Cervicovaginal Microbiota and Women's Health Outcomes

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Total Word count:
1600

Key words
Eubiosis, Dysbiosis, *Lactobacillus*, STI, Infertility.

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Abstract:

The human cervicovaginal microbiome has an important role in the health and homoeostasis of the female reproductive tract. A eubiotic microbiome is typically dominated with lactic acid producing bacteria and is categorised into five community state types. Issues arise when the microbiome becomes dysbiotic, with the microbial composition shifting to contain a greater relative abundance of strict and facultative anaerobes. This shift will lead to several adverse changes in the vaginal environment including compromised epithelial cells, cell death, inflammation, and greater susceptibility to infection. These changes are associated with various adverse outcomes including infections, preterm birth, and infertility. In this review, we discuss how the cervicovaginal microbiome influences these outcomes and possible future directions of treatment and research.

Cervicovaginal Microbiota and Women's Health Outcomes

Introduction:

The human microbiome is a unique collection of microorganisms which colonises the body and has an important role in health and homeostasis. The cervicovaginal microbiome is particularly distinctive as it is frequently dominated by *Lactobacillus* with decreased diversity of other bacteria, unlike what is seen in other sites such as the gut[1]. The cervicovaginal microbiome is extremely important to the host tissue as it maintains an acidic environment, preventing pathogenic colonisation, and modulates inflammation by cross-kingdom signalling[1]. Thus, the composition of cervicovaginal microbiome plays an important role in health outcomes for women particularly in relation to vaginal infection, pregnancy, and fertility.

The Eubiotic Microbiome:

Early culture-based studies identified *Lactobacillus* as the dominant bacteria in the vaginal microbiome and recognised that it may play a key role in maintaining the health of the female reproductive tract[2]. Molecular-based techniques, including relatively recent next generation sequencing, have been used to obtain an in-depth understanding of vaginal flora and to classify microbiota into broad profiles termed community state types (CST)[3, 4]. Four CSTs are dominated by a species of *Lactobacillus*; *Lactobacillus crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III) and *L. jensenii* (CST V). CST IV is characterised by various strict and facultative anaerobes and is typified by the absence of a dominant *Lactobacillu*s species. The CSTs have varying levels of stability and transitions between CSTs are associated with composition, menstrual cycle, and sexual activity[4]. *Lactobacillus* produces lactic acid, maintaining vaginal pH at ≤4.5, promoting a selective environment for acid tolerant bacteria whilst suppressing pathogenic colonisation (Figure 1). Lactic acid has an immunomodulatory function, acting directly on epithelial cells to promote an anti-inflammatory response via the production of interleukin(IL)-1 receptor antagonist, as well as promoting the production of pro-inflammatory mediators and antimicrobial peptides (Figure 1)[5].

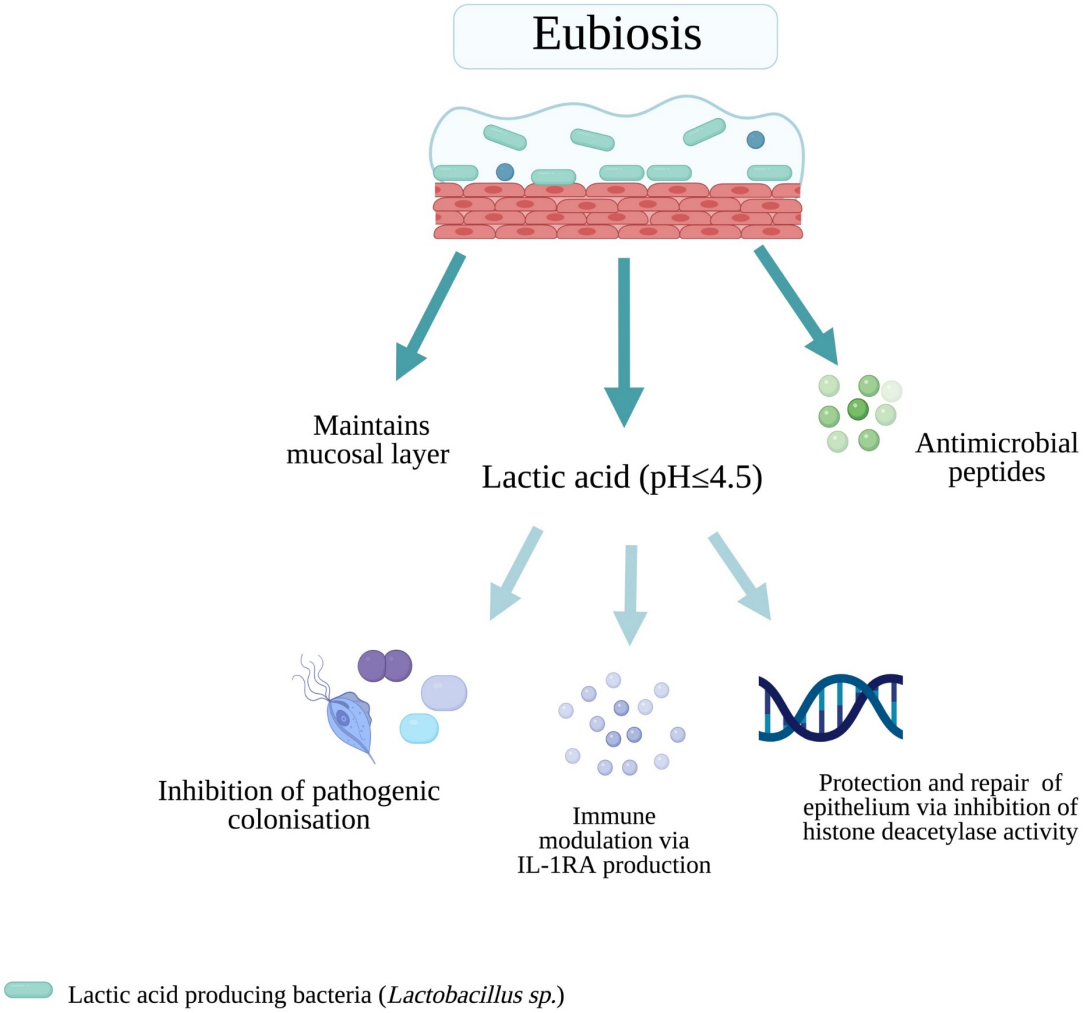


Figure 1. Eubiotic microbiome. Bacteria maintain the mucosal layer, release antimicrobial peptides, and lactic acid. Lactic acid lowers the pH, preventing pathogenic colonisation, and modulating the immune response, protecting the epithelial layer. Created with BioRender.com.

The Dysbiotic Microbiome:

Dysbiosis is defined as a change in microbiota composition relative to the community of commensal bacteria seen in a healthy state[6]. There is no specific bacteria universally seen in dysbiosis but it is frequently associated with increased relative abundance of *Gardnerella, Prevotella,* and *Atopbium*[7, 8]. This shift in composition results in a decrease in lactic acid, with an increase in short chain fatty acids, amines, and pH (Figure 2)[9]. Dysbiosis is also associated with several detrimental changes in the cervicovaginal environment including alterations in the cytoskeleton, increased cell death, an imbalance in the concentration of antimicrobial peptides and increased production of pro-inflammatory cytokines (Figure 2)[10]. These changes are thought to leave the tissue susceptible to infection and inflammation.

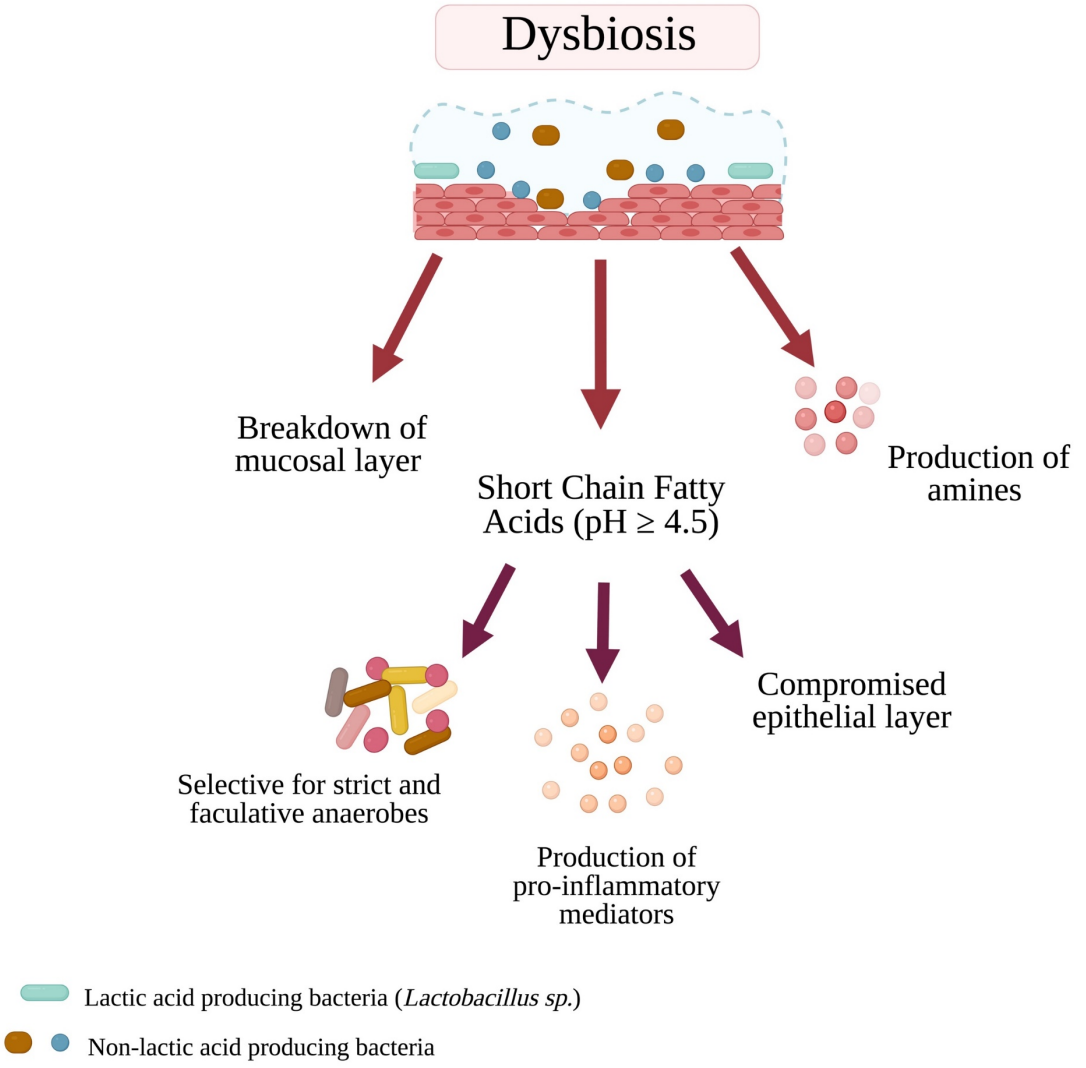


Figure 2. Dysbiotic microbiome. There is a breakdown in the mucosal layer, and the production of amines and short chain fatty acids, increasing the pH. This creates an environment selective for strict and facultative anaerobes, a pro-inflammatory response in the tissue, and compromises the epithelium. Created with BioRender.com.

Bacterial Vaginosis:

Bacterial vaginosis (BV) is the most common vaginal infection, characterised by dysbiosis and the associated metabolomic changes. BV often is asymptomatic, but women may experience symptoms such as discoloured vaginal discharge, and a 'fishy' odour. The prevalence of BV is variable between different populations but worldwide prevalence is approximately 30%, with prevalence in Australia considerably lower at 4.7%[11]. Treatment with oral or intravaginal antibiotics is only recommend for women experiencing symptoms. However, after treatment, reoccurrence is common with up to 60-80% of women experiencing reoccurrence within 12-months after treatment[12]. Recent research has now shifted to investigating the variables associated with reoccurrence, specifically microbiota composition, to improve the treatment outcomes for women with BV[13].

Sexually Transmitted Infections

Dysbiosis of the cervicovaginal microbiota is known to increase the risk of acquiring a sexually transmitted infection (STI). Numerous longitudinal studies have determined that high microbiota diversity increases the risk of acquiring an infection[14]. A possible mechanism which increases susceptibility may be an inflammatory response to diverse bacteria. Gosmann *et al.*[15] investigated the association between the microbiome, inflammation, and human immunodeficiency virus (HIV)-acquisition in a prospective cohort study of South Africa women. They determined that women with polymicrobial microbiomes dominated with anaerobes, had increased activated mucosal CD4+ T cells, and 4-fold greater risk of HIV infection. They suggested that the target cells were responding to the microbial diversity, which in turn increase host susceptibility[15]. A similar response is also hypothesised to be involved in human papillomavirus infection, but is yet to be investigated[16]. Another mechanism involved in the susceptibility is the modulation of cellular functions. Ceccarani *et al.*[17] investigated the changes in the microbiome and metabolome during *Chlamydia* infection. In comparison to a healthy state, they showed clear changes in composition occurred during infection, specifically a decrease in lactic acid. Similarly, Edwards *et al.*[18] showed D (-) lactic acid produced by the microbiome may prevent cellular proliferation, protecting against *Chlamydia* infection. They suggested that a eubiotic microbiome modulates cell function preventing *Chlamydia* infection *in vitro*. These studies support that via direct metabolic profiles and cross talk involved in host cell responses, the cervicovaginal microbiome influences the risk of STI acquisition.

Pregnancy

The composition of the cervicovaginal microbiome has been associated with increased risk of adverse outcomes in pregnancy such as preterm birth. Preterm birth is defined as either a live or still birth after 20 weeks' gestation but before 37 weeks[19]. During pregnancy, hormonal changes alters the composition of the cervicovaginal microbiota resulting in an increased abundance of *Lactobacillus*. Several studies have shown that women with a diverse, non-*Lactobacillus* dominated microbiome are at a greater risk of preterm birth[20, 21]. However, there is no defined profile of bacteria associated with adverse outcomes and results from each study greatly vary due to the population and study design. Kosti *et al.*[22] recently conducted meta-analysis to address these issues and created a microbial signature associated with preterm birth. They successfully identified a lack of *Lactobacillus* as a predictor of preterm birth,

alongside several species which had been previously reported. Interestingly, they identified an association between preterm birth and the presence of *Olsenella* and *Clostridium sensu stricto* which had not been previously reported [22]. Overall these promising results show the potential for novel diagnostics that could guide interventions to improve pregnancy outcomes for women at risk.

Infertility Treatment:

Infertility is defined as the inability to attain a clinical pregnancy after 12 months of regular unprotected intercourse [19]. *In vitro* fertilisation (IVF) is now the most common procedure used to treat a range of infertility issues [23]. However, in Australia, the success rate of IVF procedures is reported as approximately 30% with little improvement over the last five years [24]. Poor outcomes of IVF have been associated with the composition of the cervicovaginal microbiome in several studies, although these studies often have a small sample size and mixed quality of methodologies. Initially Hyman *et al.*[20] associated diverse vaginal bacteria with poor IVF outcomes and suggested that the composition of the microbiome at the time of embryo transfer may be an important factor in the success of IVF treatment. Since this initial study there have been several others that have associated increased diversity of cervicovaginal microbiota and the presence of specific bacteria, with IVF failure[25-27]. However, there is no defined profile of microbiota associated with poor outcomes in IVF treatment, mostly due to the lack of larger studies. To understand the pathogenesis of this relationship, Fu *et al*.[28] conducted a study to assess changes in the microbiome and metabolome in association with the outcomes of IVF failure. They determined that there was a lack of key metabolites necessary for embryo development and implantation such as glycerophospholipids and benzopyran in those who experienced IVF failure, and in turn these metabolite differences were associated with different compositions of microbiota. Whilst this study shows some promising results, the pathophysiology involved in this relationship is yet to be fully explored.

Future Directions:

It is clear the cervicovaginal microbiome plays a key role in health outcomes for women, with dysbiosis commonly observed in a range of adverse events. However, the mechanisms underlying these relationships are not well understood. Future microbiome and metabolome models will provide a method of representing these interactions *in vitro*. Delgado-Diaz *et al*.[29], used key metabolites associated with a *Lactobacillus* dominated microbiome and BV-associated microbiome to model the response of cells. This showed the immunomodulatory effect of lactic acid, but also showed that a lack of lactic acid and high concentrations of short chain fatty acids would stimulate increased production of pro-inflammatory cytokines. Thus, the approach of using an *in vitro* model is a promising method to better understand the microbiome and host cell interplay at a molecular level. Furthermore, large studies are necessary to determine predictive biomarkers of adverse outcomes, and to inform development of treatments such as probiotics for targeted treatment of the microbiome.

Conflicts of Interest:

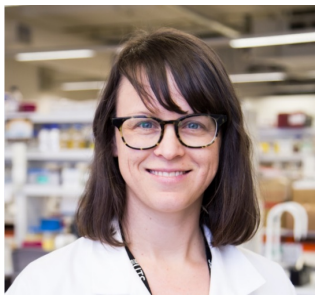
The authors declare no conflicts of interest.

Acknowledgments:

This research did not receive any specific funding.

Biographies (and picture):

Ciara J Bryant is a PhD candidate at the University of Technology Sydney. She has completed her honours degree in biomedical science and is continuing to investigate the factors involved in infertility and IVF failure in her PhD.



Catherine Burke is a Senior Lecturer at the University of Technology Sydney. She studies the human microbiome in a range of health and disease states. She was awarded her PhD in 2010 from the University of New South Wales.

Wilhelmina (Willa) M Huston is an Associate Professor at University of Technology Sydney, her research team investigates chlamydia and other STIS in the context of women's health,in order to assist with identification of improved diagnostics and therapies. She was awarded her PhD in 2004 from the University of Queensland. She was also awarded the 2020 ASM Frank Fenner Award.

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