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REVIEW

The impact of the female genital tract microbiome in women health and reproduction: a review

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Abstract

Purpose The aim of this review is to gather the available research focusing on female genital tract (FGT) microbiome. Research question focuses in decipher which is the role of FGT microbiota in eubiosis, assisted reproduction techniques (ARTs), and gynaecological disorders, and how microbiome could be utilised to improve reproduction outcomes and to treat fertility issues.

Methods PubMed was searched for articles in English from January 2004 to April 2021 for "genital tract microbiota and reproduction", "endometrial microbiome", "microbiome and reproduction" and "microbiota and infertility". Manual search of the references within the resulting articles was performed.

Results Current knowledge confirms predominance of *Lactobacillus* species, both in vagina and endometrium, whereas higher variability of species is both found in fallopian tubes and ovaries. Microbial signature linked to different disorders such endometriosis, bacterial vaginosis, and gynaecological cancers are described. Broadly, low variability of species and *Lactobacillus* abundance within the FGT is associated with better reproductive and ART outcomes.

Conclusion Further research regarding FGT microbiome configuration needs to be done in order to establish a more precise link between microbiota and eubiosis or dysbiosis. Detection of bacterial species related with poor reproductive outcomes, infertility or gynaecological diseases could shape new tools for their diagnosis and treatment, as well as resources to assess the pregnancy prognosis based on endometrial microbiota. Data available suggest future research protocols should be standardised, and it needs to include the interplay among microbiome, virome and mycobiome, and the effect of antibiotics or probiotics on the microbiome shifts.

Keywords Vaginal microbiome · Endometrial Microbiome · Infertility · Gynaecological disorders · Human-assisted reproduction · Next-generation sequencing

Introduction: studying the female genital tract microbiome

Synergistic relationships between microorganisms and hosts are found in nearly every niche of the human body, conditioning its physiology and physiopathology [1, 2]. Likewise, the female genital tract (FGT) — vagina, cervix, endometrium, fallopian tubes, and ovaries — harbours its own microbiome, which accounts for 9% of the total bacterial amount in female body [3]. Vaginal niche was assessed within the frame of the Human Microbiome Project (HMP) in 2007 [4–6], and most recently by the integrative HMP (iHMP), studying microbiome in pregnancy and preterm birth (PTB) [7]. The cervix, uterine cavity, fallopian tubes, and ovaries (being initially considered sterile [8]), harbour their own microbiota too [9–17]. Healthy vagina is dominated by *Lactobacillus* species, whereas the upper genital tract (UGT) harbours a less dense but high varied amount of bacterial strains [18, 19] (Fig. 1).

FGT microbiome is currently being analysed by next-generation sequencing (NGS) technologies based upon the analysis of hyper variable (V) regions of bacterial 16S ribosomal ribonucleic acid (rRNA) gene or whole metagenome sequencing (WMS). Such methods deepen at the species level

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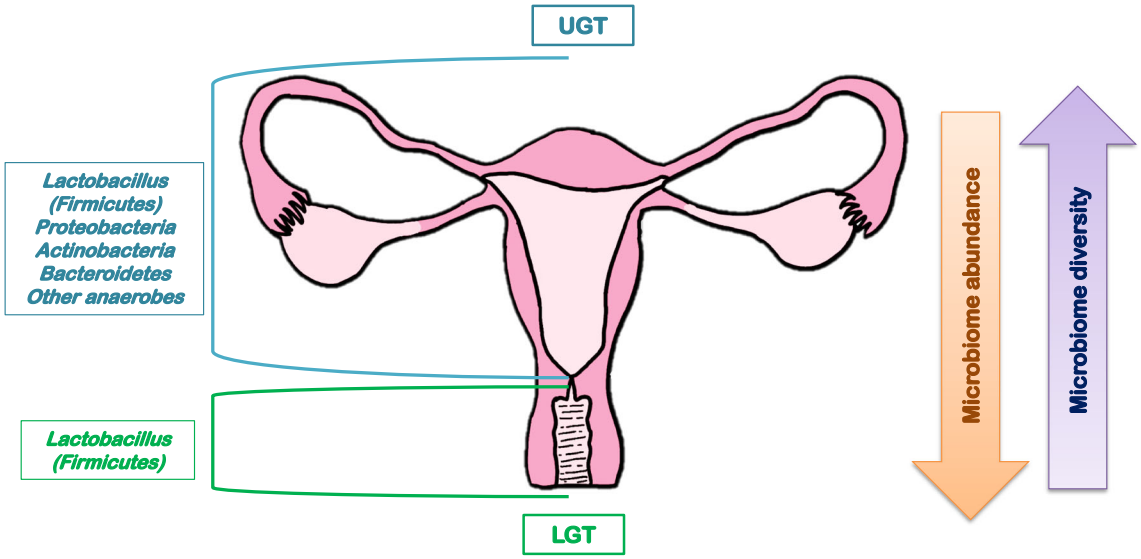

Fig. 1 Microbial composition both in upper and lower genital tract based on current knowledge. upper genital tract (UGT) comprises the cervix, the endometrium, the Fallopian tubes, and the ovaries. Lower genital tract (LGT) refers to vagina. Microbiome diversity increases from the outermost to the innermost, at the time microbial abundance decreases in the UGT compared to LGT

[20] turning the identification of bacterial DNA in metagenomic samples much accurate compared with culture-based techniques [3]. Notwithstanding, solely the presence of bacterial DNA does not mean the existence of live bacteria. For this reason, new techniques such as metatranscriptomics analyse bacterial RNA transcription processes, which may illustrate functions being accomplished in situ by particular microbial species [21, 22]. Recent studies are steadily coming up, aiming to determine the microbiome configuration and transcriptomics in specific physiological scenarios such as the different phases of the menstrual cycle [23].

From the clinical point of view, FGT microbiome is related to reproduction outcomes, gynaecological issues, or infertility [11, 12, 14–16, 20]. Understanding the FGT microbiome and its on-purpose-modulation could be useful as a clinical tool to restore the physiological state [24–26].

This review aims to bring together several FGT microbiome studies. Descriptions of eubiotic microbiome and its shifts in response to stimuli have been addressed. In addition, the microbiome role on several gynaecological disorders, reproduction, and ART has been evaluated.

Methods

PubMed was searched for articles in English from January 2004 to April 2021 for "genital tract microbiota and reproduction", "endometrial microbiome", "microbiome and reproduction", and "microbiota and infertility". Data from studies on the FGT were gathered and manual search of the references within the resulting articles was performed.

Female genital tract microbiome

From the outermost to the innermost

Vaginal microbiome

The healthy state of vaginal microbiome was studied by several groups by amplifying the 16S rRNA gene by universal primers, therefore identifying and quantifying microbial DNA in vaginal epithelium or secretions [27, 28]. Later, within the HMP frame, vaginal microbiome was characterised in healthy volunteers through 16S rRNA gene sequencing and metagenomics [4–6]. Ravel et al. depicted five community state types (CST) in vagina from healthy women, differentiated by dominant species and pH values. *L. crispatus*, *L. gasseri*, *L. iners,* and *L. jensenii* prevail in CST I, CST II, CST III, and CST V, respectively. CST IV harbours higher ratios of strictly anaerobic genera (*Prevotella*, *Dialister*, *Atopobium*, *Gardnerella*, and *Sneathia*) to the detriment of lactobacilli. Depending on the *Lactobacillus* abundance, pH average values ranged from 4.0±0.3 (CST I) to 5.3±0.6 (CST IV) [29]. This configuration was supported by Gajer and collaborators, who described the temporal dynamics of these CST in vagina. Neither variation in the composition of bacterial communities nor high levels of species diversity appeared to be related with symptoms and health misbalance in subjects [30]. Another group performed (quantitative polymerase chain reaction) qPCR using 12 different primers for bacterial DNA amplification in swab vaginal samples taken before hysterectomy, mainly detecting *Prevotella* species (spp.), *L. iners*, and *L. crispatus* [9]. Part of the research conducted by Moreno et al. analysed paired vaginal samples both from

the pre-receptive and receptive menstrual phases, showing total predominance of *Lactobacillus* in 10/13 women [11]. In 2017, Miles et al. showed predominance of *Lactobacillus*, *Streptococcus*, and *Prevotella* in vaginal samples, in agreement with previous findings [14]. Chen and colleagues considered *L. iners* and *L. crispatus* to be the vaginal microbial signature, being studied through 16S rRNA gene sequencing [13]. In line with these findings, Wee et al. depicted CST I and CST III predominance in 12/16 samples from healthy controls [31]. Another group found >97% *Lactobacillus* in healthy women (controls) [32]. Other different groups also demonstrated *Lactobacillus* dominated in vagina from healthy women, when it was sampled by using different devices or sequenced by different technological platforms [33–37].

According to current knowledge the vagina in healthy, non-pregnant women harbours high bacterial load, in which *Lactobacillus* species (spp.) prevails (Table 1).

Cervical microbiome

Likewise the vaginal canal, taking samples from the cervix, is a non-invasive process but the risk of contamination with vaginal species is higher. Table 1 summarises part of the research performed into cervical microbiome.

A study showed *Lactobacillus* (*L. crispatus*, *L. iners*) dominance in 12/13 cervical samples from healthy women (controls) and strong microbiome correlation among vaginal, cervical, and endometrial samples within an individual [31]. These findings are in accordance to previous research, which demonstrated microbiome is progressively changing from the lower genital tract (LGT) to the UGT, finding less *Lactobacillus* abundance in UGT than in LGT. In this study Lactobacilli accounted for 97.56% in cervical mucus, being *L. iners* and *L. crispatus* the most abundant species, detected through qPCR. Interestingly, intra-individual correlations between the Pouch of Douglass and cervical microbiomes were found, suggesting that cervix could be analysed to clinically draft the state of the uterus and peritoneum in the clinical realm [13]. Another publication reported that cervical mucus samples, sequenced by Illumina HiSeq (n = 25),and shotgun sequencing (n = 1), displayed *Lactobacillus* spp. dominance in cervix, as previously reported by the same group in 2017 [13, 38]. Endocervical samples were analysed by Miles et al. showing prevalence of *Lactobacillus* spp. and *Prevotella* spp. [14]. Pelzer and colleagues published *Lactobacillus* spp. was the most recovered genus in endocervical samples, followed by *Gardnerella* spp., *Veillonella* spp., *Prevotella* spp., *Sneathia* spp., or *Fusobacterium* spp. [39]. Another group examined cervical samples, publishing *Acinetobacter* was dominant (49%), followed by *Pseudomonas*, *Cloacibacterium*, and *Lactobacillus* [36]. In 2020, 67 cervix samples from women with different conditions and controls were analysed. 16S rRNA gene sequencing showed a proportion of almost 75% *Lactobacillus,* followed by >25% *Gardnerella*, *Streptococcus*, *Atopobium*, *Prevotella*, and *Pseudomonas*, among others [40]. As a brief remark, several studies have exhibited high diversity of species within cervical microbiome, along with specific genera (for instance, *Gardnerella* spp.), to be associated with human papillomavirus (HPV) risk in women [41, 42]. Such results underline the link among cervical microbiome and different gynaecological issues.

Endometrial microbiome

Endometrium harbours its unique microbiome, despite having been considered a sterile milieu for many decades (reviewed by [18, 19]). Endometrium displays a low microbial biomass, harbouring 100 to 10000 times less bacterial amount than vaginal niche [18]. Research performed on endometrium is summarised in Table 1. Several studies suggest *Lactobacillus*, if the most represented genus, indicate healthy uterine ambiance [10–12, 14, 31, 43]. Notwithstanding, there is still no consensus regarding the healthy bacterial microbiome configuration in endometrium, since some other groups have not reported *Lactobacillus* endometrial dominance [13, 17, 23, 36, 38, 44, 45].

In 2015, a cohort study assessed *L. iners*, *Prevotella* spp., and *L. crispatus* as the most recovered species in the UGT [9]. Franasiak and colleagues ascertained that *Lactobacillus* and *Flavobacterium* were the most abundant genera present in endometrial samples [10]. Another group concluded *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were dominant but *Lactobacillus* spp. could only be found in 4/19 endometrial samples [17]. Moreno et al. analysed 35 endometrial fluid samples taken from fertile women. *Lactobacillus* was the most abundant genus (71.1%), followed by *Gardnerella*, *Bifidobacterium*, *Streptococcus*, and *Prevotella* (12.6%, 3.7%, 3.2%, and 0.9%, respectively). This publication yielded a classification for samples: *Lactobacillus*-dominated (LD, >90% *Lactobacillus* spp.) and non-*Lactobacillus*-dominated microbiota (NLD, <90% *Lactobacillus* spp. plus >10% of other bacteria). This classification is valuable due to its applicability to evaluate the reproductive outcomes [25]. In 2017, Miles et al. sampled endometrial tissue from 10 subjects, concluding *Lactobacillus* was the most recovered genus [14]. Tao et al. targeted 16S rRNA gene from endometrial samples, concluding that 33/70 samples contained over 90% of *Lactobacillus*, and 50/70 samples contained over 70% of *Lactobacillus* [12]. These findings were consistent with the FGT *Lactobacillus* spp. dominance reported by Moreno et al. [11]. Conversely, Chen et al. published that *Lactobacillus* no longer dominated the endometrial milieu (that harboured 10^3 times lower biomass than vagina). *Pseudomonas* spp., *Acinetobacter* spp., *Vagococcus* spp., and *Sphingobium* spp. constituted an important fraction of

Table 1 Description of high throughput microbiome studies (mainly 16S rRNA gene sequencing, but also meta-transcriptomic) performed in the vagina, endometrium, cervix, fallopian tubes, and ovaries. Selected studies (except [52]) are performed in healthy women either undergoing surgery for non-infectious conditions or subjected for some Assisted Reproduction treatment. *AA* African American, *ART* assisted reproduction techniques, *Av.* average, *CST* community state types, *EB* endometrial biopsy, *EF* endometrial fluid, *ET* embryo transfer; *f* forward, *ICSI* intracytoplasmatic sperm injection, *IVF* in vitro fertilisation, *LD Lactobacillus* dominant, *LGT* lower genital tract, *NLD* non-*Lactobacillus* dominant, *PS* progesterone supplementation, *PTB* preterm birth, *qPCR* quantitative polymerase chain reaction, *r* reverse, *RA* Relative Abundance, *RIF* recurrent implantation failure, *RPL* recurrent pregnancy loss, *TB* term birth, *UGT* upper genital tract, *VF* vaginal fluid, *vs.* versus; *V* depicts the hyper variable region(s) of the 16S rRNA gene targeted for sequencing on each study, *yr.* years

	Publication	Study population Sample (no.) Av. age (yr.) Race/ethnicity	Diagnoses	Sampling procedure
Vagina	Zhou et al., 2004	n = 5; 28–44 yr.; Caucasian	Normal premenopausal, non-pregnant, healthy women	Mid-vaginal swab frotis
	Hyman et al., 2005	n = 20; 27–44 yr.; North American	Healthy premenopausal women, not taking contraceptive steroids, without urogenital symptoms or noticeable infections	Posterior vaginal fornix epithelium was sampled with a sterile cryoloop
	Ravel et al., 2011	n = 396; 30.6 yr.; 25% White, 26% Black, 24.5% Asian, 24.5% Hispanic	Non-pregnant women, regular menstruations, not antibiotic taken in the past 30 days	Mid-vaginal swab frotis
	Gajer et al., 2012	n = 32; reproductive age; American	Non-pregnant women	Self-collected mid-vaginal swabs and vaginal smears obtained twice-weekly for 16 weeks
	Hyman et al., 2012	n = 30 patients; n = 99 swabs; 28-44 yr.; Caucasian, Latin, Hispanic, Vietnamese, Chinese, White, Mediterranean, Asian, Japanese	IVF-ET patients with different stimulation protocols, with no cervical, uterine, or tubal infection	Vaginal swabs
	Romero et al., 2014	n = 32 non-pregnant women, n = 22 pregnant women; Reproductive age; North American	Non-pregnant women and women undergoing normal pregnancies who delivered at term (38–43 weeks)	VF samples collected by Dacron swab every four or two visits
	Mitchell et al., 2015	n = 58; 43 yr.; 79% White, 10% AA, 7% Hispanic	Women undergoing hysterectomy for non-cancer indications	Vagina flocked swab frotis collected before hysterectomy
	Digiulio et al., 2015	n = 49 divided in two groups (40 + 9); >18 yr.; North American	Pregnant women (11/40 delivered preterm, 4/9 delivered preterm)	Vaginal specimens sampled weekly until delivery and monthly for one year after delivery
	Moreno et al., 2016	n = 13 women; reproductive age; Spanish	Fertile women 2 samples/women (both from pre-receptive and receptive phases)	Vaginal aspirates with sterile catheter
	Miles et al., 2017	n = 10; 41–57 yr.; North American	Non-pregnant, non-infected women, with no immune disease. Treated with antibiotics 30min prior surgery	Vaginal samples collected with sterile swabs before surgery
	Chen et al., 2017	n = 95; 22–48 yr.; Asian	Women intervened for conditions not involving infection, cancer, or immune diseases. Not antibiotic, hormones, of vaginal/cervical treatment within a week	Vaginal nylon flocked swabs Samples taken with no prior perturbation
	Wee et al., 2018	n = 16 (cases), 37.6 yr.; n = 16 (controls), 42.75 yr. Australian	Cases: Women with history of infertility Controls: History of pregnancies with no ART procedures, healthy, hysteroscopy indication	Sterile speculum inserted into vagina, swab
	Kyono et al., 2018	n = 79 (IVF cases), 37 yr., n = 23 (non-IVF cases), 33.2 yr., n = 7 (controls), 36.6 yr.; Australian	Women enrolled for IVF treatment; infertile women not enrolled for IVF	Vaginal discharge collected with OMNIgene vaginal swab
	Brown et al., 2019	Cases: n = 38 + n = 22 subjects with high and low risk of preterm birth, 33.3 yr.; 50% Caucasian, 25% Black, 25% Asian Controls: n = 36 women with term birth; 33.8 yr.; 52% Caucasian, 21% Black, 27% Asian	Pregnant women	Vaginal swabs

Table 1 (continued)

	Liu et al., 2019	n = 50; 16–33 yr.; American	Cases: Patients with intrauterine adhesion Controls: Healthy women	Vaginal secretions sampled by swabs
	Koedooder et al., 2019	n = 192; 20–44yr.; Dutch	Fresh ET, women with no emergency IVF indications	Vaginal swabs taken prior to IVF or ICSI procedure before ET
	Kitaya et al., 2019	n = 28 (cases), 38.7 yr.; n = 18 (controls), 37.6 yr.; Asian	Cases: RIF patients; Controls: First attempt IVF patients	Vaginal discharge collected with OMNIgene vaginal swab
	Winters et al., 2019	n = 25; 45 yr.; Italian	Hysterectomy intended for fibroids (n = 23) or endometrial hyperplasia (n = 2)	Vaginal sampled by Dacron swab
	Fettweis et al., 2019	n = 135 women; 26 yr.; 78% African, 22% others	Pregnant women: n = 45 PTB (<32 weeks) + n = 90 TB (≥39 weeks)	Vaginal samples taken at 7 time points
	Bernabeu et al., 2019	n = 31 women; 40 yr.; Spanish	Women undergoing ICSI ET n = 17 achieved pregnancy, n = 14 did not achieve pregnancy	VF samples taken with a dry swab, just before the embryo transfer
	Xu et al., 2020	n = 85; 24–43 yr., Asian	Infertile women due to tubal obstruction (n = 40), normal fallopian tubes (n = 45)	Vaginal sample collected by a sterile cotton swab from the posterior fornix
	Riganelli et al., 2020	n = 24; 37 yr.; Italian	Infertile women with previous ART treatment and implantation failure	VF collected by cytobrush before starting the stimulation protocol
	Carosso et al., 2020	n = 15 35 yr. Caucasian	Women undergoing IVF with no antibiotics/probiotics/therapy	Vaginal swabs
Cervix Cervical mucus	Miles et al., 2017	n = 10 41–57 yr. North American	Non-pregnant, non-infected women, with no immune disease. Treated with antibiotics 30min prior surgery	Endocervical epithelium swabbed sterile after hysterectomy, salpingo-oopherectomy
	Chen et al., 2017	n = 95 22–48 yr. Asian	Women intervened for conditions not involved infection, cancer, or immune diseases. Not antibiotic, hormones, of vaginal/cervical treatment within a week	Nylon flocked swabs Samples taken with no prior perturbation
	Pelzer, Willner, Buttini, and Huygens, 2018	n = 145 (cases), n = 3 (virgo intacta controls); 14–52 yr.; Australian	Women with menorrhagia or dysmenorrhea undergoing hysteroscopy and/or laparoscopy	Endocervical Samples
	Wee et al., 2018	n = 10 (cases), 37.6 yr.; n = 13 (controls), 42.75 yr.; Australian	Cases: Women with history of infertility Controls: History of pregnancies with no ART procedures, healthy, hysteroscopy indication	Swabs in cervix
	Li et al., 2018	n = 137 women; 21–35 yr.; Chinese	Laparotomy or laparoscopy for non-infectious issues	Cervical mucus sampled by nylon flocked swabs
	Winters et al., 2019	n = 25; 45 yr.; Italian	Hysterectomy intended for fibroids (n = 23) or endometrial hyperplasia (n = 2)	Cervix sampled by Dacron swab
Endometrium	Mitchell et al., 2015	n = 58; 43 yr.; 79% White, 10% AA, 7% Hispanic	Women undergoing hysterectomy for non-cancer indications	Endometrial swab frotis from the contralateral endometrium
	Franasiak et al., 2016	n = 33; 35.9 yr.; 79% Caucasian , 15% Asian , 3% AA, 3% Hispanic	Women undergoing single embryo transfer of an euploide blastocyst	Distal part of IVF catheter tip
	Verstraelen et al., 2016	n = 19; 32 yr.; White Caucasian	Various reproductive conditions (RIF, RPL), with no uterine anomalies	Tao Brush TM after hysteroscopy
	Moreno et al., 2016	n = 13; reproductive age; Spanish	Fertile women 2 samples/women (both from pre-receptive and receptive phases)	EF with Wallace ET catheter
		n = 35 (cases), 25–40 yr.; n = 35 (controls), 18–35 yr.; Spanish	Cases: Infertile women undergoing IVF	
	Miles et al., 2017	n = 10; 41–57 yr.; North American	Non-pregnant, non-infected women, with no immune disease. Treated with antibiotics 30min. prior surgery	EB sampled by swabs after hysterectomy, salpingo-oopherectomy

Table 1 (continued)

Tao et al., 2017	n = 70; 36.2 yr.; 61% Caucasian, 17% Asian, 14% AA, 5.6% Hispanic	IVF patients	EB with Wallace ET catheter
Chen et al., 2017	n = 95; 22–48 yr.; Asian	Laparotomy or laparoscopy patients for conditions not involving infection, cancer, or immune diseases	Flocked nylon swabs
Pelzer, Willner, Buttini, and Huygens, 2018	n = 145 (cases), n = 3 (virgo intacta controls); 14–52 yr.; Australian	Women with menorrhagia or dysmenorrhea undergoing hysteroscopy and/or laparoscopy	EB samples taking by curette
Wee et al., 2018	n = 6 (cases), 37.6 yr.; n = 5 (controls), 42.75 yr. Australian	Cases: Women with history of infertility; Controls: History of pregnancy with no ART procedures, healthy	EB taken by endometrial curette at hysteroscopy
Kyono et al., 2018	n = 79 (IVF cases), 37 yr., n = 23 (non-IVF cases), 33.2 yr., n = 7 (controls), 36.6 yr.; Australian	Women enrolled for IVF treatment; infertile women not enrolled for IVF	EF sampled by IUI Kitazato catheter for ET
Li et al., 2018	n = 137; 21–35 yr.; Asian	Laparotomy or laparoscopy for non-infectious issues	Endometrial swab
Liu et al., 2018	n = 25; 35.2 yr.; Chinese	RPL patients	EF collected by ET catheter, endometrial tissue collected with Pipelle (paired samples)
Kyono et al., 2019	n = 92 (n = 47, LD; n = 45, NLD) 37 yr. Asian	Women who underwent frozen-thawed blastocyst transfer after endometrial microbiome analysis Antibiotics and prebiotic/probiotic supplementation in n = 9 NLD women	EF sampled by IUI Kitazato catheter for ET
Kitaya et al., 2019	n = 28 (cases), 38.7 yr.; n = 18 (controls), 37.6 yr.; Asian	Cases: RIF patients Controls: First attempt IVF patients	EF sampled by MedGyn Pipette IV
Winters et al., 2019	n = 25; 45 yr.; Italian	Hysterectomy intended for fibroids (n = 23) or endometrial hyperplasia (n = 2)	Dacron swab middle endometrial portion after hysterectomy
Younge et al., 2019	n = 10; reproductive age; North American	Women subjected for caesarean delivery. No antibiotics 48h prior intervention	Swab from the endometrial lining
Garcia-Grau et al., 2019	n = 1; 37 yr.; Spanish	Infertile RIF patient	EF sampled by Wallace ET catheter
Hashimoto and Kyono, 2019	n = 99; 35.3 yr.; Asian	IVF patients undergoing vitrified-warmed blastocyst transfer	EF sampled by Kitazato IUI catheter
Leoni et al., 2019	n = 19; 32.3 yr.; Caucasian	Women subjected for caesarean delivery at full term of normal pregnancy	5 × 5mm EB taken with new sterile scalpels
Moreno et al., 2020	n = 1; 28yr.; Spanish	Patient with primary infertility diagnostic (2 yr.) and 1 failed IVF cycle	EF transcervical aspiration by ET catheter
Riganelli et al., 2020	n = 24, infertile; 37 yr.; Italian	Women with previous ART treatment and implantation failure	EB taken by Pipelle
Kadogami et al., 2020	n = 392 (n = 216, LD; n = 176), NLD); 38.6yr.;Asian	Women diagnosed from RIF. NLD patients received different combination of oral and vaginal probiotics or oral prebiotics and antibiotics	EF sampled by Pipette IV
Carosso et al., 2020	n = 15; 35 yr.; Caucasian	Women undergoing IVF with no antibiotics/probiotics/therapy	EB taken by Guardia® Access catheter and sterile scissors
Sola-Leyva et al., 2021	n = 7; 24–31 yr.; Spanish	Women with regular menstrual cycles, no hormonal/other treatment in the last 3 months, no history of chronic disease	EBs taken by a suction curette Paired samples taken in mid-secretory and proliferative phases
Moreno et al., 2021	n = 342 patients (n = 336 EF, n = 296 EB); 36 yr.; 57.3% Caucasian, 14% East Asian , 11.4% Hispanic, 17.3% Others .	Women belonging to 13 reproductive clinics undergoing IVF (≤40yr..) or patients undertaking ovum donations (≤50yr.)	EF aspirated by flexible catheter; EB taken by a cannula of cornier

Table 1 (continued)

Fallopian tubes - Follicular fluid (ovaries)	Pelzer et al., 2011	n =71; 37 yr. (fertile), 35 yr. (infertile); Australian	Endometriosis (n = 16), PCOS (n = 14), genital tract infection (n = 9), male factor infertility (n = 18), idiopathic infertility (n = 14)	Follicular fluid collected at the time of oocyte retrieval
	Miles et al., 2017	n = 10; 41–57 yr.; North American	Non-pregnant, non-infected women, with no immune disease. Treated with antibiotics 30min prior surgery	Ampullary region sampled by swabs after hysterectomy, salpingo-oopherectomy
	Chen et al., 2017	n = 95; 22–48 yr.; Asian	Women intervened for conditions not involving infection, cancer, or immune diseases. Not antibiotic, hormones, of vaginal/cervical treatment within a week	Laparoscopy Laparotomy
	Pelzer, Willner, Buttini, Hafner, et al., 2018	n =16; 34–63 yr.; Australian	Women subjected for hysterectomy or salpingectomy/oophorectomy	Fallopian tube dissection Follicular fluid

	Analysis	Top identified phyla	Topidentified taxa and/or bacterial species	Other findings
Vagina	Big Dye version 3, 3100 PRISM Genetic analyser, universal primers 8f and 926r	*Firmicutes*	*Lactobacillus* spp. (4/5 subjects) *Atopobium* spp. (1/5 subjects)	*L. iners* (2/4 LD women), *L. crispatus* (2/4 LD women)
	Big Dye terminator, 3730 DNA analyser, universal primers 8f and 1492r	*Firmicutes*	*Lactobacillus* spp.	*Lactobacillus* spp. (10/20 subjects); *Streptococcus* spp. (2/20 subjects); *Gardnerella* spp. (1/20 subjects); *Prevotella* spp. (1/20 subjects); *Bifidobacterium* spp. (1/20 subjects)
	Roche 454 FLX V1-V2 regions	*Firmicutes*	*Lactobacillus* (different dominant species according to pH levels: lowest pH levels associated with CST I, CST III).	Vaginal microbiome classification in different CSTs CST I: *L. crispatus* CST II: *L. gasseri* CST III: *L. iners* CST V: *L. jensenii* CST IV: Anaerobes
	454 pyrosequencing V1-V2 regions (universal 27f and 338r)	*Firmicutes*	*L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III), CST IV	Vaginal microbiome classification according to CST types described by Ravel et al., 2011 Temporal dynamics of the CST: bacterial communities composition variation or high diversity levels do not necessarily imply dysbiosis
	Big Dye terminator, ABI 3730 DNA analyser, universal primers 8f and 1492r	*Firmicutes*	*Lactobacillus* spp. in 86% (85/99) swabs	3/99 swabs deficient in *Lactobacillus*. 87% *Lactobacillus* in women who had live birth, 80% *Lactobacillus* in women who did not have a live birth. *Lactobacillus* is favourable but not sufficient for successful ET
	454 FLX Titanium pyrosequencing V1-V2 (27f and 388r primers)	*Firmicutes*	*Lactobacillus* spp.	Bacterial lactobacilli-dominated CST might change from one to another within an individual undergoing a normal pregnancy. Normal pregnancy: higher abundance of *Lactobacillus vaginalis*, *L. crispatus*, *L. gasseri*, *L. jensenii*, plus lower abundance of other 22 phylotypes.

Table 1 (continued)

qPCR 12 primers* **Flavobacterium* was not tested	*Bacteroidetes*	*Prevotella spp.*(76% subjects) *L. iners* (61% subjects) *L. crispatus* (56% subjects)	
Pyrosequencing of the V3-V5 in 40 women; Illumina-based sequencing	*Firmicutes*	*L. crispatus* in stable vaginal community. *Gardnerella* and *Ureaplasma* more abundant in CST IV. Decreased *Lactobacillus* plus increased anaerobes in women with post-delivery vaginal disturbance.	CST IV (poor in *Lactobacillus*) is related with lower gestational age at delivery time
454 pyrosequencing V3-V5	*Firmicutes*	*Lactobacillus* spp.	10/13 women harboured vaginal colonisation only by *Lactobacillus* spp.
454 pyrosequencing V1-V3	*Firmicutes*	*Lactobacillus* spp. *Streptococcus* spp. *Prevotella* spp.	Microbial composition were highly related across the samples and across the subjects
Ion Torrent V5-V4 Bacterial culturing	*Firmicutes*	*L. iners L. crispatus*	Continuum microbiome changing from the vagina to the UGT
Illumina MiSeqVR V1-3 qPCR to detect *Ureaplasma*	*Firmicutes*	*Lactobacillus* spp. in 12/16 control samples; CST I (*L. crispatus*), CST III (*L. iners*), CST IV	*Ureaplasma* overrepresented in vagina of infertile women Intra-individual correlation in vagina/cervix/ endometrium
Illumina MiSeq V4	*Firmicutes*	*Lactobacillus* spp.	Average *Lactobacillus* spp.: 65.2% in IVF group, 99.4 in non-IVF group, 99.8% in controls.
V1-V2 Illumina MiSeq	*Firmicutes*	*Prevotella*, *Streptococcus*, *Peptoniphilus*, *Ureaplasma*, *Dialister* spp. Decrease in *Lactobacillus* spp. *Lactobacillus* spp. *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*	Reduced *Lactobacillus* plus increased pathogens such *Prevotella, Peptoniphilus, Streptococcus*, and *Dialister* associated with preterm birth risk and prelabor rupture of foetal membranes
V4 Illumina HiSeq 2000	*Firmicutes* (61.84%), *Actinobacteria* (24.37%), *Bacteroidetes* (8.64%), *Proteobacteria* (2.74%) *Firmicutes* (92.12%), *Actinobacteria* (5.58%), *Bacteroidetes* (0.92%), *Proteobacteria* (0.64%)	Cases: <97% *Lactobacillus* plus *Gardnerella*, *Prevotella* Controls: >97% *Lactobacillus*	Decrease in *Lactobacillus* abundance and overgrowth of *Gardnerella* and *Prevotella* in cases group. Intrauterine adhesions trigger an increase in vaginal diversity of case patients
Interespace profiling (IS-pro) molecular technique	*Firmicutes*	*Lactobacillus* spp. (classification of samples in CSTs)	High *Lactobacillus* abundance appears to be related to IVF and ICSI success >60% *L. crispatus* is not advantageous
Illumina MiSeq V4	*Firmicutes*	.*Lactobacillus* spp. Others: *Streptococcus*, *Prevotella*, *Bifidobacterium*, *Enterococcus*, *Atopobium*	Variation between RIF and controls: 19/28 (67.9%) LD in RIF vs. 8/18 (44.4%) LD in controls. EF and vaginal microbiome profiles are different
Illumina V4 qPCR for V1-V2 16S rRNA gene for *L. iners* and *L. crispatus* detection	*Firmicutes*	*Lactobacillus* spp., *Gardnerella*	
V1-V3 Illumina Miseq	*Firmicutes*	*Lactobacillus crispatus* (in TB women)	Reduced *L. crispatus* plus increased *Sneathia* and *Prevotella* in PTB women

Table 1 (continued)

	V3-V4 Illumina MiSeq	*Firmicutes*	*Lactobacillus* (92%), *Gardnerella* (5%)	Greater diversity of vaginal species is found in patients who did not achieve pregnancy
	Illumina MiSeq V4	*Firmicutes Actinobacteria Bacteroidetes*	*Lactobacillus* spp., *Gardnerella* spp., *Prevotella* spp., *Streptococcus* spp., *Atopobium* spp.	*E. coli*, *S agalactiae*, *P. intermedia* among others might be used a biomarkers for vaginal dysbiosis
	Illumina MiSeqVR V4-5	*Firmicutes*	*Lactobacillus* spp. *L. crispatus*, *L. iners*, *L. gasseri*	*Lactobacillus* spp. in pregnant women vs. not-pregnant cases. Vagina harbours less biodiversity and reducer number of species than endometrium
	Illumina MiSeqVR V3-4-6	*Firmicutes*	*Lactobacillus* spp.	*Lactobacillus* spp. (before PS.) *Lactobacillus* decrease and *Prevotella*, *Escherichia*, and *Shigella* spp. increase (after PS.)
Cervix Cervical mucus	454 pyrosequencing V1-V3	*Firmicutes*	*Lactobacillus* spp. *Prevotella* spp.	Microbial composition were highly related across the samples and across the subjects
	Ion Torrent V5-V4 Bacterial culturing	*Firmicutes*	*Lactobacillus* (RA: 97.57%) Others (RA: 2.44%)	Intra-individual continuum of bacteria that gradually changes from the outermost to the innermost
	454 pyrosequencing V5-V8	*Firmicutes*	*Lactobacillus* spp. *Gardnerella* spp. *Prevotella* spp. *Veillonella* spp. *Sneathia* spp. *Fusobacterium* spp.	*Lactobacillus* spp. dominance across all endocervical groups
	Illumina MiSeqVR V1-3	*Firmicutes*	*G. vaginalis* overrepresented in cervix of infertile women *L. crispatus, L. iners* dominance in 12/13 control samples	Intra-individual correlation vagina/cervix/endometrium
	Illumina HiSeq	*Firmicutes*	*Lactobacillus* spp.	Intra-individual continuum of bacteria that gradually changes from the outermost to the innermost
	Illumina V4 qPCR for V1-V2 16S rRNA gene for *L. iners* and *L. crispatus*	*Proteobacteria*	*Acinetobacter* spp. *Comamondaceae*, *Cloacibacterium* spp., *Pseudomonas* spp. *Lactobacillus* spp.	Relative abundance data: *Acinetobacter* = 49%, *Lactobacillus* spp. = 19.24%. Similarities between cervical and endometrial microbiomes
Endometrium	qPCR 12 primers * **Flavobacterium* was not tested	*Firmicutes Bacterioidetes*	*L. iners* (45% subjects) *L. crispatus* (33% subjects) *Prevotella* spp.(33% subjects)	95% of women harbour UGT colonisation. Endometrial cavity is not sterile in most women
	Ion 16S metagenomics V2-4-8, V3-6, V7-9	*Firmicutes Proteobacteria*	*Lactobacillus* spp.: IVF *Flavobacterium*	No microbial profile differences between successful and unsuccessful IVF
	Illumina MiSeq V1-V2	*Bacteroidetes*	*B. xylanisolvens*, *B. thetaiotaomicron*, *B. fragilis*, *Pelomonas*,	Unique microbiome dominated by *Bacteroides* in endometrium of non-pregnant women
	454 pyrosequencing. V3-5	*Firmicutes*	*Lactobacillus* spp.	9/13 women harboured endometrial colonisation exclusively by *Lactobacillus* Bacterial communities different to vagina; high proportion of *Atopobium*, *Clostridium*, *Gardnerella*, *Megasphaera*, *Parvimonas*, *Prevotella*, *Sphingomonas*, or *Sneathia*

Table 1 (continued)

	Firmicutes Actinobacteria	*Lactobacillus* spp. (RA: 71.1%), *Gardnerella* spp. (RA: 12.6%), *Bifidobacterium* spp. (RA: 3.7%), *Streptococcus* spp. (RA: 3.2%), *Prevotella* spp. (RA: 0.9%)	> 90% *Lactobacillus:* LD; < 90% *Lactobacillus:* NLD. NLD associated with poor reproductive outcome
454 pyrosequencing V1-V3	*Firmicutes*	*Lactobacillus* spp. *Corynebacterium* spp. *Staphylococcus* spp. *Acinetobacter* spp. *Blautia* spp.	Microbial composition were highly related across the samples and across the subjects
Illumina V4	*Firmicutes*	*Lactobacillus* spp., *Corynebacterium* spp., *Streptococcus*, *Staphylococcus* spp. *Bifidobacterium* spp.	*Lactobacillus* spp. was detected in all patients
Ion Torrent V5-V4	*Firmicutes*	30.6% *Lactobacillus* spp. 9.09% *Pseudomonas* spp. 9.07% *Acinetobacter* 7.39% *Vagococcus*	Endometrial microbiome cultivable 5/15 cases. Unique microbiota. Recovered biomass was 10^3 times lower in endometrium compared to vagina
454 pyrosequencing V5-V8	*Firmicutes*	*Lactobacillus* spp. *Gardnerella* spp. *Prevotella* spp. *Veillonella* spp. *Sneathia* spp. *Fusobacterium* spp. and *Jonquetella* spp. in virgo intacta subjects (n = 3)	Female upper genital tract is not sterile. *Fusobacterium* spp. and *Jonquetella* spp. normally included in the bacterial vaginosis spectrum
Illumina MiSeqVR V1-3	*Firmicutes Actinobacteria*	*Lactobacillus* dominance in 1/6 case samples *Lactobacillus* spp. *Bifidobacterium* spp. in 4/5 control samples	Need for further exploration. Lower relative abundance of species Microbiome displays lower relative abundance compared to vagina/cervix
Illumina MiSeq V4	*Firmicutes*	*Lactobacillus* spp.	Average *Lactobacillus* spp.: 63.9% in IVF group, 96.2% in non-IVF group, 99.8% in controls.
Illumina HiSeq	*Proteobacteria*	*Pseudomonas* spp., *Propionilbacterium* spp., *Streptococcus* spp., *Moraxella* spp.	Differences between LGT and UGT (*Lactobacillus* does not dominate in endometrium)
Illumina MiSeq V4	*Proteobacteria*	*Atopobium* spp., *Prevotella* spp., *Gardnerella* spp., *Bifidobacterium* spp., *Stenotrophomas* spp., *Lactobacillus* spp., *Megasphaera* spp., *Staphylococcus* spp., and *Escherichia* spp.	Authors mention EF microbiome does not reflect same configuration as tissue
Illumina MiSeq V4	*Firmicutes*	*Lactobacillus* spp., *Atopobium* spp., *Bifidobacterium* spp. *Gardnerella* spp., *Megasphaera* spp., *Sneathia* spp., *Prevotella* spp.	After intervention, 9 NLD turned LD (46 LD vs. 36 NLD) LD endometrium possibly beneficial for implantation
Illumina MiSeq V4	*Firmicutes*	*Lactobacillus* spp., *Gardnerella* spp., *Streptococcus* spp., *Prevotella* spp., *Burkholderia* spp., and *Gardnerella* spp. (RIF)	Variation between RIF and controls 18/28 (64.3%) LD in RIF vs. 7/18 (38.9%) LD in controls. *Burkholderia* only detected in EF of RIF patients
Illumina V4 qPCR for V1-V2 16S rRNA gene for *L. iners* and *L. crispatus* detection	*Proteobacteria*	*Acinetobacter* spp. *Comamondaceae*, *Cloacibacterium* spp., *Pseudomonas* spp. <1% *Lactobacillus* spp.	*Lactobacillus* does not dominate in endometrium (rarely present, does not form the microbial Core)
Illumina MiSeq V4	*Proteobacteria*	*Escherichia*, *Acinetobacter*, *Lactobacillus*, *Bacillus*	*Lactobacillus* does not dominate in endometrium

Table 1 (continued)

Ion Torrent V2-4-8, V3-6,V7, V9 NextSeq500 qPCR for *G. vaginalis*	*Actinobacteria*	*G. vaginalis*, *Lactobacillus* spp., *Pseudoalteromonas* spp., *Bifidobacterium* spp., *Rhodanobacter* spp.	*Gardnerella*, *Atopobium*, and *Bifidobacterium* persisted in endometrium
Illumina MiSeq V4	*Actinobacteria*	*Gardnerella* spp., *Atopobium* spp., *Streptococcus* spp., *Lactobacillus* spp.	IVF pregnancy rates were comparable between LD and NLD microbiome endometrial configurations (some patients achieved pregnancy with 0% *Lactobacillus*)
Illumina MiSeq V5-6	*Actinobacteria*	*Cutibacterium* spp., *Escherichia* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Streptococcus* spp., and *Corynebacterium* spp. (50% women)	*Lactobacillus* is not dominant in endometrium
Ion Torrent V2-4-8, V3-6,V7, V9) NextSeq500	*Firmicutes*	*Lactobacillus* spp., *Enterobacteriaceae* spp., *Streptococcus* spp., *Pseudomonas* spp., *Staphylococcus* spp. Before the miscarriage: *L. crispatus*	During the successful pregnancy: Lower bacterial load plus higher lactobacilli abundance in the endometrial; *L. iners* was the most abundant at early pregnancy
Illumina MiSeqVR V4-5	*Firmicutes Proteobacteria Bacteroidetes Actinobacteria*	*Kocuria dechangensis*, *L. crispatus*, *L. iners*	*Kocuria dechangensis* in women with implantation failure *Lactobacillus* spp. only in women that did not achieve pregnancy; total absence of *Lactobacillus*, presence of Lachnospiraceae and Enter obacteriaceae in the pregnant group.
Illumina MiSeq V4	*Firmicutes*	*Lactobacillus* spp., *Bifidobacterium* spp., *Gardnerella* spp., *Atopobium* spp., *Streptococcus* spp., *Prevotella* spp.,	Cure rate 78% in vaginal probiotic plus antibiotic treatments (NLD changed into LD). *Lactobacillus* endometrial proportion was <50% right after menstruation, gradually increasing to reach ~70% in luteal phase
Illumina MiSeqVR V3-4-6	*Firmicutes*	*Lactobacillus* spp., *Gardnerella* spp., *Prevotella* spp., *Propionilbacterium* spp., *Pseudomonas* spp., *Atopobium* spp., *Delftia* spp., *Pelomonas* spp., *Veillonella* spp., *Escherichia coli/Shigella*	Biodiversity increases intra individually after PS
RNAseq (meta- transcriptomics) IlluminaHiSeq 500	*Proteobacteria*	*Klebsiella pneumoniae*, *Clostridium botulinum*, and *Pasterurella multocida*	*Lactobacillus* does not seem to dominate in the endometrium from healthy women
Ion S5 XL, V2-4-8, V3-6, 7-9)	*Firmicutes*	EF and EB in patients with live birth: *Lactobacillus* spp. EF in patients with IVF failure: *Streptococcus* spp., *Chryseobacterium* spp., *Corynebacterium* spp., *Haemophilus* spp., *Bifidobacterium* spp., *Staphylococcus* spp., *Atopobium* spp., *Gardnerella* spp., *Propionilbacterium* spp. EB in patients with IVF failure: *Gardnerella* spp., *Klebsiella* spp., *Atopobium* spp., *Finegoldia* spp., *Escherichia* spp., *Propionilbacterium* spp., *Haemophilus* spp., *Anaerococcus* spp., and *Bacillus* spp.	Only in EF: *Streptomyces*, *Clostridium*, *Chryseobacterium* Only in EB: *Cupriavidus, Escherichia*, *Klebsiella*, *Bacillus*, *Finegoldia*, *Micrococcus*, and *Tepidomonas*

Table 1 (continued)

Fallopian tubes - Follicular fluid (ovaries)	Culture-based methods; 16S rRNA conventional PCR	*Firmicutes*	*L. iners, Actinomyces* spp., *Corynebacterium auromuosum, Fusobacterium* spp., *Prevotella* spp., *Staphylococcus* spp.	Left ovary displays greater colonisation than the right ovary
	454 pyrosequencing V1-V3	*Firmicutes Proteobacteria Actinobacteria Bacteroidetes*	Ovaries: *Lactobacillus* spp., *Corynebacterium* spp., *Escherichia* spp., *Blaudia* spp. Fallopian tubes: *Bacterioides* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Coprococcus* spp., *Hymenobacter* spp.	Microbial composition were highly related across the samples and across the subjects
	Ion Torrent V5-V4	*Proteobacteria*	*Acinetobacter* spp. *Comamonas* spp. *Pseudomonas* spp. *Dysgonomonas* spp. *Vagococcus* spp.	*Lactobacillus* is only found with a RA:1.69% at fallopian tubes
	454 pyrosequencing V5-V8	*Firmicutes*	*Staphylococcus* spp. *Enterococcus* spp. *Lactobacillus* spp. *Pseudomonas* spp. *L. iners, Actinomyces* spp., *C. auromuosum, Fusobacterium* spp.	*Lactobacillus* is not present in all subjects All samples harboured any kind of colonisation (positive for any bacterial species)

he endometrial microbiota [13]. Wee and colleagues examined endometrial microbiome in a case-control study. Controls (healthy fertile women, n = 5) were mostly represented by *Lactobacillus* and *Bifidobacterium*, and endometrial microbiome had lower relative abundance compared to vagina and cervix samples [31]. Another group performed endometrial microbiome shotgun sequencing, identifying *Pseudomonas*, *Propionilbacterium*, *Streptococcus,* and *Moraxella* as the top recovered taxa [38]. Kyono et al. collected endometrial fluid samples: 47 patients were LD (>90% *Lactobacillus*) and 45 were NLD (<90% *Lactobacillus* plus >10% of other bacteria). Recovered genera were *Lactobacillus*, *Atopobium*, *Bifidobacterium*, *Gardnerella*, *Megasphaera*, *Sheathia*, and *Prevotella* [43]. Another study identified *Lactobacillus*, *Pseudomonas*, *Streptococcus*, and *Atopobium* in healthy women (n = 2) [40]. Winters' group showed *Acinetobacter* (44%), followed by *Escherichia* or *Pseudomonas*, were dominant in endometrium, where <1% *Lactobacillus* was found [36]. Most recently, Sola-Leyva et al. studied samples from 7 women, in order to decipher the core microorganism composition in healthy endometrium of young women. Meta-RNA sequencing showed that *Klebsiella pneumoniae*, *Clostridium botulinum*, and *Pasterurella multocida* were the three most abundant bacterial species in endometrial biopsies, where *Lactobacillus* was not dominant [23].

Regarding UGT colonisation mechanisms, several hypotheses are put forward. UGT microbiome may derive from bacteria which ascend from the vagina, either directly or attached to the semen [46]. Communication between vagina and uterus would be facilitated or inhibited by peristaltic contractions, cervical fluid consistency, cervical folds, or the immune response [36, 47]. A publication showed that women with bacterial vaginosis (BV) had increased UGT bacterial load [48]. Conversely, other authors suggested each site displays its own community, since some vaginal species were not found in the endometrium and vice-versa [11, 16, 39]. Another hypothesis defends that UGT colonisation might arise by hematogenous spreading from gastrointestinal, airways, or oral niches [49, 50]. Finally, certain gynaecological procedures could transfer microbial load from vagina to the uterus [49].

Fallopian tubes and ovarian microbiome

Both fallopian tubes and ovaries display highly variable microbial communities among women. *Lactobacillus* spp. is present in lower ratio than in vagina or cervix (Fig. 1, Table 1). Fallopian tubes and ovaries harbour a variety of bacteria growing in mildly alkaline conditions, in contrast with the acidity of vaginal pH [13].

Chen's group showed that *Lactobacillus* comprised only 1.69% of the median relative abundance found at fallopian tubes microbiota in their cohort of 110 women [13]. Another

publication examined the composition of the microbial communities throughout fallopian tubes and ovaries of 10 women. *Bacterioides*, *Corynebacterium*, *Lactobacillus*, *Coproccocus*, or *Hymenobacter* were recovered from fallopian tube samples; whereas *Lactobacillus*, *Corynebacterium*, *Escherichia*, or *Blaudia* were recovered from ovary fluid, suggesting a high inter-individual variability among these anatomical sites [14]. Pelzer and colleagues reported that *Lactobacillus* spp. were not present all in fallopian tubes samples, but all of them were positive for at least some bacterial species [51]. Regarding microbial signature in ovarian follicular fluid, *L. iners*, *Actinomyces* spp., *Corynebacterium auromuosum, Fusobacterium* spp., *Prevotella*, or *Staphylococcus* spp. were found, being colonisation more prevalent in the left than in the right ovary [52]. Understanding the structure of these microbial communities might be helpful to propose alternative treatments for patients who would normally undergo salpingectomy for some pathologies [14].

Eubiosis and dysbiosis

Eubiosis is the healthy and balanced microbial ecosystem, whereas dysbiosis represents the non-homeostatic state [53]. If dysbiosis is the cause or the consequence of disease is unknown, but eubiotic and dysbiotic FGT configuration matters when establishing the link among bacterial communities and gynaecological and reproductive disorders [54–56]. In general, greater microbial diversity within the FT is associated with dysbiosis, linked to higher risk of negative events in the FGT physiology [7]. Overview of the main factors conditioning eubiotic and dysbiotic FGT state is depicted in Fig. 2.

Healthy vagina harbours a structured microbial ecosystem, displaying lower alpha and beta diversity than other body sites [19, 57]. As mentioned, *Lactobacillus* abundance (CST I, II, III, and V) in combination with low species diversity would be associated with better reproductive outcomes. Conversely, CST IV, rich in anaerobic species such *Gardnerella*, *Prevotella*, or *Atopobium*, could be associated to dysbiosis [20, 29, 58]. A systematic review concluded vaginal dysbiosis is a significant risk factor for early pregnancy loss in ART patients [59].

Regarding endometrium, shifts from a LD community are linked to subfertility and dysbiosis [9, 11]. LD endometrial microbiome might predict reproductive success, being considered eubiosis, but if it harbours < 90% *Lactobacillus* (NLD), the prognosis of the reproductive outcome is adverse, identified as dysbiosis [11]. Other factors such the high bacterial load or the presence of certain taxa could be linked to tissue destruction and immune over-stimulation that leads to dysbiosis [60]. This is the case of the *Fusobacterium* spp. and *Jonquetella* spp. (involved in BV too) dominance in endometrium of women with menorrhagia, which might suggest a reason for their dysbiotic state [39].

FGT microbiome shifts in response to internal and external changes

FGT microbiome may undergo shifts in response to stimuli such hormonal fluctuations, pregnancy, diseases, age, or ethnic origins, as described by several authors [29, 30, 56, 61]. A summary of the main factors affecting FGT microbiota, regarding vagina and endometrium, is outlined in Fig. 3.

Age and hormonal shifts through women lifespan

Vaginal microbiota fluctuations triggered by age are driven by hormonal shifts [30, 61, 62]. During childhood, vaginal pH is neutral, and anaerobes and *E. coli* predominate in the vaginal niche [63]. At puberty, vaginal epithelial cells, stimulated by oestrogen, start to produce glycogen, leading to the lactobacilli dominance that prevails in healthy vagina during reproductive years [63]. *Lactobacillus* spp. produce lactic acid, hydrogen peroxide, and bacteriocins, factors leading to a pH decrease, lactobacilli own adhesion to epithelial cells, and pathogenic bacteria detriment [64–66]. As oestrogen levels decrease in menopause, glycogen does too, triggering a descent in lactobacilli population [67]. Notwithstanding, there is still a predominance of *L. crispatus* and *L. iners*, as well as *Gardnerella* or *Prevotella* [68]. Figure 4 summarises vaginal microbial signature across the female life stages.

Menstrual cycle

There is a current debate about whether the microbiome remains stable during the menstrual cycle or, oppositely, if it changes throughout different phases. Gajer et al. observed high levels of species turnover and microbiota dynamism triggered by menstruation that did not dramatically affect community function [30]. Moreno et al. showed that the endometrial bacterial community did not significantly varied within the pre-receptive and receptive endometrial phases [11]. Another study analysed both intra and inter cycle fluctuations in vaginal and endometrial microbiome, showing high stability in the lactobacilli abundance [35]. On the contrary, other groups have suggested microbial communities differ between menstrual cycles. Chen et al. reported differences in the uterine microbiome between menstrual phases (*P. acnes* was more abundant in the secretory phase) [13]. Another group reported *Prevotella* spp. and *Sneathia* spp. increased, respectively, in the proliferative and secretory phases (women were suffering menorrhagia and dysmenorrhea, suggesting these bacteria might have a function such pathologies) [39]. Kadogami et al. recently found that *Lactobacillus* endometrial proportion ranged from <50% (after menstruation) to ~70% (luteal phase) [69]. Since changes in oestrogen levels trigger changes in vaginal [70, 71] and presumably in endometrial microbiome, Carosso et al. studied putative microbiome shifts

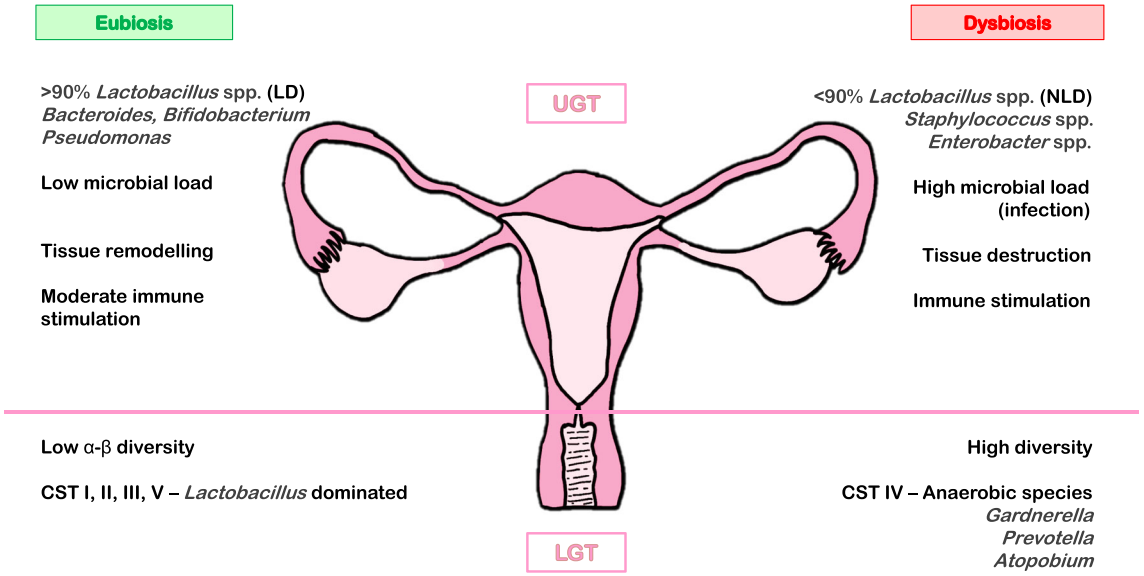

Fig. 2 Main factors that condition eubiosis and dysbiosis in UGT (upper genital tract) and LGT (lower genital tract)

after controlled ovarian stimulation and progesterone supplementation. In vagina, *Lactobacillus* decreased whereas *Prevotella*, *Escherichia*, and *Shigella* increased; in endometrium, *Lactobacillus* slightly decreased with an increase in *Prevotella* and *Atopobium* [34]. A recent publication reported endometrial microbiome to be more transcriptionally active in the mid-secretory compared to the proliferative phase, which suggest bacterial functions are regulated in a cycle-dependent way [23].

Pregnancy

Pregnancy is a condition that naturally changes the vaginal microbial signature, leading to a microbiome shift compared to the non-pregnant state. Whereas non-pregnant women might show variation in the *Lactobacillus* spp. abundance, this genus is invariable maintained, along with a reduced species diversity, during pregnancy progression in vagina [72–74]. According to Zheng and colleagues, *L. iners* abundance in

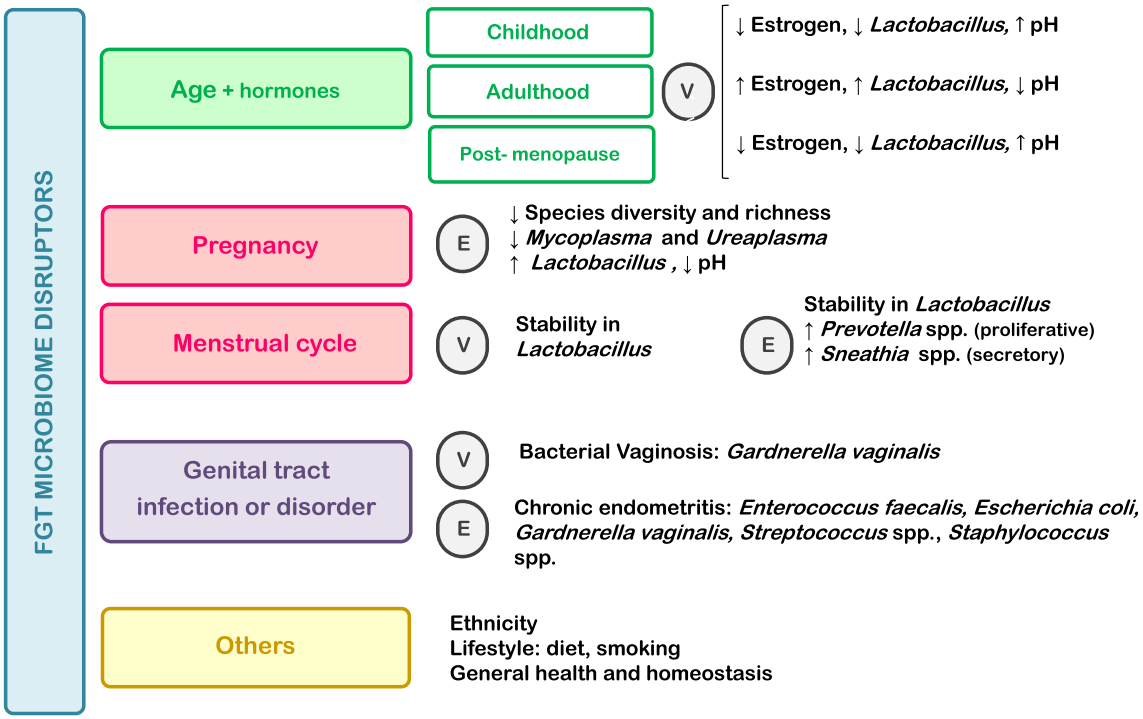

Fig. 3 Main variables involved in vaginal and endometrial microbiome shifts. Predominant taxonomy associated to each condition is depicted. (E, endometrium; V, vagina)

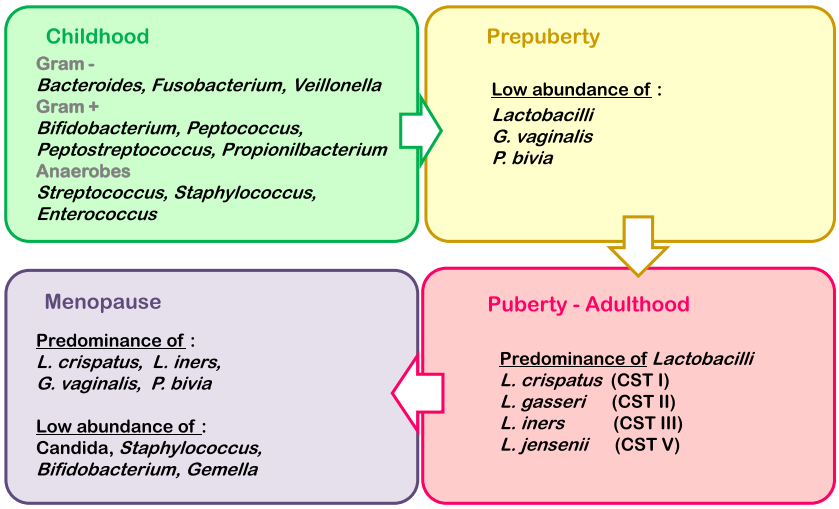

Fig. 4 Outline of the most predominant microorganisms in the vaginal niche throughout the female lifecycle

vagina decreased in second and third trimesters whereas *L. crispatus* increased in the second trimester [75]. Romero et al. reported that in spite of such lactobacilli stability and dominance, the individual CST may change from one lactobacilli-dominated CST to a different one [29, 72]. Related to this, Aagaard's group stated that vaginal microbiome harboured by women who were close to the pregnancy term kept similarities to the non-pregnant vaginal microbiome configuration. This suggest that, at some point near the delivery time, vaginal microbiome of pregnant women could return to the configuration it displayed before the gestation [74]. Freitas and collaborators reported lower *Mycoplasma* and *Ureaplasma* concentration and higher *Lactobacillus* spp. load in healthy pregnant women compared to non-pregnant cohort [76]. Endometrium was sampled at the time of caesarean delivery in ten women, finding *Escherichia*, *Acinetobacter*, *Lactobacillus*¸ and *Bacillus* as the most recovered taxa [45]. Other group found *Cutibacterium*, *Escherichia*, *Staphylococcus*, *Acinetobacter*, *Streptococcus*, and *Corynebacterium* in more than 50% of the endometrial samples (n = 19), taken from women at caesarean deliveries [44]. *Acinetobacter* and *Escherichia* were the most recovered genera in both studies [44, 45]. Interestingly, Moreno et al. sampled endometrial fluid within a successful pregnancy. According to their findings, this patient, who had suffered a spontaneous miscarriage, harboured lower bacterial load and higher lactobacilli abundance in the endometrial fluid sample taken during a successful pregnancy (sampled at pregnancy week 4) in comparison to samples taken before the miscarriage. *L. crispatus* and other species were detected before the miscarriage, whereas *L. iners* was the most abundant in the endometrium during the early pregnancy [77]. In mid stages of pregnancy, anti-inflammatory uterine milieu and microbial species harboured by the placenta (such as *Proteobacterium*, *Actinobacterium*, *Firmicutes*, *Bacteroidetes*, and *Temericutes*) may help to maintain healthy and low-risk pregnancies. This was depicted by several studies and by the iHMP, in which 1527 pregnancies were longitudinally monitored [7, 19, 78, 79]. Anti-inflammatory ambiance changes to pro-inflammatory before the onset of labour [7].

Regarding delivery and PTB, it has been suggested that high bacterial diversity (CST IV plus *Lactobacillus* depletion, and/or increased levels of *Sneathia* spp., *Prevotella* spp., among others) in vagina during the first semester of pregnancy is associated with higher risk of PTB [73, 80–82]. These hypotheses have been also supported the iHMP: women experiencing PTB were less likely to harbour *L. crispatus* dominance in vaginal microbiome [7]. Another group found *Lactobacillus* and *Bifidobacterium* richness in vaginal communities might be associated with decreased risk of early PTB [83]. An important clinical implication is the possibility of monitoring pregnancy progression using microbiome and their functions to discern between good and bad pregnancy prognosis.

Role of the female genital tract microbiome in reproduction, gynaecology and obstetrics

Microbiome and gynaecological disorders

Bacterial vaginosis

BV is usually typified by a detriment in *Lactobacillus* dominance, linked to *Gardnerella*, *Mycoplasma*, and *Prevotella* increase, being somehow comparable to CST IV configuration [84, 85]. Anaerobic bacteria dominance during BV can also be related with *Gardnerella* biofilms development [48]. Ceccarani et al. reported *L. crispatus* (present in controls, n = 21) was replaced by *L. iners*, present along with *Gardnerella*, *Prevotella*, *Megaspheaera*, *Roseburia*, and *Atopobium* in BV samples (n = 20) [86]. However, it is still unclear whether

either these are pathogens that cause BV or they are just opportunistic bacteria that take advantage of the higher pH and colonise vagina [87, 88]. Antibiotics are usually the first-line treatment to face BV [26], but novel approaches such vaginal microbiome transplants are considered to be feasible in antibiotics-nonresponsive and recurrent patients, as shown by a case series performed in 2019 [89]. A recent systematic review and meta-analysis addressed that BV was associated with early spontaneous abortion in in vitro fertilisation (IVF) patients [90].

Chronic endometritis

Chronic endometritis (CE) is the persistent inflammatory state of the endometrium, raised by an imbalance between bacterial species and female immune system [25, 91]

The endometrial microbiome in women with CE harboured *Lactobacillus*, *Enterobacter*, *Pseudomonas*, and *Gardnerella* (33.21%, 7.17%, 7.32%, and 6.91%, respectively). *Gardnerella* was not detected in control samples from healthy women [16]. Similarly, Liu et al. concluded than infertile women with CE harboured *Bifidobacterium*, *Prevotella*, and *Gardnerella*, which did not occur in non-CE infertile women [92]. Another study showed *Gardnerella*, *Klebsiella*, and *Streptococcus* were significantly increased both in endometrial fluid and biopsy samples from women who did not achieve pregnancy [93]. Since CE might go along with recurrent implantation failure (RIF) [94, 95], the development of new diagnosis tools and its treatment with antibiotics could improve pregnancy outcomes [96–98]. CE is to date the gynaecological issue which undergo the greatest improvement if treated with antibiotics, since the bacterial infection is a clear risk in this case [99]. Main bacteria triggering CE are *E. faecalis*, *Enterobacteriaceae*, *Streptococcus* spp., *Staphylococcus* spp., *G. vaginalis*, *Mycoplasma* spp., among others [98].

Epithelial ovarian cancer

Epithelial ovarian cancer (EOC) is one of the most lethal malignancies affecting women [100]. In 2017, Banerjee and colleagues identified the microbiome uniquely present in EOC, composed by *Proteobacteria* (52%), *Firmicutes* (21%), *Bacteroidetes*, *Chlamydiae*, *Spirochaetes*, and *Temericutes*. Authors suggested microbiome could influence both induction and progression of the EOC. However, it may have occurred that the tumour had generate a microenvironment in which these specific bacteria are able to persist [101]. Matching with these results, Zhou et al. analysed high-grade serous EOC samples, isolating *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* as the most represented taxa [102]. Since there is emerging evidence of the active role of microbiota in the development or metastasis of malignancies [103], establishing an unique EOC microbiome could provide useful biomarkers for EOC diagnosis or prevention [101].

Endometriosis

Endometriosis is the growth of endometrial tissue outside the uterine cavity, causing pelvic pain or infertility [104]. The pathophysiology of endometriosis is not yet fully elucidated [105], and FGT microbiome has been studied to decipher its putative involvement in endometriosis risk.

Gardnerella, *Enterococcus*, *Streptococcus*, and *Staphylococcus* were found among the pathogenic species isolated in endometriotic samples compared to controls, where *Lactobacillus* spp. was predominant [106]. *Streptococcaceae* and *Moraxellaceae* significantly increased in endometriotic samples [15]. Cregger and colleagues found *Lactobacillus*, *Barnesiella*, *Flavobacterium*, and *Pseudomonas* to be the most predominant genera in samples from women with and without endometrial lesions [107]. *Pseudomonas*, along with *Acinetobacter*, *Vagococcus,* and *Sphingobium*, were found in uterine endometriotic lesions samples [13]. Another study showed *Atopobium* was absent both in cervical and vaginal microbiota, whereas *Gardnerella* increased in cervical microbiota in the endometriosis group, in comparison with controls [57]. In 2019, Akiyama and collaborators examined cervical samples collected women with endometriosis (n = 30). Despite *Lactobacillus* being predominant, *Enterobacteriaceae* and *Streptococcus* were increased in the endometriosis group compared to controls [108]. Hernandes et al. showed different bacterial profiles in deep endometriotic lesions: reduced predominance of *Lactobacillus* and more abundance of *Alishewanella*, *Enterococcus*, and *Pseudomonas*, compared to eutopic endometrial controls [109]. Since endometrial infection triggers uterine contractility, this could explain the retrograde seeding of endometrial cells outside the uterus, allowing endometriosis progression [110, 111]. In 2018, Khan proposed the "bacterial contamination hypothesis": as the levels of *E. coli* lipopolysaccharide are increased in endometriosis, endometrial bacteria binding Toll-like receptors could elicit the immune response in endometriosis [112]. If confirmed, microbiome could be considered as a non-invasive tool in the diagnosis of endometriosis. Such is the case of *Streptococcus* or *Atopobium* [15, 40, 113], or the increase in diversity along with lactobacilli detriment in the cervical mucus and endometrial microbiomes, which have been proposed to be markers for endometriosis diagnosis [109, 114].

Endometrial cancer

It is estimated that 15% of tumours are related to infectious agents [115]. The role of the uterine microbiota in endometrial

cancer (EC) was first assessed by Walther-António, concluding that *Atopobium vaginae* and *Porphyromonas somerae* were associated with EC. The inflammatory role of bacteria (triggering immune response) was considered in the onset this pathology [116]. Later, *P. somerae* was established as a predictive biomarker of EC [117]. Another group described *Acinetobacter*, *Pseudomonas*, *Cloacibacterium*, and *Escherichia* to be predominant in EC [36]. Most recently, Lu and collaborators have published a study showing *Micrococcus* to be more abundant in the EC group (n = 25) compared to controls (n = 25, benign uterine lesions), suggesting dysbiosis in EC might be associated with this genus in particular [118]. However, the microbial configuration of EC is not fully unravelled yet.

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common cause of female endocrine-related infertility, being its prevalence among 5–20% in reproductive age women [119]. FGT microbiome is under study, although the core microbial composition that might trigger the PCOS onset has not been defined yet [120].

Colonising microorganisms in ovarian follicular fluid affected fertilisation rates in women with PCOS (n = 14), probably due to oocyte in vivo damage after being exposed to bacterial species or their metabolites [52]. Subsequent research by the same group in 49 women diagnosed of PCOS demonstrated 29% and 71% of the subjects displayed colonised or contaminated follicular fluid, respectively, which was related with a negative IVF outcome. Among the microorganisms, *Propionilbacterium* spp., *Streptococcus* spp., *Actinomyces* spp., *Staphylococcus* spp., and *Bifidobacterium* spp. were found [121].

The role of the LGT in PCOS has also been studied [120]. Vaginal and cervical biopsies from 47 PCOS patients and 50 healthy patients were analysed in a study conducted by Tu et al. Results exhibited increased abundance of pathogens such as *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Prevotella,* as well as reduced *Lactobacillus* both in vaginal and cervix in PCOS group compared to controls [122]. Similar results showed increased *Mycoplasma* and *Prevotella,* and decreased *L. crispatus* in 39 PCOS cases compared 40 healthy controls [123], indicating *Mycoplasma* could be proposed as a PCOS marker, according to these publications [122, 123].

Microbiome in reproduction and assisted reproduction techniques

Vaginal and endometrial microbiome have been studied in subfertile patients, due to the fact it might constrain pregnancy achievement and ART results [60, 124].

In the peri-implantation period, bacteria and bacterial derived fragments can elicit inflammatory immune responses (mediated by pro-inflammatory cytokines), as it also occurs in the endometrial receptivity acquisition [125, 126]. Lactic acid might benefit and support endometrial receptivity and blastocyst invasion as well, meaning lactobacilli abundance in FGT matters to achieve pregnancy [11, 127]. Regarding vaginal microbiome, if exclusively composed by *Lactobacillus*, it conforms the best scenario for a successful embryo transfer outcomes, according to several studies and experts [25, 128].

Another group ascertained that the lower *Lactobacillus* in vagina, the lower likelihood to undergo successful embryo implantation. However, >60% of *L. crispatus* was unfavourable to achieve pregnancy, which suggest the acidity is linked the implantation outcome [129]. Apart from greater lactobacilli abundance, limited diversity of species also triggered better results after embryo transfer, as concluded by another group [130]. A review and meta-analysis by Singer et al. has concluded that women with dysbiotic vaginal microbiota have 1.4 times less chance of becoming pregnant after an IVF treatment, compared with women harbouring eubiotic configuration [131].

Looking at the link between microbiome and implantation window, reproductive success was predicted based on the endometrial abundance of *Lactobacillus* spp. in IVF patients. Displaced implantation window and therefore embryo transfer failure might occur due to FGT microbiome shifts due to ovarian stimulation prior to IFV, which suggest considering transferring a frozen embryo [34], or trying to modulate FGT microbiome (see the "FGT microbiome modulation in clinical activity might help to improve reproductive results" section). >90% of lactobacilli (LD) was significantly linked to better embryo implantation results, on-going pregnancy, etc. <90% of *Lactobacillus* spp. in combination with >10% of other genera (NLD) was linked to poor reproductive outcomes in such women [11]. This was questioned by another group, which showed *Lactobacillus* abundance did not significantly correlated with the IVF result, since *Flavobacterium* was one of the most recovered taxa in endometrium [10]. Kyono and collaborators analysed the endometrial fluid from patients undergoing IVF. 15/79 patients achieved pregnancy after embryo transfer, and *Lactobacillus* average was >96% in pregnant women, according to LD endometrium condition [11, 35]. Subsequently, a pilot study analysed endometrial samples from infertile women. A total of 47 women harboured LD endometrium, whereas 45 harboured NLD, from which 9 changed to LD after antibiotics and prebiotic/probiotic treatments. Although results showed pregnancy rates did not significantly differed between LD and NLD samples, authors remarked the usefulness in deciphering *Lactobacillus* dominance before IVF [43]. Contrary to what has been previously shown, Hashimoto and Kyono concluded NLD endometrium

and taxa such *Atopobium*, *Gardnerella*, and *Streptococcus* did not impede implantation after blastocyst transfer, observing comparable pregnancy rates between LD and NLD [132]. Wee and collaborators analysed vaginal, cervical, and endometrial samples comparing fertile and infertile women. Results showed a trend by which *Ureaplasma* and *G. vaginalis* were overrepresented in vagina and cervix of infertile women, respectively [31]. Another publication depicted *Kocuria dechangensis* was present in endometrial tissue before ovarian stimulation in women with implantation failure [33]. A multicentre prospective observational study has recently sequenced endometrial microbiome from fluid and biopsy samples taken from 342 ART patients at the time of endometrial receptivity analysis (ERA [133]). *Lactobacillus* and commensal bacteria (*Cupriavia*, *Finegoldia*, *Microbacterium*, and *Tepidomonas)* were present in higher proportion in patients having live birth. Women with adverse results (clinical miscarriage or no pregnancy achievement) harboured *Bifidobacterium*, *Chryseobacterium*, *Haemophilus*, *Klebsiella*, *Neisseria*, *Staphylococcus*, *Streptococcus, Gardnerella*, and *Atopobium,* being these last two species strongly associated with BV [93]. These studies suggest the potential value of microbial species as biomarkers to predict successful reproductive outcome before embryo transfer, avoiding losing blastocysts [33, 93]

Uterine microbiome might have a role in cases of RIF or recurrent pregnancy loss (RPL) [134]. A study examined paired endometrial fluid and tissue samples from 25 patients who had suffered RPL. Results displayed *Atopobium*, *Prevotella*, *Gardnerella*, *Bifidobacterium*, *Stenotrophomas*, *Lactobacillus*, *Megasphaera*, *Staphylococcus*, and *Escherichia* as the most recovered taxa, albeit results from fluid and tissue did not fully matched [135]. Kitaya et al. studied paired vaginal and endometrial fluid samples from women with RIF, who harboured increased *Gardnerella* compared to controls, and *Burkholderia* spp. (not found in controls) [95]. These findings are consistent with a longitudinal study that showed *Gardnerella*, *Atopobium*, and *Bifidobacterium* persisted in the endometrium of a patient suffering from RIF [136].

Gathering all this knowledge, microbiome of women undergoing ART should be screened in advance, in order to improve results prior starting the treatment [25].

FGT microbiome modulation in clinical activity might help to improve reproductive results

The main and last objective of ART is to achieve healthy pregnancies and live birth in their patients. As FGT microbiome, and in particular vaginal and endometrial microbiome might condition embryo implantation and the course of pregnancy as well as the occurrence or non-occurrence of gynaecological diseases, its intended modification may be decisive in achieving the purpose of ART under some circumstances [25, 26].

In particular situations, such BV or CE, the use of antibiotics, probiotics, and/or prebiotics could be considered. Albeit antibiotic use is still controversial, and should not be used routinely to change vaginal microbial configuration, it has been suggested that their prescription could be relevant for certain patients, as it is the case of women undergoing RIF [25, 137]. Recently, the effect of different combinations of oral and vaginal probiotics or oral prebiotics and antibiotics in 176 with RIF was categorised as NLD (~25.2% *Lactobacillus* and *Bifidobacterium*, and higher concentration of *Gardnerella*, *Atopobium*, *Streptococcus*¸ and *Prevotella*). After treatment, microbiome was evaluated and there was cure rate of 78% with the combination of vaginal probiotic suppository plus antibiotics [69].

New promising strategies to improve ART outcomes or to manage gynaecological disorders related to FGT microbiome, such as vaginal microbiome transplantation [89, 138] and algorithm design for FGT microbial profile stratification [129] are currently under development.

However, microbiome configuration differs among individuals [139, 140], and samples are usually taken in women with previous medical conditions during surgical interventions, which might account for microbiome oscillations from what is considered as "normal microbiome" [141]. Moreover, sampling uterus, ovaries, and Fallopian tubes is invasive, there is risk of vaginal and cervical bacteria contamination, and their analysis is usually arduous due to the low microbial biomass harboured by the UGT [23, 26]. Due to these reasons, FGT characterisation, although needed for a preliminary diagnostic in reproduction, is still challenging. It requires a profound experimental design, a neat and standardised execution (detailing nucleic acid extraction methods, describing controls and reporting contamination, and maintaining the protocol during the whole study), and results must be interpreted with caution [142–145].

Conclusions

Microbiome impact has recently gained importance due to its role not only in pathology but also in host physiology. This review assesses current research about FGT microbiome and its implication in female health and reproduction.

General consensus depicts microbial communities change progressively from the vagina to the ovary, decreasing in abundance and increasing in diversity, from the outermost to the innermost. Rich in *Lactobacillus* vaginal and endometrial microbial profiles are linked to better reproductive outcomes. Addressing FGT microbiome provides new clinical diagnostic tool for previously misunderstood fertility issues and new approaches for ART in women. Moreover, this could be used to

design novel treatments (such probiotics or microbiome transplantations) to improve endometrial receptivity, to achieve healthier pregnancies, and to treat gynaecological disorders such endometriosis or cancer.

However, the main question still remains in unravelling if there is either a "microbial taxa core" or a "functional core" (understood as the pool of metabolic functions performed by microbiome) that leads to a eubiotic state. Bearing in mind that this configuration can vary among individuals, there is a limitation to establish a comparison among bacterial communities, and also in deciphering whether any specific configuration is eubiotic or dysbiotic. In this regard, the unification of research protocols and the standardisation of NGS techniques must be considered as a key for future research in the reproductive microbiome field.

Following steps point to FGT virome, mycobiome, and microbiome interrelation, to fully describe physiology in this body site. By pooling all this knowledge, clinical implications of microbiome in FGT could be assessed, eventually achieving a better gynaecological and reproductive health in such patients. Although the clinical recommendations for profiling and modulation FGT microbiome are yet to be fully elucidated, a new horizon in the field of personalised medicine is currently open.

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Author contribution P.P. contributed to the data collection and interpretation, E.L. revised the article and approved the final version. The first and last authors significantly contributed to the study conception and design, performed statistical analyses, and data interpretation and drafted the article.

Declarations

Conflict of interest P.P declares no conflicts of interest. E.L. received a grant from Ferring in 2020, has provided consultancy services for MSD and Ferring Pharmaceuticals, and is part of the Ferring Pharmaceuticals LIFE program and Merck Global program for Fertility Innovation Leaders. During the past 12 months, she has received honoraria from Angelini/IBSA, Merck, MSD, and Ferring Pharmaceuticals for lecturing.

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