selected articles were thoroughly reviewed in order to include other potentially related articles.

The selection of the studies was performed independently by 2 reviewers (P.B. and M.G.H.). Any inconsistency was discussed by both authors until an agreement was achieved.

Study appraisal: Of the search using the query, a total of 472 results were retrieved (Pubmed: 189, SCOPUS: 263, Cochrane Library: 20). Duplicates were removed (n=160). All articles' titles and/or abstracts were analyzed. Studies not related to the study question (n= 214), studies in animals (n=6), ongoing trials (n=6) and reviews, case reports, case series, editorials, letters to the editor, comments, corrigenda, replies, articles of opinion, book chapters and study protocols were excluded (n=48). From the 38 articles retrieved, 2 were excluded due to language and impossibility to retrieve an English version or proceed to translation (1 in Arabian and 1 in Russian); 24 articles were excluded after full text analysis either due to the absence of reference to the influence of microbiota in fertility or ART outcomes, or studies not based in NGS techniques. References search revealed 2 other studies to be included. At the end, 14 articles were selected.

The 14 articles were divided in 2 groups, according to the respective part of the reproductive tract – 10 respecting the female lower genital tract (cervix: 2 and vagina: 9) and 6 the endometrium. (Flowchart 1) Studies about the effect of microbiota in fertility (n=6) were case-control studies, and the ones about effect on ART outcomes (n=9) were cohort studies. It should be noted that the same study be included in more than one group.

This review will be divided in 2 main parts, one concerning the endometrium and the other the female lower genital tract (cervix and vagina). For each part, the impact of microbiome on fertility will be presented first, followed by the impact on reproductive outcomes after ART. Main variables analysed were: 1 – richness and diversity of species, 2 – *Lactobacillus* dominance and *Lactobacillus* various species, 3 – other species.

Tables 1 and 2 have listed all the studies included, concerning the endometrium and the inferior genital tract respectively. Tables 3 to 4 describe the main effect of each factor studied in fertility or ART outcomes, both for the endometrium and lower genital tract.

Results

Features of endometrial microbiota

Even though several factors modulate vaginal flora, such as hormonal status, endometrial microbiota was found to be stable, both inter and intra menstrual cycle. pH showed not to be a predictor of EM status. Lower rates of alpha diversity in women with *Lactobacillus spp.* dominated (LD) EM were found (lower SDI) (33,34).

Table 1: Description of included studies about the endometrial microbiota

Endometrium				
Microbiota And Infertility				
Study	**Sample**	**Aims**	**Main Results**	**Limitations**
Kyono et al. 2018 Japan Case-control and prevalence study	SAMPLE SIZE TOTAL: 109 IVF patients: 79 Non-IVF infertile: 23 Controls: 7 SAMPLE Endometrial fluid (collected by IUI catheter) LAB TECHNIQUE 16s rRNA V4 Illumina MiSeq® Greengenes database v13_8	1 – Relation between endometrial LD and infertility, in particular infertility with indication for IVF Infertility 2 – Variation of EM with menstrual cycle 3 – Description of average percentage of LD patients who achieved pregnancy 4 – Description of NLD endometrial flora	1 – Lower percentage of endometrial Lactobacillus spp. and women with LD EM in infertile patients group (especially IVF patients) 2 – EM was stable during and between menstrual cycles 3 – Median percentage of LD EM in pregnant patients was 96,5% (±34%), but 39% pregnant patients had NLD EM. 4 – Other dominant genus in NLD patients: Gardnerella, Streptococcus, Atopobium, Bifidobacterium, Sneathia, Prevotella, and Staphylococcus	Small control group Heterogeneity between groups Diversity of timing of sampling concerning menstrual cycle NR to recent use of antibiotics prior to sample collection
Kitaya et al. 2019 Japan Case-control and transversal descriptive study	SAMPLE SIZE TOTAL: 46 Cases: 28 RIF patients Controls: 18 patients no RIF SAMPLE Endometrial fluid (with a pipette during window of implantation period) LAB TECHNIQUE 16s rRNA V4 Illumina MiSeq® Greengenes database v13_8	1 - Comparison of VM and EM 2 - Relation of EM with RIF (in infertile patients)	1 –EM and VM were highly correlated. However, EM had higher: - diversity (SDI: 1,1 vs. 0,8 – p=,02) - N. of species (12.000 vs. 7.000 – p<,0001) - richness (15,3 vs. 8,6 – p<,001) 2 – No significant differences between cases and controls in percentage of patients with LD endometrium as well as the rate of detection of Gardnerella spp. Burkholderia spp. was present in the EM of 25% of the cases and no controls (p=,03)	Small sample size NR to recent use of antibiotics prior to sample collection Controls may prospectively become part of the cases in the future
Endometrium - Microbiota and Art Outcomes				
Franasiak et al. 2016 USA Cohort study	SAMPLE SIZE TOTAL: 33 patients (undergoing SET euploid blastocyst) SAMPLE Transfer catheter (Distal tip) LAB TECHNIQUE 16s rDNA V2-9 Ion 16S^{tm} Greengenes database v13_8	Relation of EM with CPR	Lactobacillus spp. and Flavobacterium spp. were the dominant species in both groups. Acinetobacter spp. and Pseudomonas spp. were the only genera with differences between groups (more frequent in pregnant group) Diversity (SDI) and richness of species (Chao1) were high and similar in both groups	Small sample size NR to recent use of antibiotics prior to sample collection Transfer catheter tip may not reflex endometrial flora No universal endometrial receptivity study

Table 1: Description of included studies about the endometrial microbiota (continue)

Endometrium				
Microbiota And Infertility				
Study	**Sample**	**Aims**	**Main Results**	**Limitations**
Moreno et al. 2016 Spain Cohort and descriptive study	SAMPLE SIZE Q1: 13 fertile women Q 2: 22 fertile women Q3: 35 candidates to IVF SAMPLE Endometrial fluid (and vaginal aspirate) LAB TECHNIQUE 16s rRNA V3-5 454 Life Sciences GS FLX+ (Roche)® Ribosomal Database Project v2.2	1 - Comparison of VM and EM 2 - Hormonal regulation of the EM 3 – Relation of EM with IVF clinical outcomes	1 – Only 7,2% of the paired samples had similar VM and EM 2 – 82% of the patients had similar EM in prereceptive and receptive phases. 3 – LD patients had higher IR (61% vs. 23%), PR (70% vs. 33%), OPR (59% vs. 13%) and LBR (59% vs. 6,7%). No relation between diversity and IR or MR. Worse outcomes if Gardnerella spp. or Streptococcus spp. were present. No relation between EM and MR.	Small sample size NR to details on sample collection No exclusion of embryo factors (PGT-a or oocyte donation) No universal number of embryos transferred
Kyono et al. 2018 Japan Cohort with small non-controlled trial and descriptive study	SAMPLE SIZE TOTAL: 92 patients (undergoing SET blastocyst) LD: 47 NLD: 45 SAMPLE Endometrial fluid (collected by IUI catheter) LAB TECHNIQUE 16s rRNA Varinos Inc®	1 – Relation between LD and pregnancy outcomes after blastocyst transfer 2 – Efficacy of treatment of NLD patients with probiotics 3 – Description of NLD flora	1A - LD defined as > 90%: no statistically significant differences in PR and MR 1B - LD defined as ≥ 80%: Higher PR and lower MR in LD group Results concerning Bifidobacterium spp. were similar. 2 – Nine patients were successfully treated with probiotics (but no differences in PR and MR) 3 - Other genus in NLD patients: Atopobium, Bifidobacterium, Gardnerella, Megasphaera, Sneathia, Prevotella, Staphylococcus and Streptococcus	Small and non controlled clinical trial about probiotics Heterogeneity between groups NR to recent use of antibiotics prior to sample collection Diversity of timing of sampling concerning menstrual cycle / IVF treatment point No exclusion of embryo factors (PGT-a or oocyte donation) No universal endometrial receptivity study NR to hypervariable region target or database
Hashimoto et al. 2019 Japan Cohort study	SAMPLE SIZE TOTAL: 99 patients (undergoing SET blastocyst) SAMPLE Endometrial fluid (collected by IUI catheter, right before embryo transfer) LAB TECHNIQUE 16s rRNA V4 Illumina MiSeq® Greengenes database v13_8	Relation between eubiotic(E)/dysbiotic(D) endometrium with IVF outcomes (Eubiosis was defined as ≥80% Lactobacillus spp. or Bifidobacterium spp.)	No differences between E and D in IR (both 53% - NS), PR (53% vs. 55% - NS) or MR (11% vs. 6% - NS). No difference in the composition of dysbiotic EM between patients who achieved pregnancy or not (dominant genera: Atopobium, Gardnerella and Streptococcus)	No exclusion of embryo factors (PGT-a or oocyte donation) No universal endometrial receptivity study

CPR: Clinical pregnancy rate, EM: Endometrial microbiota, ET: Embryo transfer, IR: Implantation rates, IUI: Intrauterine insemination, IVF: In vitro fertilization, LBR: Live birth rate, LD: Lactobacillus dominant, MR: Miscarriage rate, NLD: Non Lactobacillus dominant, NR: No reference, NS: Not significant, PGT-a: Preimplantation Genetic Test for Aneuploidies, PR: Pregnancy rate, RIF: Recurrent Implantation Failure, SDI: Shannon Index, SET: Single Embryo Transfer, VM: Vaginal microbiota

Table 2: Description of included studies about the lower genital tract microbiota

Inferior Genital Tract Microbiota And Infertility					
Study	**Sample**	**Aims**	**Main Results**	**Main Conclusions**	**Limitations**
Campisciano et al. 2016 Italy Case-control study	NUMBER OF PATIENTS TOTAL: 96 Fertile healthy: 39 Fertile with BV: 30 Infertile (idiopathic): 14 Infertile (w/ diagnosis): 13 SAMPLE Vaginal sample (5-7 days before menses) LAB TECHNIQUE 16s rDNA V3 Ion PGMT™ Vaginal 16s rDNA Ref. Database	Relation of VM with infertility, in particular idiopathic infertility	Infertile patients, especially if idiopathic infertility, had higher and richness and diversity of species. Abundance of L. gasseri, lack of L. inners and L. crispatus in VM and presence of Veillonella spp., Staphylococcus spp., Gardnerella vaginalis, Atopobium vaginae, Prevotella bivia and Ureaplasma parvum were associated with idiopathic infertility.	Idiopathic infertility was associated with abundance L. gasseri and lack of L. inners and L. crispatus in VM. Veillonella spp., Staphylococcus spp., Gardnerella vaginalis, Atopobium vaginae, Prevotella bivia and Ureaplasma parvum were associated idiopathic infertility.	Small number of infertile patients. NR to vaginal sample retrieval technique. NR to potential confounders – no baseline comparison of groups and no multivariate analysis.
Wee et al. 2017 Australia Case-control study	NUMBER OF PATIENTS TOTAL: 31 Cases (infertile): 15 Controls (fertile): 16 SAMPLE Posterior vaginal fornix Endocervical (2 independent swabs) Endometrial biopsy LAB TECHNIQUE 16s rRNA V1-3 Illumina MiSeq® Greengenes database v13_8 (qPCR - Ureaplasma spp.)	1 – Comparison of endometrial, cervical and vaginal microbiota 2 – Relation of vaginal and cervical microbiota with infertility.	The dominant microbial community was consistent in the vagina and cervix. Half of the patients had some differences between endometrial and vaginal dominant community. Infertile patients had more cervical Gardnerella vaginalis and vaginal Ureaplasma parvum (p=,04). No differences were found in richness or diversity of species.	There was consistency between endometrial, vaginal and cervical dominant flora. Cervical G. vaginalis and vaginal U. parvum were associated with history of infertility. No differences were found in richness or diversity of species.	Small sample size Heterogeneity between groups NR to recent use of antibiotics prior to sample collection Diversity of timing of sampling in respect to menstrual cycle Retrospective study – samples not collected during infertility period
Kyono et al. 2018 Japan Case-control study	NUMBER OF PATIENTS TOTAL: 109 IVF patients: 79 Non-IVF infertile: 23 Healthy controls: 7 SAMPLE Vaginal swab LAB TECHNIQUE 16s rRNA V1-V5 Illumina MiSeq® Greengenes database v13_8	Relation of VM with infertility, in particular infertility with indication for IVF	2 – No statistically significant differences between fertile and infertile patients, and between IVF and non IVF patients VM Lactobacillus spp. amount. 3 – Median percentage of LD VM in pregnant patients was 97,8%	No relation between LD in VM and fertility or indication for IVF	Small control group Heterogeneity between groups Diversity of timing of sampling concerning menstrual cycle NR to recent use of antibiotics prior to sample collection

Table 2: Description of included studies about the lower genital tract microbiota (continue)

Inferior Genital Tract Microbiota And Infertility					
Study	**Sample**	**Aims**	**Main Results**	**Main Conclusions**	**Limitations**
Graspeuntner et al. 2018 Germany Case-control study	NUMBER OF PATIENTS TOTAL: 210 Fertile: 89 Non infectious infertility: 26 Infectious infertility: 21 Female sex workers: 54 SAMPLE Cervical swabs (3 independent samples) LAB TECHNIQUE 1 – Culture 2 – PCR for main local STI 3 - 16s rRNA V3-4 Illumina MiSeq® SILVA Database	Relation of cervical microbiota with infertility, in particular infectious infertility	Cervical microbiota of infertile patients of infectious cause had less percentage of Lactobacillus spp., more diversity of species and more Gardnerella spp. L. gasseri was more frequent in infectious infertile patients, L crispatus in fertile patients and L. iners shown no differences between groups.	Cervical microbiome of patients with infectious infertility was characterized by less Lactobacillus spp., more diversity, more Gardnerella spp. L. gasseri were related to infectious infertility in contrast to L. crispatus. L. iners was stable across groups. Cervical PCR/culture, microbiota and Chlamydia serological status may be used as an algorithm to screen infectious infertility.	Small cases group NR to timing of sampling concerning menstrual cycle NR to recent use of antibiotics prior to sample collection
Amato et al. 2019 Italy Case-Control and Cohort	NUMBER OF PATIENTS TOTAL: 23 Patients undergoing IUI (Controls: Vaginal 16S rDNA Ref. Database) SAMPLE Vaginal swab (collected from posterior fornix) LAB TECHNIQUE 16s rRNA V3-4 Illumina MiSeq® Greengenes Database	1 - Relation of VM with idiopathic infertility 2 – Relation of VM with CPR after IUI	1 – No statistically significant differences between patients with idiopathic infertility and healthy controls in diversity, load of Lactobacillus spp. or Bifidobacterium spp. 2 – Lower diversity (SDI 0,8 vs.1,5 - p=,003), more LD flora (especially L. crispatus) and low Bifidobacterium spp. were associated with clinical pregnancy after IUI.	No relation between VM and idiopathic infertility. Lower diversity, more LD flora (in particular L. crispatus) and low Bifidobacterium spp. load were associated with higher CPR after IUI	Small sample size NR to timing of sampling concerning menstrual cycle NR to recent use of antibiotics prior to sample collection
Kitaya et al. 2019 Japan Case-control study	NUMBER OF PATIENTS TOTAL: 46 Cases: 28 RIF patients Controls: 18 infertile patients no RIF SAMPLE Vaginal secretion (swab of all vaginal walls, during window of implantation period) LAB TECHNIQUE 16s rRNA V4 Illumina MiSeq® Greengenes database v13_8	Relation of VM with RIF (in infertile patients)	No significant differences between cases and controls in diversity (SDI), percentage of patients with LD VM and the rate of detection of bacteria (in particular Gardnerella spp. and Burkholderia spp.)	No relationship between VM and RIF	Small sample size NR to recent use of antibiotics prior to sample collection Controls may prospectively become part of the cases in the future

Table 2: Description of included studies about the lower genital tract microbiota (continue)

Microbiota And Art Outcomes					
Hyman et al. 2012 USA Cohort study	NUMBER OF PATIENTS TOTAL: 30 SAMPLE Vaginal swab (4 different days during COS including ET day) SEQUECING 16s rDNA BigDye Terminator® Ribosomal Database Project	Relation of VM with LBR after ET	Lactobacillus spp. and Flavobacterium spp. were the dominant genus in VM of all patients, no differences between pregnant and non pregnant groups. (p=,42) Less number of bacteria (p=,034), richness (Chao1) and diversity (SDI, p=,01) in pregnant group.	Patients who achieved pregnancy had less number of bacteria, lower richness and diversity of species in WM at ET day. No differences were found in Lactobacillus spp. or Flavobacterium spp. load.	Small sample size Heterogeneity between groups (pregnant and non pregnant) Patients were submitted to routine antibiotic treatment No universal endometrial receptivity study No exclusion of embryo factors (PGT-a or donation) NR to number of embryos transferred NR to day of development of embryos at ET day NR to hypervariable region targeted
Haahr et al. 2018 Denmark Cohort study	NUMBER OF PATIENTS TOTAL: 120 Included in outcome analysis: 75 SAMPLE Vaginal swab (posterior fornix) LAB TECHNIQUE 1 - qPCR 2 -16s rRNA - V4	1 - Relation of VM with CPR and LBR after ET 2 – Comparison of qPCR and 16s rRNA for outcomes prediction	No differences in biochemical or clinical pregnancy according to the 5 CST's. Shannon index > 0,93 was associated with less clinical pregnancy and LBR. qPCR defining AVM was equally accurate compared to 16s rRNA to predict clinical pregnancy and LBR	CST's classification had no impact in pregnancy rates. Higher diversity was associated with less pregnancy rates. qPCR and 16s rRNA were equally accurate to predict pregnancy.	NR to timing of sampling concerning menstrual cycle NR to recent use of antibiotics prior to sample collection No universal endometrial receptivity study No exclusion of embryo factors (PGT-a or donation) NR to number of embryos transferred
Amato et al. 2019 Italy			See above		
Bernabeu et al. 2019 Spain Cohort study	NUMBER OF PATIENTS TOTAL: 31 Patients undergoing SET (blastocyst) after PGT-a SAMPLE Vaginal swab (collected from posterior fornix immediately before embryo transfer) LAB TECHNIQUE 16s rRNA V3-4 Illumina MiSeq® Greengenes database v13_8	Relation of VM with PR after ET	There were no statically significant differences in pregnant and non pregnant groups in alpha (SDI), beta diversity, LD flora or dominance in any bacteria (in particular Gardnerella spp.). Patients who achieved pregnancy had lower values of Chao1 index (richness of species).	Besides lower richness of species in patients who achieved pregnancy, there were no differences in diversity, Lactobacillus spp. or other bacteria abundance.	Small study sample No universal endometrial receptivity study

Table 2: Description of included studies about the lower genital tract microbiota (continue)

Microbiota And Art Outcomes					
Koedooder et al. 2019 The Netherlands Cohort study	NUMBER OF PATIENTS TOTAL: 192 Patients undergoing fresh D3 embryo transfer SAMPLE Vaginal swab (self collected by the patient before beginning IVF protocol) LAB TECHNIQUE 16-23s rRNA Interspace profiling (IS-pro)	Relation of VM with PR after ET	A load of Lactobacillus spp. < 20%, Proteobacteria spp. or Gardnerella vaginalis > 28% or L. jensenii > 35% was associated with lower PR (7 times less chance of pregnancy). L. crispatus ≥ 60% had 3 times less chance of pregnancy.	LD flora was associated with higher PR. L. crispatus, L. jensenii, Proteobacteria spp. and Gardnerella vaginalis were associated with lower PR.	Self-collected sample NR to timing of sampling concerning menstrual cycle No universal endometrial receptivity study No exclusion of embryo factors (PGT-a or donation)

AVM: Abnormal vaginal microbiota, BV: Bacterial vaginosis, CPR: Clinical Pregnancy Rate, CST: Community State Type, ET: Embryo transfer, IUI: Intrauterine insemination, IVF: In vitro fertilization, LBR: Live Birth Rate, LD: Lactobacillus dominant, NR: No reference, PGT-a: Preimplantation Genetic Test for Aneuploidies, PR: Pregnancy Rate, RIF: Recurrent Implantation Failure, SDI: Shannon Index, SET: Single Embryo Transfer, VM: Vaginal microbiota

Table 3: Impact of different microbiota on fertility and ART outcomes, according to different studies

Endometrial Microbiome And Infertility	Negative Relation with Fertility (+ Infertile Patients)	No Signficant Effect	Negative Relation with Fertility (+ Infertile Patients)
High richness of species of microbiome		Kitaya 2019 (RIF)	
High diversity of microbiome	Kitaya 2019 (RIF)		
High % of Lactobacillus spp. in microbiome		Kitaya 2019 (RIF)	Kyono 2018
Gardnerella vaginalis		Kitaya 2019 (RIF)	
Burkholderia spp.	Kitaya 2019 (RIF)		
High richness of species of microbiome		Kitaya 2019 (RIF)	
High diversity of microbiome	Kitaya 2019 (RIF)		
High % of Lactobacillus spp. in microbiome		Kitaya 2019 (RIF)	Kyono 2018
Gardnerella vaginalis		Kitaya 2019 (RIF)	
Burkholderia spp.	Kitaya 2019 (RIF)		
Endometrial Microbiome And Art Outcomes	**Negative Effect**	**No Signficant Effect**	**Positive Effect**
High richness of species of microbiome	-	Franasiak 2016 Moreno 2016	-
High diversity of microbiome	-	Franasiak 2016 Moreno 2016	-
High % of Lactobacillus spp. in microbiome	-	Franasiak 2016 Kyono 2018 (≥90%) Hashimoto 2019 (≥80%)	Moreno 2016 (≥90%) Kyono 2018 (≥80%)
Acinetobacter spp.	-	-	Franasiak 2016
Atopobium spp.	-	Hashimoto 2019	-
Gardnerella spp.	Moreno 2016	Hashimoto 2019	-
Flavobacterium spp.	-	Franasiak 2016	-
Bifidobacterium spp.	-	Kyono 2018 (≥90%) Hashimoto 2019 (≥80%)	Kyono 2018 (≥80%)
Pseudomonas spp.	-	-	Franasiak 2016
Streptococcus spp.	Moreno 2016	Hashimoto 2019	-

RIF: recurrent implantation failure (vs. infertile patients without RIF)

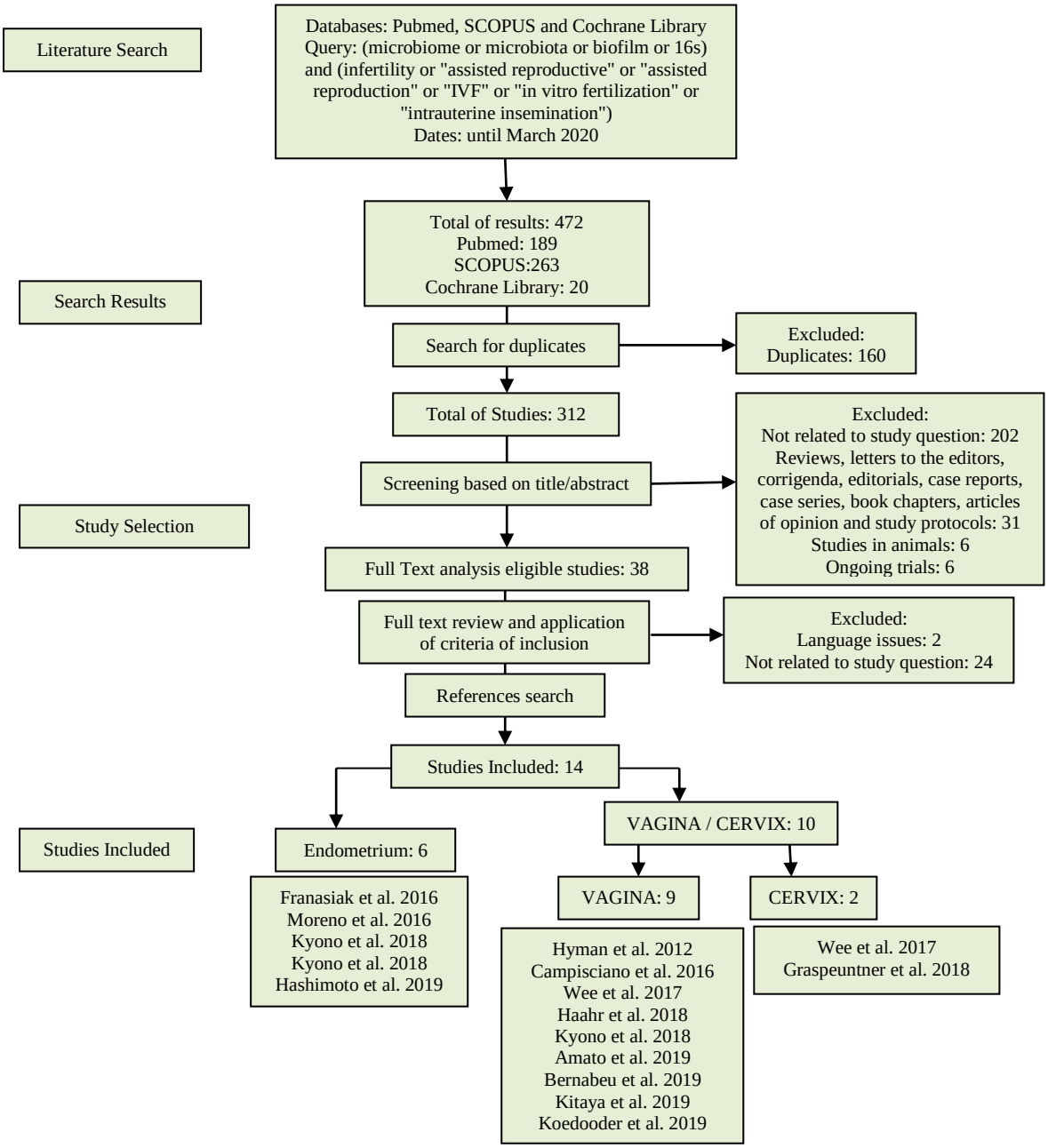
Table 4: Impact of various VM factors on fertility and ART outcomes, according to different studies

Cervical And Vaginal Microbiome And Art Outcomes	Negative Effect	No Signficant Effect	Positive Effect
High richness of species of microbiome	Campisciano 2016 (idiopathic)	Wee 2017	-
High diversity of microbiome	Campisciano 2016 Graspeuntner 2018 (infectious) (C)	Wee 2017 Amato 2019	-
High % of Lactobacillus spp. in microbiome	-	Kitaya 2017 (RIF) Kyono 2018 Amato 2019	Graspeuntner 2018 (infectious) (C)
High % of L. crispatus (CST 1)	-	-	Campisciano 2016 Graspeuntner 2018 (infectious) (C)
High % of L. gasseri (CST 2)	Campisciano 2016 (idiopathic) Graspeuntner 2018 (infectious) (C)	-	-
High % of L. iners (CST 3)	-	Graspeuntner 2018 (infectious) (C)	Campisciano 2016
High % of L. jensenii (CST 5)	-	-	-
CST 4 (diverse bacteria)	-	-	-
Ureaplasma parvum	Campisciano 2016 (idiopathic) Wee 2017	-	-
Gardnerella vaginalis	Campisciano 2016 Wee 2017 (C) Graspeuntner 2018 (infectious) (C)	Kitaya 2017 (RIF)	-
Burkholderia spp.	-	Kitaya 2017 (RIF)	-
Bifidobacterium spp.	-	Amato 2019	-
Atopobium vaginae	Campisciano 2016 (idiopathic)	-	-
Prevotella spp.	Campisciano 2016 (idiopathic) Graspeuntner 2018 (infectious) (C)	-	-
Veillonella spp.	Campisciano 2016 (idiopathic)	-	-
Staphylococcus spp.	Campisciano 2016 (idiopathic)	-	-
Sneathia spp.	Graspeuntner 2018 (infectious) (C)	-	-
Cervical And Vaginal Microbiome And Art Outcomes	**Negative Effect**	**No Signficant Effect**	**Positive Effect**
High richness of species of microbiome	Hyman 2012 Bernabeu 2019	-	-
High diversity of microbiome	Hyman 2012 Haahr 2018 Amato 2019 (IUI)	Bernabeu 2019	-
High % of Lactobacillus spp. in microbiome	-	Hyman 2012 Bernabeu 2019	Kyono 2018 Amato 2019 (IUI) Koedooder 2019
High % of L. crispatus (CST 1)	Koedooder 2019	Haahr 2018	Amato 2019 (IUI)
High % of L. gasseri (CST 2)	-	Haahr 2018	-

Table 4: Impact of various VM factors on fertility and ART outcomes, according to different studies (continue)

Cervical And Vaginal Microbiome And Art Outcomes	Negative Effect	No Signficant Effect	Positive Effect
High % of L. inners (CST 3)	-	Haahr 2018	Koedooder 2019
High % of L. jensenii (CST 5)	Koedooder 2019	Haahr 2018	-
CST 4 (diverse bacteria)	-	Haahr 2018	-
Gardnerella spp.	Koedooder 2019	Bernabeu 2019	
Bifidobacterium spp.	Amato 2019 (IIU)	-	-
Proteobacteria	Koedooder 2019	-	-
Ureaplasma spp.		Bernabeu 219	
Clostridium spp.		Bernabeu 219	
Streptococcus spp.		Bernabeu 219	

(C): Cervix | Idiopathic: refers to idiopathic infertility; Infectious: refers to infectious infertility; IUI: Intrauterine insemination; RIF: recurrent implantation failure (vs. infertile patients without RIF)



Flowchart 1: Flow diagram of study selection (according to PRISMA statement)

Whether there is any correlation between endometrial and vaginal microbiota in the same patient, is still to be defined. Studies report opposite results, some researchers founds complete inconsistency between EM and VM, others acknowledged a high level of correlation within the same woman (33–36).

Endometrial microbiota and infertility

Richness and diversity of species and fertility: Kitaya et al. compared EM of patients with history of recurrent implantation failure (RIF) and infertile patients with no history of RIF. They found a lower diversity of species in RIF patients (SDI 0,9 vs. 1,43 – p=,02), but found no significant differences in richness of species (p>,05) (35).

Lactobacillus spp. and other species and fertility: Lower amounts of endometrial Lactobacillus spp. seemed to be associated with infertility.

Kyono et al. found a lower percentage of patients with Lactobacillus dominated EM within the infertile population, especially those candidates for in vitro fertilization (IVF) (IVF 38%, non-IVF 74%, Controls

86% - p<.05). Also, these patients had a significantly lower percentage of Lactobacillus spp. in their EM (IVF 64%, infertile but non-IVF 96%, Controls 99,5% - p<.05) (33).

Respecting RIF, Kitaya et al. observed no significant differences in percentage of patients with LD endometrium (p=,13) as well as rates of detection of Gardnerella spp. (p=,53). Burkholderia spp. was present in the EM of 25% of the RIF patients but in no controls (p=,03) (35).

Endometrial microbiota and ART outcomes

Richness and diversity of species and ART outcomes: Richness and diversity of species did not show any relation with ART outcomes.

Franasiak et al. found similar high values of richness (Chao1) and diversity (SDI) of species in patients who achieved pregnancy or not, after single embryo transfer (SET) of an euploid blastocyst. Aside from these findings, Moreno et al. observed that diversity did not affect implantation rate (IR) (p=,85) or miscarriage rate (MR) (p>,32) (34,37).

Lactobacillus spp. and other species and ART outcomes: Lactobacillus dominance was found to have a different relation with fertility according to various studies – either positive or no correlation were found.

Moreno et al. reported higher rates of implantation (61% vs. 23% - p,02), pregnancy (70% vs. 33% - p,03), clinical pregnancy (CPR) (59% vs. 13% - p,02) and live birth (LBR) (59% vs. 6,7% - p,02) in patients with a Lactobacillus dominated EM (defined as a relative load ≥ 90%) compared to patients with non-Lactobacillus dominated (NLD) EM. The outcomes were worse when Gardnerella spp. or Streptococcus spp. were present in the endometrium (34).

Kyono et al., however, found no statistically significant differences in pregnancy and miscarriage rates according to Lactobacillus dominance, if this was defined as ≥ 90% of the flora, but they found higher pregnancy rates and lower miscarriage in LD patients if cut-off was reduced to 80% (PR: LD - 61%, NLD – 40% - p=,05) (33,38). Based on these findings, in a later study, they defined 2 groups – eubiotic and dysbiotic - being eubiosis characterized by an EM of at least 80% of the bacteria belonging to genera Lactobacillus or Bifidobacterium. This time, the authors found no differences in pregnancy rate, implantation rate or miscarriage rate between both groups (p>,05). Among dysbiotic patients, the most abundant genera were Atopobium, Gardnerella and Streptococcus, but their proportion didn't have any impact on PR. They reported 1 pregnancy in a patient with no Lactobacillus spp. at all in the endometrium (39).

Franasiak et al. also found high loads of Lactobacillus spp. and Flavobacterium spp. but they observed no relation with PR (p=,75 and p=,45). Acinetobacter spp. and Pseudomonas spp., in turn, were significantly more frequent in pregnant group (p=,04 and p=,004).(37) No impact of EM in miscarriage rates was described (33,34,39).

Treatment with probiotics: Kyono et al. treated NLD patients with probiotics with success, all of the 9 patients became LD, however, this had no statistically significant impact on PR, maybe due to the small sample size (38).

Vaginal / cervical microbiota and fertility

Richness and diversity of species and fertility: Results concerning richness and diversity of species in the vagina/cervix and fertility are diverse – either higher levels were associated with infertility or no association was found.

In respect of the vagina, Campisciano et al. found that infertile patients (especially those with idiopathic infertility) had higher richness and diversity of species than healthy controls (Chao1: Control – 419, Idiopathic – 579 - p<,05; Simpson's index: Control - 1,5, Idiopathic – 2,4, Infertile – 2,6 – p<,05) (40). In contrast, Amato et al. found no statistically significant differences in diversity between infertile patients and controls (41). Likewise, Kitaya et al. found no differences in diversity between patients with history of RIF showed compared to other infertile patients (35).

As concerns cervical microbiome, Graspeuntner et al. showed that the diversity (Simpson's index) was significantly and progressively higher from fertile patients - 0,21, to patients with non-infectious infertility (nIF) - 0,52, patients with infectious fertility (IIF) - 0,57 and female sex workers (FSW) - 0,69 (p<,05). They included in the infectious infertility group patients with history of pelvic inflammatory disease with or without tubal occlusion (42). Another study found no differences in cervical microbiome richness or diversity of species between fertile and infertile patients, maybe due to its small sample size (36).

Lactobacillus spp. and fertility: Vaginal / cervical Lactobacillus spp. influence on fertility was unclear. Broadly, L. crispatus and L. iners were more frequent in fertile population and L. gasseri in infertile patients.

Unlike the results with the endometrium, Kyono et al. found no correlation between Lactobacillus dominance in the vagina and fertility (33). Kitaya et

al. also reported no relation between vaginal LD and history of RIF (35).

At the species level, Campisciano et al. reported that L. gasseri was more abundant in infertile patients, especially those with idiopathic infertility, On the other hand, L. inners and L. crispatus were more common in controls .The authors suggest that it's the synergic action of different bacteria together with the imbalance of Lactobacillus spp. flora in disfavour of L. iners and L. crispatus that may be a cause for some of the idiopathic infertility, rather than isolated bacteria dominance (40). Amato et al. found a similar trend but with no statistical significance, maybe due to the small size of the sample (41).

Concerning cervical microbiome, Graspeuntner et al. found that the percentage of Lactobacillus spp. was significantly higher in fertile patients - 78% and non-infectious infertility - 69%, when compared to infectious infertility - 58% and FSW -42%. At the species level, significant differences were found: L. gasseri was more frequent in infectious infertility, L. inners was stable across groups, while L. crispatus was more frequent in controls and non-infectious infertility (42).

Other species and fertility: Ureaplasma parvum (especially patients with idiopathic infertility), Gardnerella vaginalis, Atopobium vaginalis, Veillonella spp. and Staphylococcus spp. were more frequent in VM of infertile patients (36,40,42). No differences were found in Bifidobacterium spp. composition of VM between infertile and healthy patients (41).

No relation was found between rates of detection of various other bacteria and RIF, in particular Gardnerella spp. or Burkholderia spp (35).

Regarding cervical microbiome, the relative count of Gardnerella spp. was similar in fertile and patients with non-infectious infertility, but patients with infectious infertility had the double (p<,05). A similar trend was observed with genera Prevotella and Sneathia (42).

Algorithms for predicting fertility: Graspeuntner et al. proposed a model to diagnose infectious cases of infertility, using cervical PCR or culture results addressing sexually transmitted infections (STI), Serologic status of Chlamydia trachomatis and the first 10 taxa more abundant in cervical microbiome sequencing. Based on their data, the model could accurately predict most of the cases of infectious infertility, but further assessment is need to validate these findings (42).

Vaginal / cervical microbiota and art outcomes

Richness and diversity of species and ART outcomes: Overall, lower richness and diversity of species in VM have been associated with higher PR after ART.

Amato et al. reported lower diversity in VM in patients who achieved pregnancy after IUI (mean SDI of 1,5 in pregnant group and 0,8 in non-pregnant group p=,003) (41). Likewise, Haahr et al. found that a Shannon index higher than 0,93 in VM was associated with less clinical pregnancy and LBR after IVF (odds ratio of pregnancy = 0,1 - p=,01) (43). Hyman et al. reported lower richness and diversity of species (Chao1 index and SDI – p=,001, respectively) in the group with live birth (44). Bernabeu et al. revealed a lower richness of species (p=,04) in VM in patients who achieved pregnancy after SET (euploid embryos), but they found no differences in alpha or beta diversity (p=,09), maybe due to the small sample size (45).

Lactobacillus spp. and ART outcomes: Data concerning the role of Lactobacillus dominance and the various Lactobacillus spp. in modulating ART outcomes is inconsistent.

Amato et al. found that IUI failure was more frequent in patients with less Lactobacillus spp (41).

Regarding patients undergoing FIV/ICSI (intracytoplasmic sperm injection), results are somewhat incoherent. Koedooder et al. studied 192 patients undergoing fresh embryo transfer and showed that a low relative load of Lactobacillus spp. (<20%) was associated with lower PR. (46) In Kyono et al. study, patients who achieved pregnancy had apparently a high average percentage of Lactobacillus spp. in VM (97,8%), but no comparison was made to non pregnant patients (33). On the contrary, Hyman et al. had previously found no relation between the load of Lactobacillus spp. and LBR (p=,42), with high levels of vaginal Lactobacillus spp. in both groups (pregnant and non pregnant). Bernabeu et al. had similar results (p=,2) (44,45).

At the species level, according to Koedooder et al., the percentage of women who did not achieved pregnant differed according to the CST group: CST 3 - 55,4%, CST2 - 62,5%, CST1 - 68,3%, CST4 - 70,8% and CST5 - 100%. They reported that high relative loads of L. jensenii (> 35%) or L. crispatus were associated with poor reproductive outcome. Patients with L. crispatus relative load ≥ 60% had poorer IVF outcomes (24% of patients with this profile got pregnant compared to 53% in the opposite group – p=,0003). That is to say that women with a low L. crispatus load had a one and a half times higher chance to become pregnant after the first fresh

ET, while women with a high L. crispatus profile had a third times lower chance of becoming pregnant compared to the overall pregnancy rate. In contrast, women with a relative load of L. iners ≥ 60% had 50% chance of getting pregnant (vs. an overall rate of 35%). (Koedooder et al. 2019) Other researchers, though, had opposite results. Haahr et al. observed no differences in biochemical or clinical pregnancy rates according to CST in vaginal microbiome (43) Amato et al. found better outcomes in patients with dominance of L. crispatus IUI cycles. They acknowledge L. crispatus as the species that mostly differentiated the VM between IUI successful and non successful groups (p=,0002). Contradicting Koedooder et al., these authors pointed vaginal L. crispatus as a potential promoter of favourable environment for pregnancy (41).

Other species and ART outcomes: A correlation between Bifidobacterium spp. in VM and worse IUI outcomes was found (41). Likewise, Koedooder et al. observed poorer IVF outcomes with high relative loads of Proteobacteria. They found the same relation with a load of Gardnerella vaginalis > 20%. However, Bernabeu et al. found no statistically significant association (p=,11). (45,46)

The presence of Ureaplasma spp., Clostridium spp. or Streptococcus spp. revealed no statistically significant effect on ART outcomes (45).

Algorithms for predicting ART outcomes: In order to predict ART outcomes, Haahr et al. proposed the concept of abnormal vaginal microbiota (AVM) based on the rates of G. vaginalis, A. vaginae and Lactobacillus spp. (L. crispatus, L. inners, L. gasseri and L. jensenii) by qPCR. They concluded that this was as accurate as deep microbiome analysis based on 16s rRNA (43).

Koedooder et al. propose a predicting algorithm based on 3 factors: patients with relative Lactobacillus load<20%, relative load of L. jensenii > 35%, presence of G. vaginalis or Proteobacteria > 28% of total bacterial load would be classified as patients with unfavourable profile. According to the same study, these patients had a seven times lower chance of achieving pregnancy compared to women who had a favourable vaginal microbiome profile. This model had very good specificity (97%) but low sensitivity (26%) (46).

Discussion

Microbiota has shown to have an important role in regulating many of human body functions. If so, it would be logical to think that endometrial microbiota would have an impact on fertility and reproductive outcomes, in particular those related to ART.

It's not clear whether the EM richness or diversity of species have an impact in fertility. However, infertility may somehow be linked to the endometrial load of Lactobacillus spp., as a lower percentage of Lactobacillus spp. was found in this population (33). No relation was found between EM and RIF (35).

Concerning the impact of the EM on ART clinical outcomes, richness and diversity of species shown no relation at all. Regarding Lactobacillus spp., one group found that an endometrial load of Lactobacillus spp. above 90% was associated with higher pregnancy rates (34). Thereafter, another group found differences in PR only if this cut-off was reduced to 80%, suggesting that this would be the minimal value of Lactobacillus spp. (together with Bifidobacterium spp.) to achieve optimal ART outcomes (33). However, the same group redid the study with a slightly bigger sample and found no differences in PR. The same happened with other bacteria – G. vaginalis, A. vaginae, Streptococcus spp. and Burkholderia spp (39).

Treatment of NLD patients with probiotics was successful converting their EM to LD but it had no impact on ART outcomes. One must be aware that this was based in a non controlled trial with a very small sample (38).

In spite of the higher number of studies about the VM (probably because vaginal sampling is less invasive compared to endometrium), in some points data is incoherent.

Data regarding richness and diversity of species of the VM is inconsistent, either pointing an adverse effect of high levels of this features on fertility and ART outcomes, or pointing no association at all.

Concerning the total amount of Lactobacillus spp. in VM, no conclusion may be drawn as well. Apparently the load of Lactobacillus spp. in VM did not show any relationship with infertility (35,40,42). The only study with IUI showed better results in patients with higher levels of Lactobacillus spp (41). relative load < 20% as a predicting factor of bad outcomes, or reporting no significant association at all between ART outcomes and Lactobacillus spp. load in VM (46,47). Studies evaluating IVF/ICSI results had different results, either pointing a Lactobacillus spp.

At the species level, the incoherence between studies was even higher. Koedooder et al. found statistically significant differences between CST

groups in VM and pregnancy rates; Haahr et al., however, found no association between these variables and ART outcomes. The former group also reported that patients with VM dominated by L. crispatus or L. jensenii had significantly worse results (46,47). In total conflict with these statements, Amato et al. found that L. crispatus was the species associated with better outcomes (41).

Regarding other genera of bacteria, Gardnerella spp. in the vagina, in particular G. vaginalis, tended to have a negative effect on fertility and ART outcomes. (46) Other entities such as Ureaplasma parvum, Atopobium vaginalis, Veillonella spp. and Staphylococcus spp. may also have a negative impact on fertility but the evidence was lower (40). Concerning ART outcomes, a possible negative effect of Bifidobacterium spp. and Proteobacteria was pointed (41).

In respect to cervical microbiome, it seems that it may be predictor of infertility of infectious cause, but its direct impact on fertility is unclear (42).

Finally, some authors proposed algorithms to predict infectious infertile or ART outcomes based on Lactobacillus loads and dominant Lactobacillus species as well as other potentially detrimental species. Based on their own results and the analysis of this review, it seems hasty and somewhat inappropriate to consider them at this point (43,46).

There are some important limitations that must be noted. The number of studies addressing genital microbiota, fertility and ART outcomes is still low. Most of the studies were based in small samples - the largest study about the endometrium had 109 patients, including controls.

There was a considerable variation between the methods used to quantify results, either concerning microbiota - diversity (using different indexes), Lactobacillus dominance (some used percentage of Lactobacillus spp., others used percentage of women with LD microbiota), number and type of species considered – or related to the outcomes – some addressed RIF, others infectious infertility (which has not a clear definition). Some groups weren't able to assure homogeneity between cases and controls regarding diverse variables, such as age or sexual habits, and some studies did not have into account many confounding factors such as gynaecological history, cause of infertility or recent use of antibiotics.

The sampling methodology was not always well defined, in particular with respect to the timing of collection of samples (time point of fertility treatment or menstrual cycle). Even though the EM seems to be stable over time, it would be preferable and certainly more accurate to study EM always at the time of embryo transfer. Most of the authors reinforce that a careful endometrial sampling was performed in order to avoid contamination by cervical or vaginal microbiota, but in fact that's impossible to assure with a transcervical sampling.

The laboratory methodology was quite variable between studies. Researchers used different kits, targeting different hypervariable regions and using different background databases.

The evidence of the effect of microbiota on fertility was all based in retrospective case controls studies. In most of the studies, samples were collected in patients that had suffered infertility in the past, not during the time patients were facing fertility problems.

Most of the studies concerning ART outcomes did not had into account 4 factors of upmost importance - the quality of the embryos (either by PGT-a or based on cycles with oocyte donation), the day of embryo development at transfer, the endometrial receptivity (e.g. ERA test ®) and the number of embryos transferred.

The main limitation was the incoherence between conclusions of most of the studies.

This review has its own limitations. Two studies could not be considered due to language issues. Only a systematic review was performed, without metanalysis, because the paucity of data, the small size of samples, potential bias associated with some studies and especially the different variables considered by different authors limits the interest of a metanalysis.

Besides all the limitations described, with this review it is possible to conclude that the impact of female genital microbiome in fertility, and consecutively in ART outcomes, is still unclear. Few studies until date had addressed this matter, most of them with considerable bias and based on small samples. Due to the paucity of evidence and the incoherence of the results of the various studies, it's still not possible to firmly state the influence of genital microbiota in fertility and ART outcomes.

Conclusion

Despite the inconsistency of the studies, it seems that vaginal, cervical and endometrial may eventually play a role. Whether high richness and diversity of species, low amounts of Lactobacillus spp. or the presence of other bacteria, such as Gardnerella spp., may adversely affect reproductive outcomes, is not clear.

In future, it would interesting to direct research

not only to the merely description of microbiota, but also the interaction between microbes, the formation of biofilms and the interaction of microorganisms with human cells, to be able to fully understand the role of microbiome.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The authors have no conflict of interests to report.

References

1. Young VB. The role of the microbiome in human health and disease: An introduction for clinicians. BMJ 2017; 356: j831.
2. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. Microbiome 2015; 3: 31.
3. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014; 6: 237ra65.
4. Tita A, Cliver S, Goepfert A, Goldenberg R, Conner M, Andrews W. Characteristics of the endometrial microbial flora. Am J Obstet Gynecol 2006; 195: S234.
5. Stewart EJ. Growing unculturable bacteria. J Bacteriol 2012; 194: 4151–60.
6. Bonk F, Popp D, Harms H, Centler F. PCR-based quantification of taxa-specific abundances in microbial communities: Quantifying and avoiding common pitfalls. J Microbiol Methods 2018; 153: 139–47.
7. Prince AL, Chu DM, Seferovic MD, Antony KM, Ma J, Aagaard KM. The perinatal microbiome and pregnancy: Moving beyond the vaginal microbiome. Cold Spring Harb Perspect Med 2015; 5: a023051.
8. Balvočiute M, Huson DH. SILVA, RDP, Greengenes, NCBI and OTT - how do these taxonomies compare? BMC Genomics 2017; 18: 114.
9. Moya AS. Microbiome and next generation sequencing. Rev Esp Quimioter 2017; 30: 305–11.
10. Yang B, Wang Y, Qian PY. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinformatics 2016; 17: 135.
11. Van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing Technology. Trends Genet 2018; 34: 666–81.
12. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun 2016; 469: 967–77.
13. Reese AT, Dunn RR. Drivers of Microbiome Biodiversity: A Review of General Rules, Feces, and Ignorance. MBio 2018; 9: e01294-18.
14. Hagerty SL, Hutchison KE, Lowry CA, Bryan AD. An empirically derived method for measuring human gut microbiome alpha diversity: Demonstrated utility in predicting health-related outcomes among a human clinical sample. PLoS One 2020; 15: e0229204.
15. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207–14.
16. Donders GGG. Definition and classification of abnormal vaginal flora. Best Pract Res Clin Obstet Gynaecol 2007; 21: 355–73.
17. Amabebe E, Anumba DOC. The vaginal microenvironment: The physiologic role of Lactobacilli. Front Med (Lausanne) 2018; 5: 181.
18. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 2011; 108: 4680–7.
19. Xu J, Bian G, Zheng M, Lu G, Chan W-Y, Li W, et al. Fertility factors affect the vaginal microbiome in women of reproductive age. Am J Reprod Immunol 2020; 83: e13220.
20. Borovkova N, Korrovits P, Ausmees K, Türk S, Jõers K, Punab M, et al. Influence of sexual intercourse on genital tract microbiota in infertile couples. Anaerobe 2011; 17: 414–8.
21. García-Velasco JA, Menabrito M, Catalán IB. What fertility specialists should know about the vaginal microbiome: a review. Reprod Biomed Online 2017; 35: 103–12.
22. Neal SA, Tao X, Sun L, Hanson BM, Kim JG, Osman EK, et al. High concordance between vaginal and cervical microbiome assessments with increasing microbial diversity negatively impacts pregnancy outcomes following transfer of a single euploid blastocyst. Fertil Steril 2019; 112: Poster Session, Issue 3, Supplement : E192.
23. Koedooder R, Mackens S, Budding A, Fares D, Blockeel C, Laven J, et al. Identification and evaluation of the microbiome in the female and male reproductive tracts. Hum Reprod Update 2019; 25: 298–325.
24. Moreno I, Simon C. Relevance of assessing the uterine microbiota in infertility. Fertil Steril 2018; 110: 337–43.
25. Verstraelen H, Vilchez-Vargas R, Desimpel F, Jauregui R, Vankeirsbilck N, Weyers S, et al. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. Peer J 2016; 4: e1602.
26. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore

U, Bulla R. Uterine immunity and microbiota: A shifting paradigm. Front Immunol 2019; 10: 2387.
27. Franasiak JM, Scott RT. Endometrial microbiome. Curr Opin Obstet Gynecol 2017; 29: 146–52.
28. Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. Reprod Med Biol 2018; 18: 40–50.
29. Moreno I, Franasiak JM. Endometrial microbiota—new player in town. Fertil Steril 2017; 108: 32–9.
30. Liu Y, Wong KK-W, Ko EY-L, Chen X, Huang J, Tsui SK-W, et al. Systematic Comparison of Bacterial Colonization of Endometrial Tissue and Fluid Samples in Recurrent Miscarriage Patients: Implications for Future Endometrial Microbiome Studies. Clin Chem 2018; 64: 1743–52.
31. Franasiak JM, Scott Jr RT. Introduction Microbiome in human reproduction. Fertil Steril 2015; 104: 1341–3.
32. Benner M, Ferwerda G, Joosten I, van der Molen RG. How uterine microbiota might be responsible for a receptive, fertile endometrium. Hum Reprod Update 2018; 24: 393–415.
33. Kyono K, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. Reprod Med Biol 2018; 17: 297–306.
34. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol 2016; 215: 684–703.
35. Kitaya K, Nagai Y, Arai W, Sakuraba Y, Ishikawa T. Characterization of microbiota in endometrial fluid and vaginal secretions in infertile women with repeated implantation failure. Mediators Inflamm 2019; 2019 : 4893437.
36. Wee BA, Thomas M, Sweeney EL, Frentiu FD, Samios M, Ravel J, et al. A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. Aust N Z J Obstet Gynaecol 2018; 58: 341–8.
37. Franasiak JM, Werner MD, Juneau CR, Tao X, Landis J, Zhan Y, et al. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. J Assist Reprod Genet 2016; 33: 129–36.
38. 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: 72–82.
39. Hashimoto T, Kyono K. Does dysbiotic endometrium affect blastocyst implantation in IVF patients? J Assist Reprod Genet 2019; 36: 2471–9.
40. Campisciano G, Florian F, D'Eustacchio A, Stanković D, Ricci G, De Seta F, et al. Subclinical alteration of the cervical–vaginal microbiome in women with idiopathic infertility. J Cell Physiol 2017; 232: 1681–8.
41. Amato V, Papaleo E, Pasciuta R, Viganò P, Ferrarese R, Clementi N, et al. Differential Composition of Vaginal Microbiome, but Not of Seminal Microbiome, Is Associated With Successful Intrauterine Insemination in Couples With Idiopathic Infertility: A Prospective Observational Study. Open Forum Infect Dis 2020; 7: ofz525.
42. Graspeuntner S, Bohlmann MK, Gillmann K, Speer R, Kuenzel S, Mark H, et al. Microbiota-based analysis reveals specific bacterial traits and a novel strategy for the diagnosis of infectious infertility. PLoS One 2018; 13: e0191047
43. Haahr T, Humaidan P, Elbaek HO, Alsbjerg B, Laursen RJ, Rygaard K, et al. Vaginal Microbiota and In Vitro Fertilization Outcomes: Development of a Simple Diagnostic Tool to Predict Patients at Risk of a Poor Reproductive Outcome. J Infect Dis 2019; 219: 1809–17.
44. Hyman RW, Herndon CN, Jiang H, Palm C, Fukushima M, Bernstein D, et al. The dynamics of the vaginal microbiome during infertility therapy with in vitro fertilization-embryo transfer. J Assist Reprod Genet 2012; 29: 105–15.
45. Bernabeu A, Lledo B, Díaz MC, Lozano FM, Ruiz V, Fuentes A, et al. Effect of the vaginal microbiome on the pregnancy rate in women receiving assisted reproductive treatment. J Assist Reprod Genet 2019; 36: 2111–9.
46. Koedooder R, Singer M, Schoenmakers S, Savelkoul PHM, Morré SA, De Jonge JD, et al. The vaginal microbiome as a predictor for outcome of in vitro fertilization with or without intracytoplasmic sperm injection: A prospective study. Hum Reprod 2019; 34: 1042–54.
47. Haahr T, Zacho J, Bräuner M, Shathmigha K, Skov Jensen J, Humaidan P. Reproductive outcome of patients undergoing in vitro fertilisation treatment and diagnosed with bacterial vaginosis or abnormal vaginal microbiota: a systematic PRISMA review and meta-analysis. BJOG 2019; 126: 200–7.

Citation: Brandão P, Gonçalves-Henriques M. **The Impact of Female Genital Microbiota on Fertility and Assisted Reproductive Treatments.** J Fam Reprod Health 2020; 14(3): 131-49.