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Review

What fertility specialists should know about the vaginal microbiome: a review

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KEY MESSAGE
The vaginal microbiome composition varies between women, and has the capacity to fluctuate throughout a woman's life. The abundance of various bacterial species in the vagina greatly impacts a woman's reproductive health and pregnancy outcomes. The vaginal microbiome may be altered by exogenous factors that may impact reproductive health outcomes.

A B S T R A C T

Our understanding of the composition of the microbial communities that inhabit the human body, known as the 'microbiome', is aided by the development of non-culture-dependent DNA sequencing. It is increasingly apparent that the balance of microbial species greatly affects the health of the host. Disturbances in the composition of bacterial communities have been shown to contribute to various disease states, and there is a growing body of evidence that the vaginal microbiota, which is unique to each woman, plays an important role in determining many facets of reproductive health. The purpose of this review is to investigate what is currently known about the composition of the vaginal microbiome, including what is considered 'normal' in terms of bacterial species and abundance. We will investigate the impact of vaginal microbiome composition on reproductive outcomes within the context of infertility treatments, and the implications this has been shown to have on assisted reproductive technology procedures.

Introduction

The sequencing of the human genome in 2001 (Venter et al., 2001), while a notable achievement in the biological and physiological worlds, is considered by some to be only a partial physiological blueprint for humans. The human microbiota, i.e. the community of microorganisms that populate us, both inside and out, is understood to contribute to this blueprint, and plays a significant role in determining the health status of the individual. Until recently, the primary focus

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http://dx.doi.org/10.1016/j.rbmo.2017.04.005

Table 1 – Definitions.

Term	Definition
Microbiota	The microbial taxa that are associated with an environment and are revealed using molecular techniques such as 16S rRNA sequencing (Cho and Blaser, 2012; Ursell et al., 2012).
Microbiome	Refers to the habitat as a whole, thus incorporating the biotic and abiotic factors, encompassing host and microorganism genomes and environmental conditions (Cho and Blaser, 2012).
Metagenome	The collection of genomes and genes from the inhabitants of a microbiome (Marchesi and Ravel, 2015).
Metatranscriptome	The total content of gene transcripts in a community at a specific sampling time (Parro and Paz, 2015).
Metaproteomics	Interrogation of the entire protein complement of a community at a given time point (Wilmes and Bond, 2004).
Culturomics	Method allowing the determination of microbial community composition by high-throughput culture (Greub, 2012).
Biofilm	A structured consortium attached to a living or inert surface formed by microbial cells adherent to each other and surrounded by the self-produced extracellular polymeric matrix (de la Fuente-Nunez et al., 2013; Hall-Stoodley et al., 2004).
Dysbiosis	Qualitative and quantitative changes, their metabolic activity and their local distribution (Holzapfel et al., 1998).
Probiotic	Live microorganisms which when administered in adequate amounts confer a health benefit on the host (Sanders, 2008).
Prebiotic	A non-digestible food ingredient that benefits the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995).

rRNA = ribosomal RNA.

of human microbiotal characterizations has been within the context of a disease state; however, it is now recognized that the indigenous microbiota also serve to maintain human health (Zhou et al., 2010).

Prior to the advent of high-throughput DNA sequencing, culture-based methods were employed to identify bacterial species found in the body. However, comprehensive large-scale microbiome sequencing has been facilitated by advances in gene sequencing in the past two decades (Gonzalez et al., 2011; Peterson et al., 2009). Thus, culture-based data, while still informative, must be interpreted within the limits imposed by those paradigms. They detect only a small proportion of (mostly aerobic) organisms, and are not truly representative of the clinically relevant spectrum.

Most bacterial communities coexist in mutualistic relationships with the human host, and it is known that our microbiota evolved together with our genome (Moran and Sloan, 2015). However, it is also clear that microbial dysbiosis can result in disease, and the outgrowth of opportunistic pathogens can threaten the health and life of the human host.

In order to navigate and explore this exciting micro-ecosystem and its inhabitants, we need an extensive roadmap. The importance of such a roadmap is shown by the global research effort and funding dedicated to creating genetic profiles of the entire human microbiome (Mullard, 2008). It has been suggested that the human microbiome may be thought of as the 'second human genome', and recent data published in the field have revealed that it is just as complex (Franasiak and Scott, 2015a, 2015b). In recent years, the microbiota in various body sites, such as the gastrointestinal (GI) and urogenital tracts, have been investigated, where it has been established that the urogenital site contributes 9% of the whole human microbiome, compared with the GI tract, which makes up 29% of the total (Peterson et al., 2009).

To understand the ever-growing metagenomic lexicon, the terminology frequently used in the field is explained in **Table 1**.

Technological advances in DNA/RNA, protein and metabolite analytic platforms, combined with the development of 'big data' computing capabilities, have revealed that the true diversity of the microbiome had been greatly underestimated, as less than 1% of bacteria grow and form colonies on agar plates (Sirota et al., 2014). **Figure 1** depicts the multiple methods by which the microbiota of a species can be interrogated.

Profiles of the vaginal microbiome (VMB) were generated via 16S ribosomal RNA (rRNA) profiling (16S rRNA gene sequencing via PCR and pyrosequencing) (Human Microbiome Project Consortium, 2012), Sanger sequencing or via whole genome sequencing (Sirota et al., 2014). Recent findings from the Human Microbiome Project demonstrate that while many different species of *Lactobacillus* are present in the vaginal tract, there are a few that predominate; indeed, in comparison with the GI microbiome, the microbial diversity within the reproductive tract is relatively narrow (Gonzalez et al., 2011; Peterson et al., 2009). These advances have transformed the field of microbial community analysis and subsequently the areas of health that may be directly or indirectly influenced by it, such as fertility and reproduction. Indeed, this transformation is shown by the exponential increase in the number of publications describing the influence of the composition and structure of the inhabiting microbial communities on reproductive health and fertility outcomes (Green et al., 2015; Haahr et al., 2016; Ma et al., 2012; Nuriel-Ohayon et al., 2016; van de Wijgert and Jespers, 2016).

The objective of this review is to provide the reader with a clear understanding of the terminology used in this field, which is sometimes unknown to reproductive health and IVF specialists. This review will also examine the impact of a dynamic, adaptable microbiota on assisted reproductive technology procedures and reproductive outcomes, and the impact of such procedures on the composition of the microbiota itself.

The VMB of healthy women

The VMB has been recognized as an important factor involved in the protection of the host from various bacterial, fungal and viral pathogens. Moreover, the VMB of the mother plays an essential role in the initial colonization of newborn babies, which has consequences for the immune system and neurodevelopment (Dominguez-Bello et al., 2010).

A 'healthy' VMB is generally defined as a lack of symptoms and various infections, and is associated with good pregnancy outcomes. The 'normal' microbiome of the vagina in non-pregnant healthy women predominantly includes a variety of *Lactobacillus* species, which promote a healthy, supportive environment for the embryo in the pre- and peri-conceptual period (Sirota et al., 2014). In healthy individuals, *Lactobacillus* species dominate this ecosystem at a concentration of 10^7–10^8 colony forming units per gram of vaginal fluid. Via the metabolism of carbohydrates and sugars, *Lactobacillus* species generate

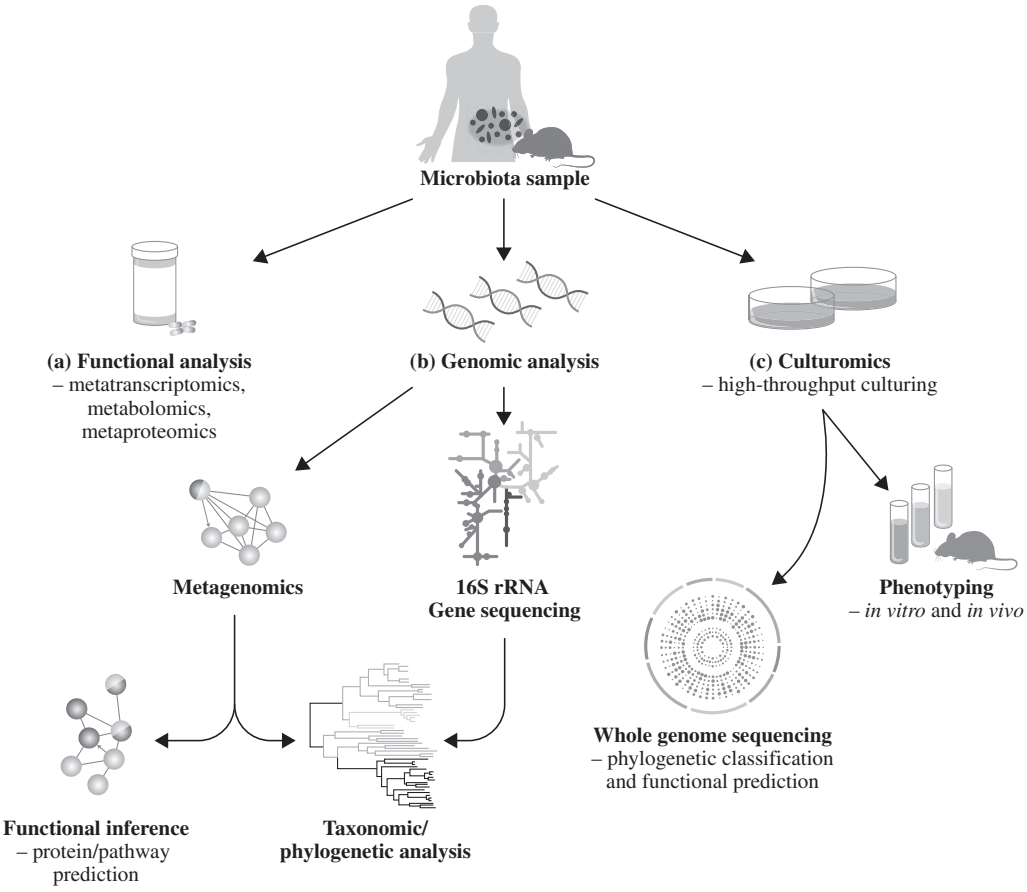

Figure 1 – Overview of some of the most common techniques used to study the human microbiota. Image adapted from Pham and Lawley (2014).

lactic acid in the vagina (Boskey et al., 2001). Lactic acid production is a hallmark feature of normal VMB, as the resulting lower vaginal pH creates an unfavourable environment for the growth of pathogenic bacteria (O'Hanlon et al., 2013; Petrova et al., 2015). Lactic acid not only creates an inhospitable environment for the growth of pathogenic bacterial vaginosis (BV)-associated microbes, but this vaginal environment is also related to lower infection rates upon vaginal exposition to human immunodeficiency virus (Witkin, 2015), thereby promoting the growth of a low-pathogenic microbiota (O'Hanlon et al., 2013).

Species-level classification of the VMB

In 2005, Verhelst and colleagues were one of the first groups to categorize the VMB of 197 pregnant women into several grades or ecological communities, using a combination of Gram staining and 16S rRNA sequencing (Verhelst et al., 2005). Subsequent studies have further characterized the VMB in various ethnic groups based on microbiota composition (again identified by 16S rRNA sequencing and vaginal pH), revealing significant differences between ethnicities (Ravel et al., 2011; Zhou et al., 2007). Strikingly, Ravel and colleagues demonstrated that there is no 'core VMB' common to all women, rather their analysis of 396 asymptomatic White (24.8%), Black (26.2%), Asian (24.5%) and Hispanic (24.5%) women showed that there are in fact multiple core microbiomes that vary according to ethnicity, which can be divided into categories similar to those proposed by Verhelst and colleagues (Ravel et al., 2011; Zhou et al., 2007). This observation is important because differences in microbial composition may radically influence how vaginal communities respond to infections or other imbalances.

Broadly, the VMB may be classified into different community groups, or grades. The five main grades characterized by Ravel and colleagues, designated as I, II, III, IV and V, contained 104, 25, 135, 108 and 21 microbial taxa, respectively. Grade I, characterized by a dominance of *Lactobacillus crispatus*, was found in 26.2% of the sampled population, while Grades II (6.3%), III (34.1%) and V (5.3%) were identified by a dominance of *Lactobacillus gasseri*, *Lactobacillus iners* and *Lactobacillus jenseii*, respectively. These four main groups were isolated primarily from White and Asian women. Grade IV, found mainly in Black and Hispanic women, was classified as non-*Lactobacillus* dominated, and included *Gardnerella*, *Pretovella*, *Corynebacterium*, *Atopobium*, *Megasphaera* and *Sneathia*. This study could constitute the first step towards personalized medicine for women's reproductive health related to the microbiome, wherein differences between the VMB of individuals would be taken into account in risk assessment and for disease diagnosis and treatment (Ravel et al., 2011).

These findings were partially confirmed by Smith et al. (2012). Their study, comparing the VMB of 10 Costa Rican women over a 7-year period, did not identify a VMB in which *Lactobacillus jenseii* dominated. However, they did identify two further microbial community grades, characterized by the presence of *Gardnerella vaginalis* (Grade VI), and even

distribution of *Gardnerella vaginalis* and *Lactobacillus* species (Grade VII) (Smith et al., 2012).

Dysbiosis/infections

Gardner and Dukes named the vaginal disorder *Haemophilus vaginalis* vaginitis and described 'clue cells' in 1955 (Gardner and Dukes, 1955). The first diagnostic criteria for BV were published by Amsel et al. (1983). Later, Eschenbach and colleagues determined that the essential factors for the presence of BV is a lack of H_2O_2-producing *Lactobacilli* associated with an overgrowth of *Gardnerella vaginalis* and other anaerobic Gram-negative rods and anaerobic Gram-positive cocci (Eschenbach et al., 2000).

In addition to species variation, the relative abundance of individual microbes in the vagina influences the reproductive health status of the individual. A link between perturbations in the 'normal' vaginal flora (specifically, a relative scarcity of *Lactobacillus* and an overgrowth of anaerobic bacteria) and disease states was observed as early as the mid-1900s (Gardner and Dukes, 1955). In addition, 16S rRNA Illumina sequencing of isolates from 14 BV patients revealed a huge diversity in inter-patient species, implying that no single pathogen is responsible for the development of the disorder. Rather, BV may be described as an 'ecological disorder of the vaginal microbiota' (Shipitsyna et al., 2013), which prompts questions regarding the suitability of metronidazole or clindamycin, the antibiotics usually selected to treat it. Given that the VMB serves as first-line defence against pathogenic organisms, an understanding of its ecological diversity will undoubtedly contribute to the characterization of various disease states.

In clinical settings, BV is typically diagnosed using Amsel criteria [three of the following four criteria should be present: (i) clue cells on wet mount microscopy; (ii) a 'fishy' odour after adding 10% potassium hydroxide to vaginal secretions; (iii) vaginal pH.4.5; and (iv) thin, homogenous vaginal discharge (Amsel et al., 1983; Money, 2005)]. In research settings, BV is also often defined by Gram stain Nugent scoring, which is based on microscopic visualization of three bacterial morphotypes (a Nugent score of 0–3 is considered normal, 4–6 intermediate microbiota, and 7–10 BV) (Nugent et al., 1991). BV is the most common vaginal disorder in women of reproductive age. In a systematic review and meta-analysis of 12 studies, van Oostrum and colleagues reported that the incidence of BV was significantly higher among women with tubal infertility compared with women with non-tubal infertility [odds ratio (OR) = 2.77, 95% confidence interval (CI) 1.62–4.75] (van Oostrum et al., 2013). While BV was not associated with decreased conception rates (OR = 1.03, 95% CI 0.79–1.33), it was associated with a significantly elevated risk of preclinical pregnancy loss (OR = 2.36, 95% CI 1.24–4.51) (DiGiulio et al., 2015; van Oostrum et al., 2013). These results should be interpreted with care, as some of the studies included were carried out using the Gram stain technique rather than with 16S RNA sequencing.

Interestingly, the first biofilms in gynaecology, specifically on the endometrium and Fallopian tubes, were described in women with BV by Swidsinski et al. (2005). The BV biofilm comprises mainly *Gardnerella vaginalis* and *Atopobium vaginae*, with lower concentrations of *Lactobacilli* present (Mendling et al., 2014). Neither the immune system nor antibiotics eradicate all of the microbes within these biofilms (Cerca et al., 2006; Machado et al., 2015); therefore biofilm-related infections may be persistent and women with BV tend to have high recurrence rates (Bradshaw et al., 2006).

Intrauterine infection is observed in many cases of premature birth. Microbes may gain access to the amniotic cavity via several mechanisms – by ascending from the vagina and cervix, haematogenous transfer through the placenta, accidental inoculation and colonization through invasive procedures, or via retrograde transfer from the Fallopian tubes (Goldenberg et al., 2000). The most common pathway leading to intrauterine infection is the ascending route, and although most researchers believe that ascent occurs during the second trimester, the timing is largely unknown; some women may harbour asymptomatic endometrial colonization before becoming pregnant (Goldenberg et al., 2008).

Physiological and environmental factors

Menstrual cycle-induced fluctuations in circulating oestrogen levels impact upon the VMB, and as such, the decline in oestrogenic levels during the menopause is associated with a decrease in *Lactobacillus* species in the vaginal tracts of post-menopausal women (Cribby et al., 2008). The high levels of circulating oestrogens in reproductive-age women causes glycogen deposition in the vaginal epithelium (Jakobsson and Forsum, 2008; Yamamoto et al., 2009; Zhou et al., 2007), and the increasing glycogen favours the growth of glucose-fermenting micro-organisms, such as *Lactobacillus* species (Yamamoto et al., 2009). Research has revealed that not only is the diversity of the VMB unique to each individual woman, but each woman's VMB composition fluctuates throughout her reproductive lifespan (Cribby et al., 2008; Ravel et al., 2011; Yamamoto et al., 2009).

The microbiome of the vagina continually evolves as the woman encounters and responds to various physiological and environmental challenges (Braundmeier et al., 2015). Before the menarche, the vaginal microbiota is an unsteady mix of skin and gut microbes, which may include some *Lactobacilli* (Fettweis et al., 2012). After puberty, the environmental conditions for *Lactobacilli* species are improved by oestrogens and progesterone with the start of and during the reproductive phase. Oestrogens support the proliferation of the vaginal epithelium and the development of intraepithelial glycogen, while progesterone supports the cytolysis of epithelial cells, which release glycogen. Lactobacilli and other bacteria are able to metabolize this glycogen to glucose and maltose and further to lactic acid. This leads to a vaginal pH of 3.8–4.4, which is defined as normal.

Age, pregnancy, sexual activity, smoking and exogenous hormones are some of the factors that influence the composition of the VMB (Cherpes et al., 2008; DiGiulio et al., 2015; Romero et al., 2014; Ryckman et al., 2009; Sirota et al., 2014). It is not known how genetic variations affect VMB constitution and maintenance, but it has been postulated that genetic polymorphisms, which disrupt normal signalling of the innate immune system, may be associated with less healthy flora (Genc and Onderdonk, 2011). Genc and colleagues demonstrated that an allelic polymorphism in the intron of a gene in the interleukin-1 cytokine family, specifically IL1RN2, is associated with elevated vaginal pH and, in Black women, a decrease in *Lactobacillus* species levels.

Effects of assisted reproductive technology procedures on the VMB

Hormonal and methodological influences during IVF

IVF protocols provide an opportunity to study fluctuations of the VMB in the context of defined hormonal shifts that are representative of

the hormonal variations throughout a woman's lifespan, with very low oestrogen levels at the beginning of the cycle and increasing during pregnancy. Endometrial implantation of the embryo is the singular most important event that determines the success of IVF-embryo transfer (Fanchin et al., 1998). Successful embryo transfer hinges upon many factors, including the presence of microbial colonization of the upper genital tract (Kroon et al., 2012). Uterocervical microbial colonization has been suspected to influence conception rates, with possible causes including an association between cervical microbial species and a pre-existing uterine infection, or colonization of the endometrium or the embryo during transport through the colonized cervix (Salim et al., 2002).

Indeed, the nature of uterocervical colonization has been shown to be an independent and significant factor determining assisted reproductive technology success (Salim et al., 2002). Egbase and colleagues first demonstrated this link in a study of 110 women undergoing IVF-embryo transfer and intracytoplasmic sperm injection (ICSI), in which the endocervical swabs at time of embryo transfer and embryo transfer catheter tips were analysed for microbial colonization (Egbase et al., 1996). A significantly higher clinical pregnancy rate per transfer was observed in the group with no microbial growth, compared with the group whose cultures were positive for microbial colonization (57.1% and 29.6%, respectively, $P < 0.005$) (Egbase et al., 1996). Further correlations between bacterial transfer via embryo transfer catheter tip and pregnancy outcomes have been reported by Moore et al. (2000). In their prospective clinical trial in 91 women, an increase in live-birth rate was associated with the recovery of H_2O_2-producing *Lactobacilli* from the vagina ($P = 0.01$) and the embryo transfer catheter ($P = 0.01$), while a reduction in live-birth rate was associated with recovery of *Streptococcus viridans* from the embryo transfer catheter tip ($P = 0.04$) (Moore et al., 2000). In clinics, great care is taken to reduce the risk of microbial transfer into the cervix during embryo transfer by cleaning the external os of the cervix with culture medium and by avoiding touching the vaginal walls and external cervix with the catheter tip. Again, using culture-based identification methods, Fanchin and colleagues investigated the impact of cervical micro-organisms on IVF-embryo transfer outcomes (Fanchin et al., 1998). In this study, which involved 265 women undergoing ovarian stimulation for IVF-embryo transfer, significantly lower clinical pregnancy rates, ongoing pregnancy rates and implantation rates were observed in those who tested positively for the presence of cervical micro-organisms (24% versus 37%, $P < 0.02$; 17% versus 28%, $P < 0.04$; and 9% versus 16%, $P < 0.01$, respectively). Among the bacterial species identified in the positive culture group were *Escherichia coli*, including both *Staphylococcus* and *Streptococcus* strains. It is worth mentioning that the studies performed by these authors used culture-based methods for identification of bacterial strains (Egbase et al., 1996; Fanchin et al., 1998; Salim et al., 2002).

Examining the role of the vaginal microbiota in reproduction and assisted reproductive technology procedures is in its relative infancy; while there is a growing literature that supports its relevance, many questions remain unanswered. Several studies have supported the hypothesis that the reproductive tract microbiome on the day of embryo transfer affects pregnancy outcome. Recently Franasiak and colleagues published an encouraging study using next-generation 16S ribosome sequencing (Franasiak et al., 2016). After consecutive single embryo transfer of euploid embryos, the most distal 5 mm portion of the transfer catheter was placed in a DNA-free tube under sterile conditions. Next-generation sequencing was performed on 35 samples from 33 patients and two *Escherichia coli* controls, allowing genus and species calls for micro-organisms. Of the 33 patients, 18 (54.5%) had ongoing pregnancies and 15 (45.5%) did not. There was a total of 278 different genus calls present across patient samples. The microbiome at time of transfer for those patients with ongoing pregnancy compared with those without ongoing pregnancy was characterized by top genera by sum fraction. *Lactobacillus* species were the top species for both outcomes. These data show that the microbiome at the time of embryo transfer can be successfully characterized without altering standard clinical practice. This novel approach, both in specimen collection and analysis, is the first step towards the goal of determining physiological from pathophysiological microbiota at the time of embryo transfer and its impact on pregnancy outcome (Franasiak et al., 2016).

Contraceptives

Choice of contraceptive method has been shown to have an effect on the composition of the VMB (Gupta et al., 2000). A study involving 331 women initiating a birth control method revealed that cervical cap and diaphragm–spermicide use were associated with an increase in *Escherichia coli*, enterococci and anaerobic Gram-negative rod colonization of the vaginal microenvironment (Gupta et al., 2000). In contrast, vaginal colonization by *Escherichia coli* and *Candida* species decreased in women prescribed the oral contraceptive pill (Gupta et al., 2000), although this change was noted as minimal by the investigators. A study of 30 women beginning a combined oral contraceptive regimen showed a nominal effect of such medication on the vaginal microflora; however, a small decrease in the number of women who screened positively for H_2O_2-producing *Lactobacilli* following 2 months of oral contraceptive treatment was observed (Eschenbach et al., 2000). The relative paucity of large-scale studies examining the effects of contraceptive methods on the VMB, using RNA sequencing profiling techniques, makes it difficult to outline definitive conclusions on this relationship at this time.

Ovarian stimulation

Notably, circulating hormones impact greatly upon the VMB and susceptibility of the woman to infection (Hyman et al., 2012). Jakobsson and Forsum (2008) evaluated the changes in the predominant human *Lactobacilli* during IVF, with the aim of characterizing the normal cultivable vaginal flora present at differing oestradiol levels in plasma during ovarian stimulation as part of an IVF treatment. Culture media and sequencing techniques were combined in these studies to identify the bacterial strains.

The hormone-dependent microbiotal shifts may then be related to pregnancy outcomes, as investigated by Hyman et al. (2012). Using 16S rRNA deep sequencing, the authors reported an IVF-embryo transfer induced shift in the VMB in some, but not all, of the women sampled, with novel bacterial strains found in 33% of women screened during the treatment cycle. Further larger scale and controlled studies are required to investigate the effects of defined hormonal shifts on the vaginal milieu and subsequent IVF-embryo transfer outcomes.

Down-regulation with gonadotrophin-releasing hormone analogues

As previously discussed, exogenous hormones impact upon the vaginal environment and the composition of the VMB. A study investigating the effects of a gonadotrophin-releasing hormone agonist (GnRHa)

in the context of endometriosis therapy revealed that the vaginal pH increased significantly above 4.5 both in control women and women with clinically identified endometriosis following 4–6 months of GnRHa treatment ($P = 0.0004$ and $P = 0.003$, respectively) (Khan et al., 2014). The authors also reported a significant shift towards intermediate vaginal microflora and a decrease in 'normal' vaginal microflora in women with endometriosis ($P = 0.05$ and $P = 0.007$, respectively). Similar, but non-significant, shifts were seen in the control group (Khan et al., 2014). Bacteriological examination of endometrial samples from all women revealed significantly higher colonization of *Gardnerella* and *Escherichia coli* in both groups of women. Application of the antibiotic levofloxacin reduced the growth of cultured *Escherichia coli* isolated from vaginal smears, prompting the authors to postulate that administration of probiotics and/or antibiotics may be considered useful to treat subclinical vaginal or uterine infections (Khan et al., 2014). This further emphasizes the importance of vaginal microbial profiling in the context of IVF-embryo transfer treatments, as endometriosis is associated with infertility, and this study provides evidence that treatment for endometriosis may affect the VMB.

Altering the VMB

Antibiotics

If we accept the proposition that an altered VMB may have an influence on fertility and assisted reproductive technology outcomes, it is necessary to investigate possible therapeutic alternatives. It is conceivable that the administration of prophylactic oral antibiotics prior to embryo transfer in IVF/ICSI may have an impact on pregnancy rates by reducing the level of microbial colonization of the upper genital tract (Kroon et al., 2012). However, conflicting research findings have been reported to date. Results from a single randomized controlled trial have shown that reduced genital tract colonization following antibiotic treatment with doxycycline was not associated with an increase in clinical pregnancy rate or a reduction in H_2O_2-producing *Lactobacilli* or virulent bacteria (Moore et al., 2000). In contrast, other groups have demonstrated that the administration of prophylactic antibiotics, ceftriaxone and metronidazole, 48 h prior to oocyte retrieval for IVF-embryo transfer, was associated with significantly increased implantation ($P < 0.01$) and clinical pregnancy rates ($P = 0.01$). In one such study, women were subjected to mock embryo transfer prior to antibiotic administration and were classified into three groups based on the presence of bacterial colonization on the embryo transfer catheter tip at both mock and actual embryo transfer: Group 1 were negative for bacterial culture at both procedures; Group 2 tested positive for bacterial presence on catheter tip at mock embryo transfer, but, following antibiotic administration, tested negative; and Group 3 tested positive for bacteria on the embryo transfer catheter tip both at mock and actual embryo transfer (Egbase et al., 1999). It is worth noting that these studies used culture-based bacterial identification methods.

Transcervical procedures, such as hysterosalpingography, saline infusion sonography and hysteroscopy, and transvaginal procedures, such as oocyte retrieval, may potentially seed the endometrium, Fallopian tubes or peritoneal cavity with micro-organisms from the endocervix or upper vagina. Up to now, the use of antibiotics during and after IVF treatments has been associated with risk reduction in these procedures. But there are perhaps several questions we should ask ourselves: what is the impact of these antibiotic treatments on the VMB? Can this have an impact on IVF outcomes? To the best of our knowledge, nothing has been published related to the microbiome's alteration and the utilization of antibiotics during IVF.

Jakobsson and Forsum (2008), as part of their protocol prior to IVF treatment, treated women with prophylactic antibiotics; doxycycline 200 mg on day 1, and 100 mg daily for a further 8 days, and metronidazole three times daily for a week. However, 16 of the patients started IVF treatment 1–2 months later; therefore, the impact of these antibiotics on the microbiome of these patients could not be assessed. The other point to consider when using an antibiotic is their appropriate use in order to reduce infectious post-operative complications and minimize the development of antibiotic-resistant organisms.

As part of the Jakobsson and Forsum (2008) study, male partners of the patients were concurrently given the same antibiotic prophylaxis. Semen contains micro-organisms along with other constituents, such as male reproductive proteins and markers of inflammation. Thus, semen serves as a medium for the transmission of micro-organisms between men and women (Hou et al., 2013). At some IVF clinics, men are also treated with antibiotics prior to sperm sample collection, and, despite its importance for men and their partners, the male genital tract microbiota has been much less frequently studied than the female genital microbiota. It is important for clinicians to appreciate when antibiotic prophylaxis is indicated and when it is inappropriate (Pereira et al., 2016).

Probiotics

As previously discussed, the hallmark of a healthy VMB, in most women, is a relative abundance of *Lactobacillus* species (Ravel et al., 2011; Sirota et al., 2014; Verhelst et al., 2005). The beneficial effects of probiotic supplementation on human health are becoming increasingly recognized by clinicians and, given the abundance and impact of micro-organisms in the reproductive tract, it stands to reason that these effects could potentially be harnessed within the context of reproductive health. Due to the large proportion of bacteria inhabiting the gut, research efforts have thus far focused on determining the impact of oral probiotic treatment on GI health. In 1992, Hilton and colleagues examined the efficacy of treating recurrent candida vulvovaginitis with a daily oral dose of 250 g yoghurt containing *Lactobacillus acidophilus* for 6 months. In women treated with yoghurt, the mean rate of vaginal recurrence was 0.38 compared with 2.5 in the control arm ($P = 0.001$) (Hilton et al., 1992). Subsequently, various bacterial species have been investigated. *Lactobacillus rhamnosus Lcr 35* (Coudeyras et al., 2008a, 2008b) was shown to inhibit the growth of *Gardnerella vaginalis* and *Candida albicans* in vitro via increased glycogen metabolism and lactic acid production.

In recent years, the health benefits of probiotic supplementation have been the subject of much research, and while there is currently no evidence of a direct beneficial effect of probiotics on reproductive health outcomes, oral *Lactobacillus rhamnosus* and *Lactobacillus fermentum* supplementation have been shown to restore healthy vaginal flora in up to 82% of women with previous vaginal dysbiosis, specifically an increase in *Lactobacillus* species (Reid et al., 2001). Macklaim et al. (2015), using 16S rRNA gene sequencing, reported that tandem treatment of BV with antimicrobial therapy and probiotic supplementation, namely *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, increased the abundance of indigenous *Lactobacillus iners* and *Lactobacillus crispatus*. This observation opens up the possibility of harnessing specific bacterial strains in order to prime the vaginal microbiota in the pre- and peri-conceptual periods;

however, further investigations are necessary to elucidate the optimum combination, dosing regimen and route of administration of such treatments to improve women's health (Cribby et al., 2008). Crucially, probiotics containing *Lactobacillus* may be used over a long period, an attractive trait of an alternative to antibiotics, particularly within the context of high infection recurrence rates (Mastromarino et al., 2013).

Looking to the future

Throughout a woman's lifespan, the VMB undergoes dramatic shifts that coincide with hormonal and lifestyle changes. The complexity of these communities and the genetic and environmental factors that influence these changes have yet to be fully described, although the use of next-generation DNA sequencing technologies should further expand knowledge in this area. A healthy vaginal microbiota can help to prevent urogenital conditions such as urinary tract infections and BV; therefore, studies leading to a better understanding of the VMB will facilitate the discovery of improved treatments and diagnostics for such conditions. The composition of the vaginal microbiota also has a notable impact on pregnancy and neonatal outcomes. A recent study by Moreno and colleagues examined whether the composition of the endometrial microbiota influenced pregnancy outcomes in women undergoing IVF. Interestingly, the authors found a correlation between adverse pregnancy outcomes and an endometrial microbiota that was poor in *Lactobacillus* species (Moreno et al., 2016). The authors concluded that the negative effect of non-*Lactobacillus*-dominated endometrial microbiota should be considered as one of the causes of poor reproductive outcomes, implantation failure and loss of pregnancy.

By better understanding the VMB and its dynamics throughout pregnancy, researchers hope to promote reproductive health and reduce the risk of infertility, spontaneous abortion and preterm birth. Crucially, a mother's VMB may serve as the evolutionary role of seeding the microbiome of her baby at birth, which may influence lifelong microbiome composition and health (Neu and Rushing, 2011). Indeed, one of the primary determinants of a newborn's microbiotal colonization is the method of delivery (Dominguez-Bello et al., 2010). Large-scale epidemiological studies have reported an increased incidence of chronic health deficiencies in infants delivered via Caesarean section, while infants born via the birth canal develop microbiotal communities resembling those of the maternal VMB (Huh et al., 2012; Sevelsted et al., 2015). To that end, a recent pilot study by Dominguez-Bello and colleagues investigated whether the microbiota of infants born via Caesarean section could be manipulated by direct inoculation of the newborn with the vaginal fluid of the mother following Caesarean delivery. The investigators found the newborn skin, gut and oral microbiotas were enriched with VMB species during the first 30 days of life, in a similar manner to those born naturally, demonstrating the possibility of restoring the microbiota of Caesarean-born infants (Dominguez-Bello et al., 2016). Longitudinal studies will be instrumental in determining whether this method of inoculation at birth carries through later in life, and how the long-term health status of these infants is affected.

NIH-funded research currently under way in the Ravel Laboratory at the University of Maryland School of Medicine will determine the link between bio-behavioural and social factors, birth gestation and the VMB. Specifically, the study follows 400 women (recruited from areas associated with higher than average rates of preterm birth) from 20 weeks' gestation until birth. This work will help to elucidate the effect of modifiable social and bio-behavioural factors on the VMB during pregnancy (http://ravel-lab.org/vaginal-microbiome-and-preterm-birth/). Interestingly, as of December 2016, there are 28 ongoing clinical trials registered on clinicaltrials.gov that include the search terms 'pregnancy' and 'microbiome' (http://clinicaltrials.gov).

Because microbial contamination at embryo transfer may influence implantation rates, more prospective studies are required to examine whether eradication of endocervical micro-organisms is possible, and whether their eradication will improve implantation rates. A systematic review, investigating whether consistent patterns of VMB could be determined across various population subgroups (van de Wijgert et al., 2014), identified only two studies in which the population being examined consisted of women undergoing IVF treatment. The increased availability and accessibility of high-throughput sequencing technology will no doubt aid further studies in this area, which will lead to increased understanding of normal physiology, pathological development of disease in dysbiotic states and the influence of the VMB on reproductive outcomes (Franasiak and Scott, 2015a, 2015b). In addition, while prophylactic antibiotics are commonly used as part of IVF-embryo transfer protocols to prevent infections after invasive pelvic procedures, their effect on implantation in the setting of IVF-embryo transfer still remains to be determined. Recently, following a review of the literature, Pereira et al. (2016) recommended antibiotic prophylaxis prior to oocyte retrieval in patients with a history of endometriosis, pelvic inflammatory disease, ruptured appendicitis or multiple prior pelvic surgeries (Pereira et al., 2016).

Although extensive basic research has been done in this area and very interesting data are being published, there is still a lack of translational data that delineate the role of the VMB in assisted reproductive technology procedures and in clinical practice. Overall, there is a growing body of evidence that both embryo-fetal environmental factors and the maternal microbiome may play an important role in natural fertility and assisted reproductive technology outcomes. Improving knowledge in this area could inform future research about modifications to the individual's microbiome as a potential strategy to prevent adverse outcomes and to foster the development of a healthy microbiome in the mother and their offspring.

Conclusions

The composition of the vaginal microbiome differs greatly between women, and has the capacity to fluctuate throughout an individual woman's life. The abundance of various bacterial species in the vagina greatly impacts a woman's reproductive health and pregnancy outcomes. The vaginal microbiome in turn may be altered by exogenous factors that may lead to improved pregnancy outcomes, however much research is still needed to elucidate the balance of bacterial species that result in optimal reproductive health outcomes for each woman.

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 5 April 2017
Accepted 6 April 2017

Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
Assisted reproductive technology
Dysbiosis
IVF
Pregnancy outcome
Probiotics
Vaginal microbiome

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