REVIEW

Microbiome as a predictor of implantation

Xu Shan Gao[a], *Joop Laven*[a], *Yvonne Louwers*[a], *Andries Budding*[c], *and Sam Schoenmakers*[b]

Purpose of review
Review the latest research on the female urogenital microbiome as a predictor of successful implantation.

Recent findings
Lactobacillus crispatus seems to be beneficial species in a healthy female genital tract, although the presence of anaerobic bacteria and their impact has yet to be determined. The vaginal microbiome is associated with assisted reproductive technology (ART) outcome in terms of successful implantation and pregnancy. Approaches restoring a dysbiotic vaginal microbiome seem promising. It is questionable if a unique endometrial microbiome exists, given the low bacterial biomass, the invasiveness of endometrial sampling, and its associated high contamination risk. Future studies should focus on the whole microbiome using proteomics and metabolomics, as well as the virome to get a more holistic understanding of its role in reproduction.

Summary
The vaginal and endometrial compartments are being studied to determine a healthy and unhealthy microbiome composition. Defining a healthy composition could provide insight into physiological processes related to the success of embryo implantation. The vaginal microbiome is easily accessible and its composition can be reliably assessed and can be associated with ART outcome. The existence of an endometrial or uterine microbiome is still debated, due to the combination of low biomass and unavoidable high risk of contamination during sampling.

Keywords
fertility, implantation, IVF outcome, microbiome, reproductive outcome

INTRODUCTION

The female genital tract microbiome undergoes important changes in its composition during the reproductive lifespan that are directly related to fluctuating sex-steroid hormone levels [1▪]. Of all the different anatomical compartments of the female reproductive tract, the vagina is easily assessable and sampling of local microbes can occur virtually noninvasive with a very low risk of microbial contamination from the surrounding vulvar skin. Recent studies have revealed that the vaginal microbiome composition is associated with assisted reproductive technology outcome, more specifically with the chance of implantation and achieving a pregnancy [2]. Herein, we will review the latest evidence on the female genital tract microbiome with a focus on its predictive role in blastocyst implantation (Table 1).

GENERAL

Human microbial colonization during the life course

The female reproductive tract, excluding the vagina, has historically been considered sterile with the cervix acting as a barrier against external pathogens. However, over the last decade, several studies have postulated the presence of bacterial microbiome in the placenta, amniotic fluid, and meconium in pregnancies [3,4]. In contrast, recent evidence has questioned in-utero colonization and previous results are most likely based on contamination [5,6▪▪]. These studies could not detect viable bacteria in amnion fluid or placental samples [6▪▪,7], and its presence in the fetal meconium, has been challenged [8]. de Goffau *et al.* [9▪▪] reanalyzed these data, reported a batch effect that was not taken into consideration in the earlier analysis, and concluded colonization was not supported by these data.

[a]Division of Reproductive Endocrinology and Infertility, [b]Division of Obstetrics and Fetal Medicine, Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam and [c]Inbiome, Amsterdam, The Netherlands

Correspondence to Xu Shan Gao, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, Erasmus University Medical Centre, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands. Tel: +31 1070 33760; e-mail: x.gao@erasmusmc.nl

Curr Opin Obstet Gynecol 2022, 34:122–132
DOI:10.1097/GCO.0000000000000782

KEY POINTS

- Future studies on the microbiome of the female reproductive genital tract should use uniformly classified community state types to make microbiome datasets comparable.
- In contrast to pregnancy, the female microbiome seems to remain relatively constant during ART treatment.
- The vaginal microbiome is predictive for IVF outcome and can predict those women who do not become pregnant with great certainty.
- To improve the outcomes of assisted reproduction, it is important to detect a dysbiotic microbiome before initiation of ART treatment.

Molecular techniques used to study low microbial biomass, such as fetus-related or endometrial microbiome often detect bacterial contaminants, resulting in false-positive results [10]. It is currently impossible to eliminate contamination in a clinical setting when collecting samples from the uterine environment.

During the fetal-to-neonatal transition, a horizontal bacterial transfer occurs without a doubt. Throughout childhood, the vaginal microbial community seems to be dominated by *Escherichia coli* and anaerobes, which change around puberty to a *Lactobacilli* dominant microbiome [11]. During the reproductive life span, the vaginal microbial community changes with menarche, sexual activity, menstrual cycle, contraceptive use, and pregnancy. Finally, after menopause, a less diversified microbiome occurs due to estrogen depletion [12]. Increasing the estrogen level with hormone replacement therapy seems to be effective to restore the premenopausal microbiome [12].

Composition and dynamics of the microbiome during the reproductive age

The vaginal microbiome of healthy, reproductive-aged women has low diversity and is dominated by *Lactobacillus* species. Recently, the group of Ravel *et al.* [13▪] has analyzed the taxonomic profiles of vaginal microbial communities by VALENCIA, which is the most extensive database so far. VALENCIA classifies samples according to their diversity using the nearest centroid method, allowing researchers to characterize the vaginal microbiome uniformly by allocating any microbial community to one of 13 community state types (CST).

Women with a healthy vaginal microbiome predominantly harbor *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacilli iners,* and *Lactobacillus jensenii* [14], classified as CST I, II, III, and V, respectively. CST IV is characterized by the presence of a non-Lactobacilli dominance, such as *Prevotella, Gardnerella, Streptococcus, Bifidobacterium, Enterococcus,* and *Staphylococcus*. Although the transition between different CSTs can occur, CST I, II, III, and V are relatively stable. CST IV, however, is highly variable and often changes into one of the other CSTs. Importantly, these findings demonstrate that single assessments only provide a temporary microbiome profile.

Lactobacilli play a protective role in the female reproductive tract and a fundamental role in the ecological balance of the vagina. The physiological role of *Lactobacilli* in the female reproductive tract is to maintain a eubiotic vaginal environment by the production of lactic acid, hydrogen peroxide, and bacteriocins as well as promoting autophagy [15]. After menstruation or exposure to seminal fluid, the vaginal pH tends to rise, reducing the antimicrobial effect of lactic acid and creating the conditions under which the vaginal microbiome is prone to change [16]. Recently, Kadogami *et al.* [17] found that *Lactobacillus* endometrial proportions ranged from less than 50% (day 5 menstruation) to almost 70% during the luteal phase in patients with a regular menstrual cycle. This suggests that sampling on specific days after menses would improve comparability between studies.

However, not all *Lactobacilli* species have a protective role, since the vaginal microbiome with *L. iners* dominance (CST III) is associated with the presence of bacterial vaginosis [13▪]. The composition of the vaginal microbiome varies depending on factors such as ethnicity and biogeographical locations, with White or Asian women more likely to have an *Lactobacillus*-dominant (LD) microbiome (CST-I) [18].

The introduction of next-generation sequencing (NGS) has enabled quick, large-scale microbiome investigation and has led to the discovery in every – traditionally considered sterile – body compartment (despite 100 years of scientific consensus that these compartments are sterile). The uterus is no exception. As compared with the vagina, the uterine cavity contains a very low level of microorganisms and harbors 10 000 times fewer bacteria [19]. Aging, a history of abortion and the mode of delivery, all have a substantial impact on the composition of the uterine microbiome [20▪▪].

Due to the low biomass of bacteria combined with the invasiveness of endometrial sampling and the unavoidable high contamination risk, it remains questionable whether a unique endometrial microbiome exists [21▪]. Other groups attempting to

Table 1. Overview of scientific publications of the female reproductive tract (January 2020–January 2022)

Anatomical region						
Cervicovaginal						
First author, Year	Title	Article type	Anatomical region	Population + ethnicity if reported	Sampling + timing of sampling if reported	Conclusion
Amato, 2020	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	Original	Vagina	First IUI treatment ($n = 23$) Caucasian	Vaginal swabs, seminal fluids	VM and not seminal microbiome in idiopathic infertile couples was different compared with healthy controls Vaginal *Lactobacillus crispatus* dominance was associated with successful implantation
Fu, 2020	Alterations in Vaginal Microbiota and Associated Metabolome in Women with Recurrent Implantation Failure	Original	Vagina	First frozen ET in RIF patients ($n = 27$) and control ($n = 40$)	Vaginal swabs on the day of ET	Higher alfa diversity between RIF patients and those who were pregnant in first frozen embryo cycle Vaginal *L. crispatus* dominance was positively correlated with pregnancy rate
Hao, 2021	Association of the Cervical Microbiota With Pregnancy Outcome in a Subfertile Population Undergoing In Vitro Fertilization: A Case–Control Study	Original	Cervico-vaginal	IVF population ($n = 124$)	Cervical swabs before ET	*Lactobacillus* plays a limited role in the prediction of clinical pregnancy
Karaer, 2021	The Vaginal Microbiota Composition of Women Undergoing Assisted Reproduction: A Prospective Cohort Study	Original	Vaginal	IVF population ($n = 223$)	Vaginal swab prior to ET	*Lactobacillus* does not significantly distinguish the pregnant from the nonpregnant group A relatively higher abundance of *Streptococcus* was associated with a lower ART success rate
Kong, 2020	The Disordered Vaginal Microbiota is a Potential Indicator for a Higher Failure of In Vitro Fertilization	Original	Vagina	IVF population ($n = 475$)	Vaginal secretion collected with suction tube before IVF	Higher abundance of *Lactobacillus* was found in the pregnancy group Age, endometrial thickness, reduction in *Lactobacillus* and higher abundance of *Gardnerella, Atopbium* and *Prevotella* were associated with IVF failure
Xu, 2020	Fertility Factors Affect the Vaginal Microbiome in Women of Reproductive Age	Original	Vagina	Infertile due to tubal obstruction $n = 40$ and controls ($n = 45$) Asian	Vaginal swab	VM dysbiosis is correlated with infertility: High abundance of *Escherichia coli* was found in women with tubal obstruction compared with women with normal fallopian tubes

Table 1 (*Continued*)

Anatomical region						
Zhao, 2020	Characterization of the Vaginal Microbiome in Women with Infertility and Its Potential Correlation with Hormone Stimulation during In Vitro Fertilization Surgery	Original	Vagina	IVF population with secondary infertility $n=30$, Healthy women $n=92$	Vaginal swabs (fornix posterior) 1st sample during first 3 days of follicular phase; 2nd after GnRH agonist + r-HCG	During the follicular phase, the VM of infertile women have a decreased diversity compared with healthy women, whereas the microbiome of healthy women fluctuates during ovulation No alterations were observed in microbiome composition after ovulation induction in infertile IVF patients *Bifidobacterium* seems correlated with negative IVF outcome
Skafte-Holm, 2020	The Association between Vaginal Dysbiosis and Reproductive Outcomes in Sub-Fertile Women Undergoing IVF-Treatment: A Systematic PRISMA Review and Meta-Analysis	Review	Vagina			When compared with patients with a normal vaginal microbiome, vaginal dysbiosis was associated with a significantly lower clinical pregnancy rate
Endometrium						
Chen, 2021	Identification of Uterine Microbiota in Infertile Women Receiving in vitro Fertilization With and Without Chronic Endometritis	Original	Endometrium	IVF population: CE ($n=25$) and non-CE group ($n=69$) Asian	Endometrial fluid with IUI catheter, 3–5 days after the secretory phase	IVF patients with CE have a significantly lower chance of conceiving compared with non-CE patients *Gardnerella* was associated with a negative IVF outcome and was found in the CE group as well
Diaz-martÚnez, 2021	Impact of the Vaginal and Endometrial Microbiome Pattern on Assisted Reproduction Outcomes	Original	Endometrium and vagina	IVF-population ($n=48$)	Vaginal swabs, Transcervical endometrial tissue (Tao Brush IMC Endometrial Sampler)	Alfa and beta diversity were comparable between pregnant and nonpregnant women in vaginal and endometrial samples The RIF group and the control group had significantly different compositions of the endometrial fluid microbiota. Higher abundance of *Prevotella* was detected in RIF patients

Table 1 (*Continued*)

Anatomical region						
Wang, 2021	Translocation of Vaginal Microbiota is Involved in Impairment and Protection of Uterine Health	Original	Endometrium and vagina	Healthy women and women with CE ($n=145$) Rodent model with VMT Asian	Vaginal swabs, transcervical endometrial tissue Sample collection: in follicular phase	VM could translocate to the uterine cavity, cause dysbiosis and influence reproductive health Intrauterine inflammation can cause a lower uterine microbiome diversity and a higher VM diversity Aging, delivery mode, and abortion history have a substantial impact on the composition of uterine microbiome VMT from CE patients in vaginas of rodent models can induce inflammation in the uterine cavity
Ichiyama, 2021	Analysis of Vaginal and Endometrial Microbiota Communities in Infertile Women with a History of Repeated Implantation Failure	Original	Endometrium and vagina	RIF ($n=145$) and controls ($n=21$) Asian	Vaginal swabs, transcervical endometrial tissue with Pipette on day 5–7 after ovulation	The VM of the RIF group had lower levels of *Lactobacillus* than in controls, whereas endometrial microbiome composition was similar among both There is a higher alfa diversity in endometrial microbiome than in VM Dysbiotic microbiota from the RIF group consisted of significantly higher levels of 14 other genera
Carosso, 2020	Controlled Ovarian Stimulation and Progesterone Supplementation Affect Vaginal and Endometrial Microbiota in IVF Cycles: A Pilot Study	Original	Endometrium and vagina	IVF-population ($N=15$) Caucasian	Vaginal swab, embryo transfer catheter tip Sample collection: 6 months prior to IVF treatment	In endometrial samples, an increase in *Prevotella* and *Atopobium* with a decrease in *Lactobacillus* following controlled ovarian stimulation and progesterone supplements could affect the implantation
Kadogami, 2020	Use of a Vaginal Probiotic Suppository and Antibiotics to Influence the Composition of the Endometrial Microbiota	Original	Endometrium	RIF women ($n=392$) Asian	Transcervical endometrial fluid using Pipette	*Lactobacillus* abundance changes during the menstrual cycle and was associated with the stage of follicular development Combination of oral and vaginal antibiotics along with vaginal probiotic may represent an effective treatment for RIF patients with non-*Lactobacillus* dominance microbiome (no medication-related adverse effects were observed)

Table 1 (*Continued*)

Anatomical region						
Moreno, 2022	Endometrial Microbiota Composition is Associated with Reproductive Outcome in Infertile Patients	Original	Endometrium	IVF-population ($n=342$)	Endometrium fluid with catheter + syringe and biopsy with Pipelle	High abundance of *Lactobacillus* is associated with live birth outcome A dysbiotic microbiota profile composed of *Atopobium, Bifidobacterium, Chryseobacterium, Gardnerella, Haemophilus, Klebsiella, Neisseria, Staphylococcus,* and *Streptococcus* was associated with unsuccessful outcomes
Riganelli, 2020	Structural Variations of Vaginal and Endometrial Microbiota: Hints on Female Infertility	Original	Endometrium and vagina	Infertile women with a history of implantation failure ($N=34$) Caucasian	Vaginal fluid with cytobrush, endometrial biopsy collected with IUI catheter inside Pipelle Collected at day 21 of menstrual cycle before start of IVF	Higher diversity in the endometrial microbiome was found compared with the VM *Lactobacillus* was only detected in the group with unsuccessful IVF *Kocuria dechangensis* was found in high abundance in the endometrium of women who failed to conceive after IVF NB: low pregnancy rate was observed (4/34)
Saxtorph, 2020	Assessing Endometrial Receptivity After Recurrent Implantation Failure: A Prospective Controlled Cohort Study	Original	Endometrium and vagina	RIF ($n=86$) and controls ($n=37$)	Vaginal swabs, transcervical endometrial tissue	In the RIF group, a higher prevalence of CE was revealed compared with the group with no but no correlation with a decrease of endometrial lactobacilli was found
Sola-Leyva, 2021	Mapping the Entire Functionally Active Endometrial Microbiota	Original	Endometrium	Healthy women $n=7$	Endometrial biopsies in mid-secretory phase and proliferative phase	Mapping the whole functionally active endometrial microbiota revealed a presence of >5300 microorganisms (including bacteria, viruses, archaea, and fungi) The level of *Lactobacillus* fluctuates throughout the menstrual cycle; with a low abundance after menstruation and a peak during the luteal phase The endometrium of healthy women is non-*Lactobacillus* dominant

Table 1 (*Continued*)

Anatomical region				
Elnashar, 2021	Impact of Endometrial Microbiome on Fertility	Review	Endometrium	A healthy microbiome is becoming increasingly important for all reproductive processes It is possible to sequence the microbiome and gain insight into its makeup, but not its function
Jimenez, 2021	The Impact of the Female Genital Tract Microbiome in Women Health and Reproduction	Review	Endometrium, vagina and fallopian tubes	Overview of the female genital tract microbiota and its importance as a tool for diagnosis and treatment of reproductive outcomes, infertility, or gynecological diseases
Sehring, 2022	Human Implantation: The Complex Interplay between Endometrial Receptivity, inflammation, and The Microbiome	Review	Endometrium and vagina	Understanding the challenging aspects of endometrial receptivity would provide treatment to infertile patients and implantation failure
Vitale, 2022	The Role Of Genital Tract Microbiome In Fertility: A Systematic Review	Review	Endometrium, cervix, and vagina	To compare studies, standardization of the sampling methods is needed to determine the relationship between female genital tract and fertility
Tomaiuolo, 2020	Microbiota and Human Reproduction: The Case of Female Infertility	Review	Endometrium and vagina	Microbiota is continuously under influence of several internal and external factors. Specific endometrial or vaginal dysbiosis could be linked to worse IVF outcomes
Toson, 2022	The Endometrial Microbiome and Its Impact on Human Conception	Review	Endometrium	Investigation in larger cohorts and standardized protocols is essential to compare studies and understand physiological and dysbiotic endometrial microbiota on reproductive outcome

ART, assisted reproductive technology; CE, chronic endometritis; GnRH agonist, gonadotropin-releasing hormone agonist; IUI, intrauterine insemination; r-HCG, recombinant human chorionic gonadotropin; RIF, recurrent implantation failure; VM, vaginal microbiome; VMT, vaginal microbiota transplantation.

determine the endometrial microbiome of women of reproductive age via surgical methods did not find *Lactobacillus* predominance. Winters *et al.* [22] obtained endometrial samples after hysterectomy and did not find *Lactobacilli*, but an endometrial microbiome mainly comprised of *Acinetobacter, Pseudomonas, Cloacibacterium, and Comamonadaceae*, which are common contaminants of sequencing reagents [23,24]. An older study of Verstraelen *et al.* [25] analyzed the endometrial samples obtained with a transcervical device of 19 nonpregnant women. They showed that the uterine microbiome mainly consisted of *Firmicutes, Proteobacteria* with *Bacteroidetes* being the most dominant phylum. However, since species generally found in the vaginal microbiome, were also found in the endometrial samples, contamination cannot be ruled out, indicating that the endometrial microbiome may be an artifact.

The microbiome and its role in implantation

Successful embryonic implantation is the result of a synchronized process between a healthy embryo and a receptive endometrium. It seems that the mother's endometrium is capable of sensing if an embryo is metabolically active, with increased amino acid turnover due to (genetic) defects in that embryo, and subsequently refuses to accept that particular embryo [26]. Implantation failure can be caused by miscommunication between endometrial immune cells, a window of implantation that is out of phase, or aneuploidy of the embryo resulting in pregnancy loss [27]. Endometrial receptivity during the implantation window can be affected by multiple factors. Although compounds such as cytokines, lipids, adhesion molecules, and growth factors are identified, their exact roles remain unclear [28].

Cervicovaginal microbiota

Of all the different anatomical compartments of the female reproductive tract, the vagina is readily accessible, and sampling of the microbiome can occur virtually noninvasive with a very low risk of microbial contamination from the surrounding vulvar skin. The study from Kong *et al.* [29] with 475 infertile patients showed that the vaginal microbiome with LD was associated with the occurrence of clinical pregnancy. In addition, they showed that a lower abundance of *Lactobacillus* and a higher abundance of *Gardnerella* and *Prevotella* were found in the vaginal samples from nonpregnant IVF patients. However, Karaer *et al.* [30] found that *Lactobacillus* does not seem to distinguish the pregnant and nonpregnant groups from each other, while a relatively higher abundance of *Streptococcus* was associated with poor assisted reproductive technology (ART) outcomes. Hao *et al.* [31] showed that *Lactobacillus* was in a higher abundance in the nonpregnant group compared with the pregnant group in cervicovaginal samples at the time of fresh embryo transfer. The dominance of anaerobic bacteria seems to have a negative impact on IVF outcome, whereas *L. crispatus* seems to be positively correlated with pregnancy [29,32▪]. In women with recurrent implantation failure (RIF) a significant relative lower and higher abundance was found for respectively *Firmicutes* and *Bacteroidetes* at the phylum level [32▪]. Similarly, Zhao *et al.* [33] reported that vaginal presence of non-*Lactobacillus* bacteria, such as *Atopobium, Aerococcus,* and *Bifidobacterium,* in subfertile women were found to be associated with IVF failure. In summary, it seems that a more diverse reproductive microbiome is associated with ART failure, whereas LD microbiota is associated with ART success [34]. To gain more insight into the pathophysiology of IVF failure, Fu *et al.* [32▪] performed a study to analyze alterations in the microbiome and metabolome concerning IVF outcomes. The group with an IVF failure appeared to lack important metabolites required for embryo growth and implantation, such as glycerophospholipids and benzopyran. Moreover, the differences in metabolites were linked to the diversity of the microbiome. Hence, one explanation for a link between the local microbiota and reproductive outcomes could simply be the fact that embryonic survival and local bacteria are dependent on similar substrates.

The impact of ovarian stimulation on different microbiome compositions is currently being studied as well. Although estrogen levels are believed to be positively associated with *Lactobacillus* levels, ovarian stimulation did not influence the vaginal microbiome composition of infertile women who underwent IVF [33]. In contrast, Carosso *et al.* [35▪▪] found that ovarian stimulation altered the composition of the vaginal and endometrial microbiome, hereby affecting endometrial receptivity and implantation. Although the latter study suggests that ovulation induction might alter the microbiome, the timing of sampling might be crucial as well.

Intrauterine microbiome

As mentioned before, due to the very high risk of contamination, it is still unclear whether a (low bacterial biomass) microbiome exists in the uterus. Many studies that have claimed to have detected a microbiota in bodily niches that were traditionally thought to be sterile have now been shown to have mistakenly interpreted contaminants as a resident microbiome [5,9▪▪]. This should serve as a warning

in interpreting studies that claim the presence of uterine microbiota. A recent study involving 342 infertile patients revealed *Lactobacillus* is the most abundant species in endometrium and positively correlates with live birth, while the presence of *Atopobium, Bifidobacterium, Chryseobacterium, Gardnerella, Haemophilus, Klebsiella, Neisseria, Staphylococcus*, and *Streptococcus* were associated with adverse outcomes after ART [36▪]. However, samples with a low or absent bacterial load are often contaminated with bacteria from reagents from other laboratory sources. These contaminants are often Gram-negative bacteria, which could also explain the finding of bacteria that are generally uncommon to the urogenital tract, such as *Chryseobacterium, Haemophilus,* and *Neisseria* [37]. Hence, an endometrial LD microbiome appeared to be a predictive biomarker for a successful reproductive outcome [36▪,38]. However, other studies did not find a significant association between LD endometrial microbiome and successful reproduction outcomes [39,40]. In a study of 34 infertile women, paired vaginal, and endometrial samples were obtained before IVF stimulation. Endometrial tissue obtained transcervical showed that *Lactobacillus* was only found in the group with failed IVF In vaginal samples, *Lactobacilli* were associated with positive pregnancy outcomes, although not statistically significant [39]. Furthermore, in a study of 48 infertile women analysis of vaginal and endometrium samples obtained during the IVF cycle showed no significant species diversity between those who got pregnant and those who did not [40]. There is clear evidence that the reported endometrial microbiome seems to be composed of vaginal bacteria (contamination during sampling) and bacteria that are very unusual to find in humans (laboratory or reagent contamination) [24].

Recurrent implantation failure and microbiome

RIF has an estimated prevalence of 15% [41]. RIF is frustrating for both patients and physicians, while the pathogenesis of RIF remains unclear [27] and interventions to prevent RIF have not been proven beneficial [42]. Alternatively, some women may lack the ability to distinguish good versus poor quality embryos and therefore accept every embryo. Hence, the low-quality embryos may be eliminated later on during later occurring integrity checks resulting in higher miscarriage rates [43].

It is suggested that endometrial microbiota might have a role in the occurrence of RIF. The vaginal and endometrial microbiome of 145 women with RIF and 21 healthy controls with male factor infertility were compared in a Japanese study. The vaginal microbiome of the RIF group had lower levels of *Lactobacillus*, although the endometrial microbiome composition was similar among both [44]. In addition, the RIF group had significantly higher levels of 14 other genera *(Atopobium, Megasphaera, Gardnerella, Prevotella, Enterococcus Schlegelella, Delftia, Burkholderia, Sphingobacterium, Dietzia, Micrococcus, Ralstonia, Leucobacter,* and *Hydrogenophaga)*. Of note, the latter nine genera are common contaminants questioning again whether a core uterine microbiome exists [23]. Finally, a prospective cohort study of 48 infertile women undergoing cryopreserved embryo transfer, showed that patients with RIF had higher levels of species as *Prevotella*, *Lactobacillus Helveticus*, *Sneathia amnii,* while pregnant women showed higher levels of *L. iners* and *L. Jensenii* [40]. Of note, women without RIF had an alteration in the vaginal microbiome diversity, whereas women with RIF had a stable microbiome pattern over time. Authors suggest this lack of dynamics in vaginal microbiome pattern may indicate an inability to adapt to endometrium physiology, and hence a poor prognosis for embryo implantation [40].

FUTURE PERSPECTIVES

Current knowledge about the urogenital microbiome and its association with ART outcome is limited: certain CST are related to poor reproductive outcomes although with no current evidence-based treatment or microbiome restoration options. Restoring a healthy urogenital microbiome is challenging due to several reasons. First, a healthy microbiome, defined as a s collection of microbial taxa, with genomic and functional characteristics that are unique to a particular host or environment [45], has yet to be established. Second, administration routes of probiotics are challenging due to the low pH of the upper gastrointestinal tract (oral route) and the subsequent necessity to deliver probiotics to the right colonization location via the physical ascending path (vaginal route) or through the bloodstream (oral or vaginal route) [46]. The combination of oral and vaginal antibiotics (metronidazole) and vaginal probiotics by increasing the *Lactobacillus*' proportion may be a successful treatment for RIF patients with non-*Lactobacillus*-dominant (NLD) microbiota [17]. Finally, until we discover a tool for microbiome recolonization, restoring and targeting these bacteria remains difficult.

In addition, microbiome analyses are mostly conducted using NGS: with short-read sequencing of the hypervariable regions (V1–V9) of the bacterial 16S rRNA gene [47]. Long-read sequencing using nanopore sequencing technology is also feasible to detect urogenital microbiota [48]. Nanopore sequencing is used to sequence reads longer than

2 Mbp, which can be used to improve bacterial species identification. Another approach is using the IS-pro technique, a eubacterial technique based on detecting and identifying bacteria by a combination of sequence polymorphisms of the 16S rDNA region and length polymorphisms of the 16S-23S rRNA gene intergenic spacer regions, highly specific for different microbial species [49]. Finally, future studies should also focus on a more holistic approach of the microbiome including proteomics, metabolomics as well as the virome and its role in reproduction [50].

CONCLUSION

The current literature aims either define the healthy or dysbiotic female genital tract microbiome to provide insights into physiological processes related to successful embryo implantation and which allow prediction of ART outcomes. The healthy vaginal microenvironment is a dynamic *Lactobacilli* dominant ecosystem and alterations could impact its function and ability to prevent pathogens to establish an infection. Determining the vaginal microbiome prior to an ART treatment might predict ART outcome and pregnancy. Moreover, repeated implantation failure seems to be associated with a more diverse non-*Lactobacilli* dominant urogenital microbiome. The presence of a unique endometrial microbiome remains debatable, given its potential low bacterial biomass, the invasiveness of endometrial sampling, and the unavoidable high contamination risk. Evidence supports that contamination from the lower reproductive tract and reagents are considered components of the endometrial microbiome, resulting in an outcome that is an artifact. Future studies should also focus on the microbiome and its population (proteomics, metabolomics, virome) to get a holistic view of its role in reproductive outcomes.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

X.G., Y.L., and S.S. have nothing to declare. J.L. reports unrestricted research grants from Ferring, the Dutch Heart Association, ZonMw. He also received consultancy fees from Ferring, Gedeon Richter, and Titus Healthcare, outside the submitted work. A.B. holds a position at ArtPred BV, a company investigating and selling assays to predict the success rate of ART procedures based on vaginal microbiota composition.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. ■ Schoenmakers S, Laven NJ. The vaginal microbiome as a tool to predict IVF success. Curr Opin Obstet Gynecol 2020; 32:169–178.

Previous review on the recent microbiome research and findings within the field of reproductive medicine and its relation with the outcome of assisted reproductive technology.

2. Koedooder R, Singer M, Schoenmakers S, *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; 34:1042–1054.
3. Aagaard K, Ma J, Antony KM, *et al.* The placenta harbors a unique microbiome. Sci Transl Med 2014; 6:237ra65.
4. Stinson L, Hallingstrom M, Barman M, *et al.* Comparison of bacterial DNA profiles in mid-trimester amniotic fluid samples from preterm and term deliveries. Front Microbiol 2020; 11:415.
5. Kennedy KM, Gerlach MJ, Adam T, *et al.* Fetal meconium does not have a detectable microbiota before birth. Nat Microbiol 2021; 6:865–873.
6. ■■ Kuperman AA, Zimmerman A, Hamadia S, *et al.* Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. BJOG 2020; 127:159–169.

Analyses using microbiological and molecular methods did not find evidence of bacteria in human placentas.

7. de Goffau MC, Lager S, Sovio U, *et al.* Human placenta has no microbiome but can contain potential pathogens. Nature 2019; 572:329–334.
8. Rackaityte E, Halkias J, Fukui EM, *et al.* Viable bacterial colonization is highly limited in the human intestine in utero. Nat Med 2020; 26:599–607.
9. ■■ de Goffau MC, Charnock-Jones DS, Smith GCS, Parkhill J. Batch effects account for the main findings of an in utero human intestinal bacterial colonization study. Microbiome 2021; 9:6.

The authors identified a batch effect that violates the underlying assumptions of the bioinformatic method used to remove contamination in the study from Rackaityte *et al.* [8].

10. Milani C, Duranti S, Bottacini F, *et al.* The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 2017; 81:e00036–e00017.
11. Farage M, Maibach H. Lifetime changes in the vulva and vagina. Arch Gynecol Obstet 2006; 273:195–202.
12. Gliniewicz K, Schneider GM, Ridenhour BJ, *et al.* Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. Front Microbiol 2019; 10:193.
13. ■ France MT, Ma B, Gajer P, *et al.* VALENCIA: a nearest centroid classification method for vaginal microbial communities based on composition. Microbiome 2020; 8:166.

VALENCIA classifies samples according to their diversity using the nearest centroid method, allowing researchers to characterize the vaginal microbiome uniformly by allocating any microbial community to one of 13 community state types.

14. Ravel J, Gajer P, Abdo Z, *et al.* Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 2011; 108(Suppl. 1):4680–4687.
15. Amabebe E, Anumba DOC. The vaginal microenvironment: the physiologic role of lactobacilli. Front Med (Lausanne) 2018; 5:181.
16. O'Hanlon DE, Moench TR, Cone RA. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. PLoS One 2013; 8:e80074.
17. Kadogami D, Nakaoka Y, Morimoto Y. Use of a vaginal probiotic suppository and antibiotics to influence the composition of the endometrial microbiota. Reprod Biol 2020; 20:307–314.
18. Fettweis JM, Brooks JP, Serrano MG, *et al.* Differences in vaginal microbiome in African American women versus women of European ancestry. Microbiology (Reading) 2014; 160(Pt 10):2272–2282.
19. Chen C, Song X, Wei W, *et al.* The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat Commun 2017; 8:875.
20. ■■ Wang J, Li Z, Ma X, *et al.* Translocation of vaginal microbiota is involved in impairment and protection of uterine health. Nat Commun 2021; 12:4191.

Study showed that vaginal microbiome transplantation from chronic endometritis patients in vaginas of rodent models can induce inflammation in the uterine cavity. Aging, delivery mode and abortion history have a substantial impact on the composition of the uterine microbiome.

21. ■ O'Callaghan JL, Turner R, Dekker Nitert M, *et al.* Re-assessing microbiomes in the low-biomass reproductive niche. BJOG 2020; 127:147–158.

The assignment of uterine microbiota is difficult due to the low biomass and high risk of amplification bias from contaminating bacteria.

22. Winters AD, Romero R, Gervasi MT, *et al.* Does the endometrial cavity have a molecular microbial signature? Sci Rep 2019; 9:9905.
23. Salter SJ, Cox MJ, Turek EM, *et al.* Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 2014; 12:87.
24. de Goffau MC, Lager S, Salter SJ, *et al.* Recognizing the reagent microbiome. Nat Microbiol 2018; 3:851–853.

25. Verstraelen H, Vilchez-Vargas R, Desimpel F, *et al.* Characterisation of the human uterine microbiome in nonpregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. PeerJ 2016; 4:e1602.
26. Teklenburg G, Salker M, Heijnen C, *et al.* The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. Mol Hum Reprod 2010; 16:886–895.
27. Franasiak JM, Alecsandru D, Forman EJ, *et al.* A review of the pathophysiology of recurrent implantation failure. Fertil Steril 2021; 116:1436–1448.
28. Governini L, Luongo FP, Haxhiu A, *et al.* Main actors behind the endometrial receptivity and successful implantation. Tissue Cell 2021; 73:101656.
29. Kong Y, Liu Z, Shang Q, *et al.* The disordered vaginal microbiota is a potential indicator for a higher failure of in vitro fertilization. Front Med (Lausanne) 2020; 7:217.
30. Karaer A, Doğan B, Günal S, *et al.* The vaginal microbiota composition of women undergoing assisted reproduction: a prospective cohort study. BJOG Int J Obstet Gynaecol 2021; 128:2101–2109.
31. Hao X, Li P, Wu S, Tan J. Association of the cervical microbiota with pregnancy outcome in a subfertile population undergoing in vitro fertilization: a case-control study. Front Cell Infect Microbiol 2021; 11:654202.
32. ■ Fu M, Zhang X, Liang Y, *et al.* Alterations in vaginal microbiota and associated metabolome in women with recurrent implantation failure. mBio 2020; 11: e03242–e03219.

The authors analyzed alterations in the microbiome and metabolome concerning IVF outcomes and found that important metabolites are required for embryo growth and implantation.

33. Zhao C, Wei Z, Yang J, *et al.* Characterization of the vaginal microbiome in women with infertility and its potential correlation with hormone stimulation during in vitro fertilization surgery. mSystems 2020; 5:e00450–e00420.
34. Garcia-Velasco JA, Budding D, Campe H, *et al.* The reproductive microbiome – clinical practice recommendations for fertility specialists. Reprod BioMed Online 2020; 41:443–453.
35. ■■ Carosso A, Revelli A, Gennarelli G, *et al.* Controlled ovarian stimulation and progesterone supplementation affect vaginal and endometrial microbiota in IVF cycles: a pilot study. J Assist Reprod Genet 2020; 37:2315–2326.

The current study describes the effect of ovarian stimulation on the composition of the vaginal and endometrial microbiome. This observation may have important consequences since hormones can alter the microbiome composition.

36. ■ Moreno I, Garcia-Grau I, Perez-Villaroya D, *et al.* Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Microbiome 2022; 10:1.

The current study showed that *Lactobacillus* is associated with live birth outcomes.

37. Glassing A, Dowd SE, Galandiuk S, *et al.* Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. Gut Pathog 2016; 8:24.
38. Toson B, Simon C, Moreno I. The endometrial microbiome and its impact on human conception. Int J Mol Sci 2022; 23:485.
39. Riganelli L, Iebba V, Piccioni M, *et al.* Structural variations of vaginal and endometrial microbiota: hints on female infertility. Front Cell Infect Microbiol 2020; 10:350.
40. Diaz-Martinez MDC, Bernabeu A, Lledo B, *et al.* Impact of the vaginal and endometrial microbiome pattern on assisted reproduction outcomes. J Clin Med 2021; 10:4063.
41. Busnelli A, Reschini M, Cardellicchio L, *et al.* How common is real repeated implantation failure? An indirect estimate of the prevalence. Reprod Biomed Online 2020; 40:91–97.
42. Busnelli A, Somigliana E, Cirillo F, *et al.* Efficacy of therapies and interventions for repeated embryo implantation failure: a systematic review and meta-analysis. Sci Rep 2021; 11:1747.
43. Weimar CH, Kavelaars A, Brosens JJ, *et al.* Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. PLoS One 2012; 7:e41424.
44. Ichiyama T, Kuroda K, Nagai Y, *et al.* Analysis of vaginal and endometrial microbiota communities in infertile women with a history of repeated implantation failure. Reprod Med Biol 2021; 20:334–344.
45. Neu AT, Allen EE, Roy K. Defining and quantifying the core microbiome: challenges and prospects. Proc Natl Acad Sci U S A 2021; 118: e2104429118.
46. Lopez-Moreno A, Aguilera M. Probiotics dietary supplementation for modulating endocrine and fertility microbiota dysbiosis. Nutrients 2020; 12:757.
47. Altmae S, Rienzi L. Endometrial microbiome: new hope, or hype? Reprod Biomed Online 2021; 42:1051–1052.
48. Oberle A, Urban L, Falch-Leis S, *et al.* 16S rRNA long-read nanopore sequencing is feasible and reliable for endometrial microbiome analysis. Reprod BioMed Online 2021; 42:1097–1107.
49. Singer M, Koedooder R, Bos MP, *et al.* The profiling of microbiota in vaginal swab samples using 16S rRNA gene sequencing and IS-pro analysis. BMC Microbiol 2021; 21:100.
50. Sola-Leyva A, Andres-Leon E, Molina NM, *et al.* Mapping the entire functionally active endometrial microbiota. Hum Reprod 2021; 36:1021–1031.