



# Exploring Immunome and Microbiome Interplay in Reproductive Health: Current Knowledge, Challenges, and Novel Diagnostic Tools

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## Abstract

### Keywords

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- interactions

The dynamic interplay between the immunome and microbiome in reproductive health is a complex and rapidly advancing research field, holding tremendously vast possibilities for the development of reproductive medicine. This immunome–microbiome relationship influences the innate and adaptive immune responses, thereby affecting the onset and progression of reproductive disorders. However, the mechanisms governing these interactions remain elusive and require innovative approaches to gather more understanding. This comprehensive review examines the current knowledge on reproductive microbiomes across various parts of female reproductive tract, with special consideration of bidirectional interactions between microbiomes and the immune system. Additionally, it explores innate and adaptive immunity, focusing on immunoglobulin (Ig) A and IgM antibodies, their regulation, self-antigen tolerance mechanisms, and their roles in immune homeostasis. This review also highlights ongoing technological innovations in microbiota research, emphasizing the need for standardized detection and analysis methods. For instance, we evaluate the clinical utility of innovative technologies such as Phage ImmunoPrecipitation Sequencing (PhIP-Seq) and Microbial Flow Cytometry coupled to Next-Generation Sequencing (mFLOW-Seq). Despite ongoing advancements, we emphasize the need for further exploration in this field, as a deeper understanding of immunome–microbiome interactions holds promise for innovative diagnostic and therapeutic strategies for reproductive health, like infertility treatment and management of pregnancy.

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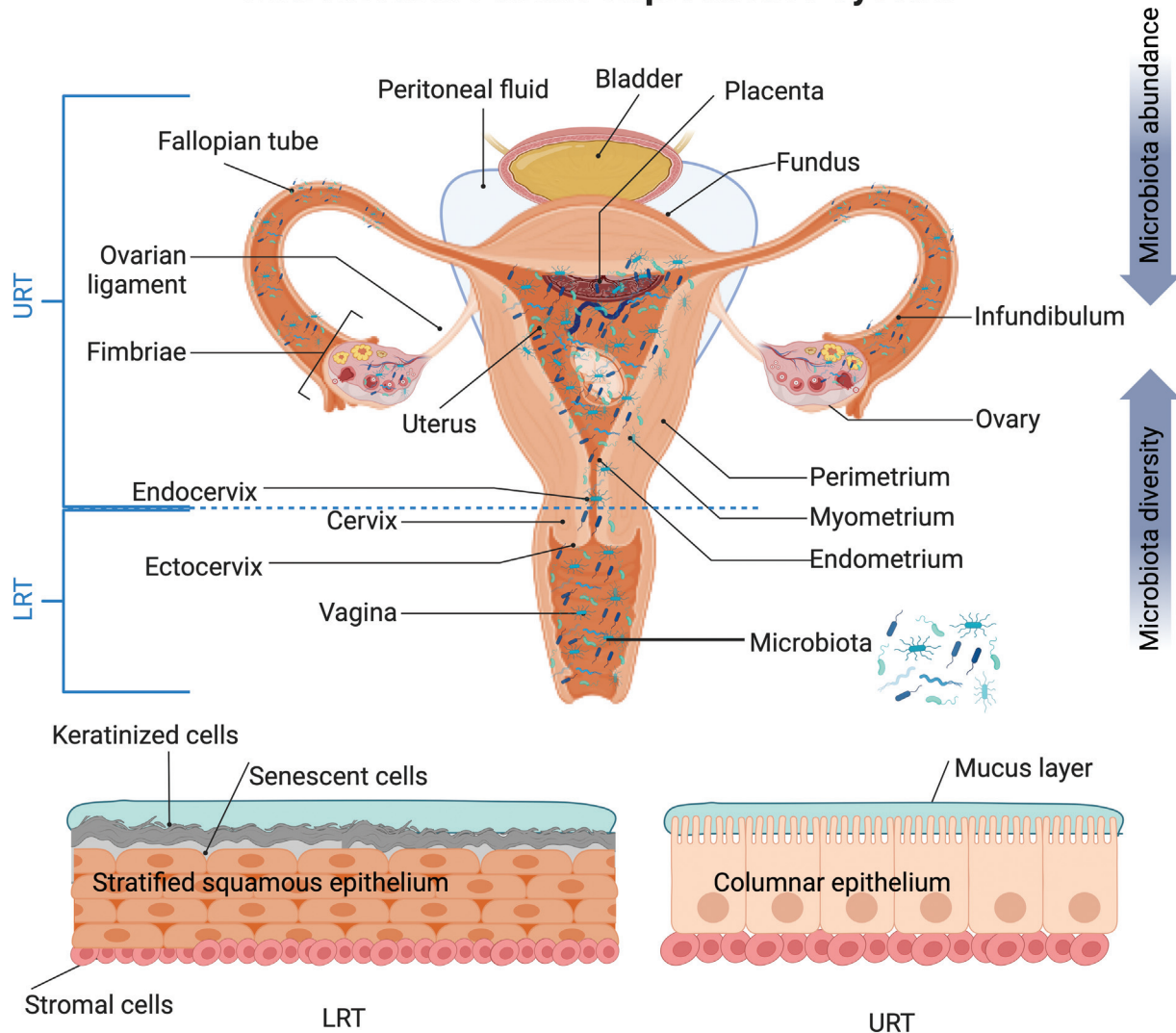
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## Microbiota in Female Reproductive System



**Fig. 1** Distribution of microbiota in the female reproductive tract (FRT). This figure illustrates the microbial composition in the FRT, which is divided into upper and lower reproductive tracts (URT and LRT). The LRT includes the vagina and ectocervix, both lined with a stratified squamous epithelium. This epithelium originates from the basement membrane and culminates in fully keratinized senescent cells. The URTs comprise the endocervix, uterus with endometrium, fallopian tubes, ovaries, peritoneal fluid, and placenta and features a monolayer columnar epithelium. In particular, the transition from stratified squamous epithelium to single columnar epithelium occurs in the “transformation zone” between the ectocervix and endocervix. Microbial abundance was generally higher in the LRT than in the URT, whereas microbial diversity increases from the outermost to the innermost regions. This figure has been adapted from BioRender.com (2023). Source: <https://app.Biorender.com>.

### Overview of the Female Reproductive Tract Microbiota

The human body is known to host trillions of microorganisms, forming our microbiome, a complex ecosystem that significantly affects various aspects of health and disease.<sup>1,2</sup> This so-called microbiome-dominated world is often referred to as a “second genome.”<sup>3</sup> Gut microbiome has been extensively studied, along with the findings emphasizing the role of the female reproductive tract (FRT) microbiota in reproductive health.<sup>4–6</sup> The FRT microbiota comprises bacteria, fungi, viruses, archaea, and protozoa and is collectively referred to as the reproductive tract (RT) microbiota. FRT accounts for ~9% of the total bacterial burden in the

body.<sup>7</sup> Microorganisms produce biologically active substances interacting with endocrine, immune, nervous, metabolic, and reproductive systems, contributing to healthy development by maintaining homeostasis. Changes in the microbiota composition, known as dysbiosis, have been suggested to affect metabolism, immunity, gene expression, epigenetics, and disease risk by altering metabolic enzymes, hormones, and disease risks.<sup>8</sup>

The term FRT refers to both the upper reproductive tract (URT), which includes the endocervix, endometrium, uterus, fallopian tubes, ovaries, peritoneal fluid, and placenta, and the lower reproductive tract (LRT), consisting of the ectocervix, vagina, and vulva (see ▶Fig. 1). Previous studies have revised the notion of sterility in URT, revealing the presence of

microorganisms in locations such as the ovarian follicles, fallopian tubes, uterus, and placenta.<sup>9–16</sup> Each segment of the FRT contains its own microbiome composition, which can vary based on factors such as age, presence of pregnancy, physiological state, lifestyle, and environmental elements. Microorganisms play an essential role in reproductive well-being by protecting against infections, encouraging fertility, and supporting an uncomplicated pregnancy. However, changes in FRT microbiome are suggested to be associated with reproductive disorders, including infertility, endometriosis, recurrent pregnancy loss, and preterm birth.<sup>9,17–20</sup> Therefore, understanding the interactions between microbiome and reproductive health is crucial for designing personalized treatments.<sup>8,17</sup>

## The Role of the Microbiota in Reproductive Health

The microbiome plays an integral role in shaping reproductive health by intricately balancing microbial communities within the female reproductive organs. Notably, the vaginal microbiome, characterized by diverse bacteria, fungi, and viruses, is paramount for vaginal health and infection prevention.<sup>11,21</sup> Previous research has identified five “community state types (CSTs)” within the vaginal microbiome, primarily characterized by the prevalence of *Lactobacillus*-dominant species. More specifically, four out of five CSTs demonstrated dominance by distinct lactobacilli, while CST-IV was characterized by the prevalence of bacteria associated with bacterial vaginosis (BV).<sup>22,23</sup> Factors like hormonal fluctuations, sexual activity, and contraceptive use exert an influence on the vaginal microbiome.<sup>24–26</sup> Lactic acid production by *Lactobacillus* sp. plays a central role in neutralizing pathogens, including *Chlamydia trachomatis*, *Escherichia coli*, and herpes simplex virus 1 (HIV-1).<sup>27–29</sup> Dysbiosis leads to BV, linked to adverse outcomes like sexually transmitted infections (STIs), infertility, and pregnancy complications.<sup>9,30–35</sup> Variations in the vaginal microbiome are evident in conditions such as polycystic ovary syndrome (PCOS) and during pregnancy, predisposing to adverse pregnancy outcomes.<sup>28,36–38</sup>

The cervix connects uterus and vagina, and its microbiota is essential for female reproduction. Despite being sparsely studied, the cervical microbiome includes *Lactobacillus* spp., *Gardnerella vaginalis*, and various bacteria.<sup>39–43</sup> However, it is important to note that sampling methods can impact the results, as they may introduce potential contamination from the vaginal microbiome. Pervious research established connections between cervical microbiota, cervical health, and outcomes such as preterm birth, cervical ripening, and labor.<sup>44,45</sup> Certain cervical bacteria can strengthen the immune response against human papillomavirus, potentially offering effective protection against infection.<sup>46</sup> Microbiota of endometrium is known to have lower biomass and greater diversity than vaginal and cervical tissues as a result of factors such as blood flow and increased pH.<sup>28,47–49</sup> *Lactobacillus* sp., prevalent in the vagina, exhibit variations in abundance in the endometrium.<sup>47,50–52</sup> Studies have suggested that *Lactobacillus*-dominated endometrial microbiota may improve repro-

ductive outcomes; however, conflicting reports have been published.<sup>49,53–58</sup> Imbalances in endometrial microbiota are linked to various reproductive issues, including repeated implantation failure (RIF), pregnancy loss, endometriosis, endometritis, endometrial polyps, hyperplasia, and endometrial cancer (EC).<sup>49,59–63</sup> For instance, *Fusobacterium* infection of the endometrium causes inflammation, leading to conditions such as endometriosis.<sup>19</sup>

In contrast to earlier assumptions that fallopian tubes (FT) are sterile, recent research has demonstrated the presence of host specific microbiome, including *Lactobacillus*, *Staphylococcus*, and *Enterococcus* sp.<sup>9,64</sup> Additionally, variations in bacterial composition may exist between the right and left FTs. *Staphylococcus* spp. was more abundant on the right, while *Lactobacillus* spp., *Enterococcus* spp., and *Prevotella* spp. were more prevalent on the left.<sup>9,64,65</sup> Reproductive-aged women exhibit overlapping bacterial profiles between FTs and endometrium, emphasizing shared microbial fingerprints.<sup>64,66</sup> Concerns have been expressed regarding *G. vaginalis* biofilms migrating from the vagina to fallopian tubes and disrupting pregnancy.<sup>67</sup> However, lactobacilli produce biosurfactants that counteract pathogenic biofilm formation.<sup>68</sup> Further investigations should explore the impact of FT microbiota on reproductive health.

Ovaries, once thought to be completely sterile, harbor distinct microbiome associated with various medical conditions.<sup>15,69</sup> *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* predominate in the ovarian environment, with specific bacteria associated with ovarian cancer.<sup>70,71</sup> The source of the ovarian microbiota remains debated, with possible migration through the vaginal passageway or bloodstream.<sup>72,73</sup> Unfortunately, the ovarian microbiota has not been studied as extensively as other reproductive organs, and the focus of these studies has been given more on certain specific health issues and diseases rather than those of healthy and fertile state, emphasizing the need for further exploration and understanding. The placenta has traditionally been considered sterile. However, certain studies have revealed its richness in microbial composition. Nevertheless, its exploration poses challenges due to the low biomass involved.<sup>74–77</sup> Contrarily, some recent studies have revealed that healthy placenta has no microbiome. Almost all bacterial signals were related either to acquisition of bacteria during labor and delivery or contamination of laboratory reagents with bacterial DNA.<sup>75,78–82</sup> However, considering the conflicting results of previous studies, new and better-planned research is still necessary, as the placental microbiota provides a new frontier that may affect the health outcomes of pregnancy and newborns. Understanding the intricate interactions between reproductive organs and their microbiota is crucial for advancing women's and children's health.

## Importance of the Immune System in Female Reproductive Health

The immune system is an intricate network composed of innate and adaptive components that work together to respond effectively and overcome various health threats. Immune cells provide defense mechanisms against potential pathogens, while tolerating self-antigens to avoid harmful immune

reactions.<sup>83</sup> Simultaneously, the microbiota of FRT interacts with immune cells in an unpredictable environment to modulate immune responses and maintain balance.<sup>84</sup> However, our knowledge of the interaction between microbiota and immune cells in RT remains incomplete, necessitating further investigation. The acquisition of diverse microbiota coincides with the development of the immune system, suggesting a co-evolutionary relationship and creating a symbiotic partnership.<sup>85</sup> A notable component of this symbiotic relationship is the role of maternal secretory (s) immunoglobulin (Ig) A (sIgA) in reproductive health. sIgA plays an essential role in restricting immune activation and inhibiting microbial attachment by binding to nutritional and microbial antigens, contributing to healthier host-microbe interactions in infant intestines.<sup>86</sup> FRT maintains its balance through a complex network comprising epithelial defenses, natural killer cells (NKC), macrophages, dendritic cells (DCs), and T lymphocytes. Epithelial defenses include physical barriers, commensal bacteria with IgA antibodies, and antimicrobial peptides.<sup>87,88</sup> These commensal bacteria provide complex protection, known as colonization resistance.

It is suggested that even subtle disruptions in the delicate relationship between the microbiota and immune system can dramatically affect reproductive health, potentially leading to infertility, miscarriage, or premature birth.<sup>17,88,89</sup> Such imbalances may result in dysbiosis, inflammation, and various reproductive disorders.<sup>17,90</sup> Recent advances in our understanding of immune system dynamics have shed light on their intricate relationship with microbiomes, prompting further research on reproductive health. For example, *Fusobacterium* infections cause an innate immune response, activate transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling pathways, and promote the transformation of endometrial fibroblasts to myofibroblasts, ultimately contributing to endometriotic lesions.<sup>19</sup> This study established, for the first time, a solid link between the microbiome, immune dysfunction, and endometriosis development.<sup>19</sup> Similarly, women whose vaginal microbiome predominantly contained *Lactobacillus crispatus* showed higher IgA levels, suggesting increased immune protection.<sup>32,91</sup> Moreover, contradictory immune regulation and inflammation have been associated with reproductive/pregnancy complications, such as preeclampsia, fetal growth restriction (FGR), gestational diabetes, and maternal weight gain.<sup>92-94</sup> Therefore, understanding the intricate interactions between immune function and the microbiome is critical in unraveling the biological mechanisms that can enhance fertility and contribute to more effective disease treatments.

## Rationale for Studying the Microbiota-Immunome Interaction

This comprehensive review examines the current knowledge of microbiome-immunome interactions in reproductive health using available technologies. We will evaluate the existing literature to understand better how microbiota affects and regulates the immune system in the RT. Understanding this interaction of microbiota-immunome interactions in the context of reproductive health is crucial to unravel the mechanisms

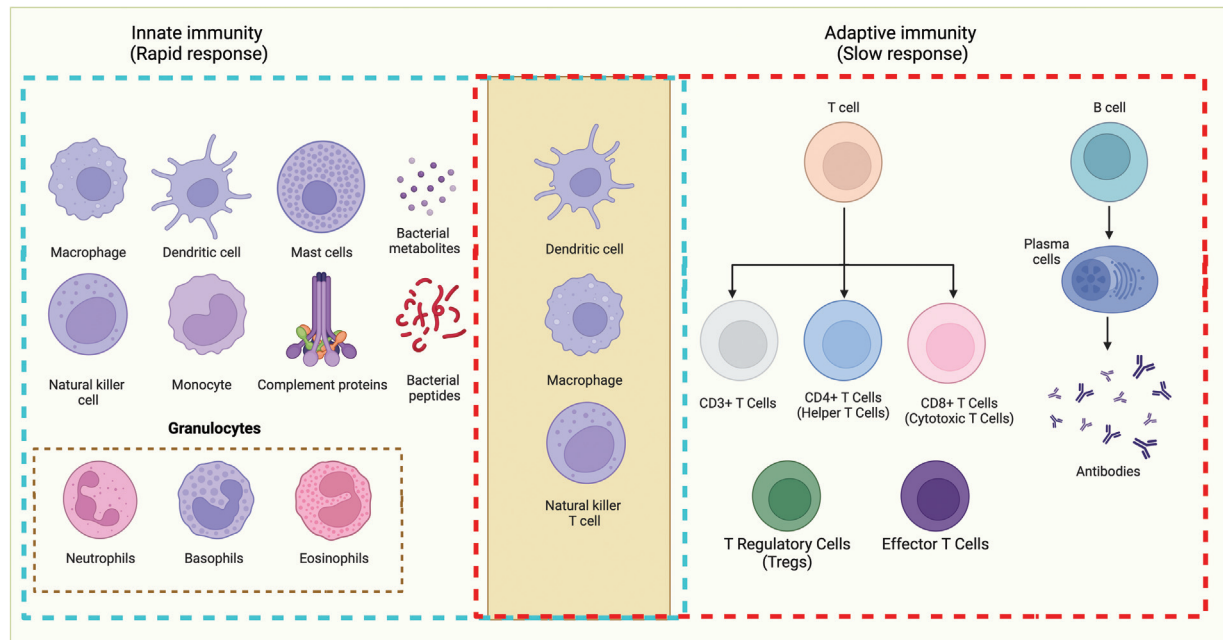
underlying disease etiology, identify biomarkers, develop targeted therapies, and implement preventive strategies. Recent advances in multi-omics technologies, such as metagenomics, 16S rRNA sequencing, Phage ImmunoPrecipitation Sequencing (PhIP-Seq), and Microbial Flow Cytometry coupled to Next-Generation Sequencing (mFLOW-Seq), have allowed for a more comprehensive analysis of microbiota-immunome interactions. This provides valuable insight into personalized interventions and improved reproductive health outcomes, while creating opportunities to protect or restore reproductive health outcomes. Integrating knowledge of microbiota-immunome interactions with advanced technologies has great potential to improve our understanding of reproductive outcomes and enable personalized interventions.

## Immunome-Microbiota Interplay in Female Reproductive Health

The FRT engages in intricate interactions with the external environment, involving resident immune cells and microbiota.<sup>95-98</sup> Pattern recognition receptors (PRRs), including Dectin-1, toll-like receptors (TLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors, detect commensal bacteria in both URTs and LRTs environments.<sup>96,99-102</sup> After exposure to microbes, these receptors activate signaling pathways that result in secretion of proinflammatory cytokines, such as interleukins (IL)-1 $\beta$ , IL-6, IL-8, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). This further triggers the activation and mobilization of various immune cell types, including NK cells, macrophages, CD4+ T cells, CD8+ cytotoxic T cells, and B lymphocytes (see ►Fig. 2).<sup>102-107</sup> Genetic variants in PRR genes, including TLR4, TLR9, IL-1R2, and TNF- $\alpha$ , can influence women's responses to microbiological challenges and pregnancy outcome.<sup>104</sup> PRRs are present in both the squamous epithelial cells lining the vagina and the columnar cells in the upper FRT.<sup>95,96</sup> (see ►Fig. 3). Commensal FRT bacteria contribute to an effective antimicrobial barrier by producing antimicrobial peptides (AMPs) and mucins, which reinforce tight junctions.<sup>84,108</sup> These bacteria also influence immune responses in similar ways as intestinal bacteria by impacting CD4+ T cell differentiation as well as pro- and anti-inflammatory responses.<sup>109,110</sup> Regulatory T (Treg) cells, which are essential for commensal bacterial tolerance, also possess anti-inflammatory properties.<sup>111-113</sup> Increased endocervical Tregs have been linked to reduced proinflammatory cytokine and CD4+ T cell counts.<sup>114</sup>

Antigen-presenting cells (APC), such as DCs, can contribute to Treg selection through the TGF- $\beta$  and retinoic acid (RA) signaling pathways.<sup>115,116</sup> Furthermore, microbiota can activate TLR4 at lower FRT to further support immune tolerance.<sup>89</sup> Immune-microbiota interactions involve leukocyte subsets, plasma cells, interleukin regulation, and antibodies of various classes (IgA, IgM, and IgG).<sup>104</sup> Defense mechanisms include epithelial cells, AMPs, mucous layers, IgA antibodies, commensal bacteria, and immune cells, such as NK cells, macrophages, DCs, and T lymphocytes.<sup>88,99,117</sup> Immune balance protects against pathogens while assuring fetal antigen tolerance during pregnancy.<sup>99,118</sup> Estrogen and progesterone steroid hormones





**Fig. 2** Schematic representation of innate and adaptive immune systems. The immune system comprises two main components: innate and adaptive immunity. Innate immunity, depicted on the left, provides a rapid, nonspecific initial defense against various pathogens, and includes cell types like macrophages, dendritic cells, granulocytes, natural killer cells, and mast cells. The adaptive immune system, shown on the right, offers highly specific protection and involves T and B lymphocytes, with CD8+ T cells (cytotoxic T-lymphocytes) and CD4+ T cells (helper T cells) among its key components. In the middle, the activation of T and B cells is predominantly orchestrated through the stimulation of dendritic cells, macrophages, and natural killer T cells, underscoring the central role of innate immunity in modulating adaptive immune responses. Figure created using BioRender.com (2023). Source: <https://app.Biorender.com>.

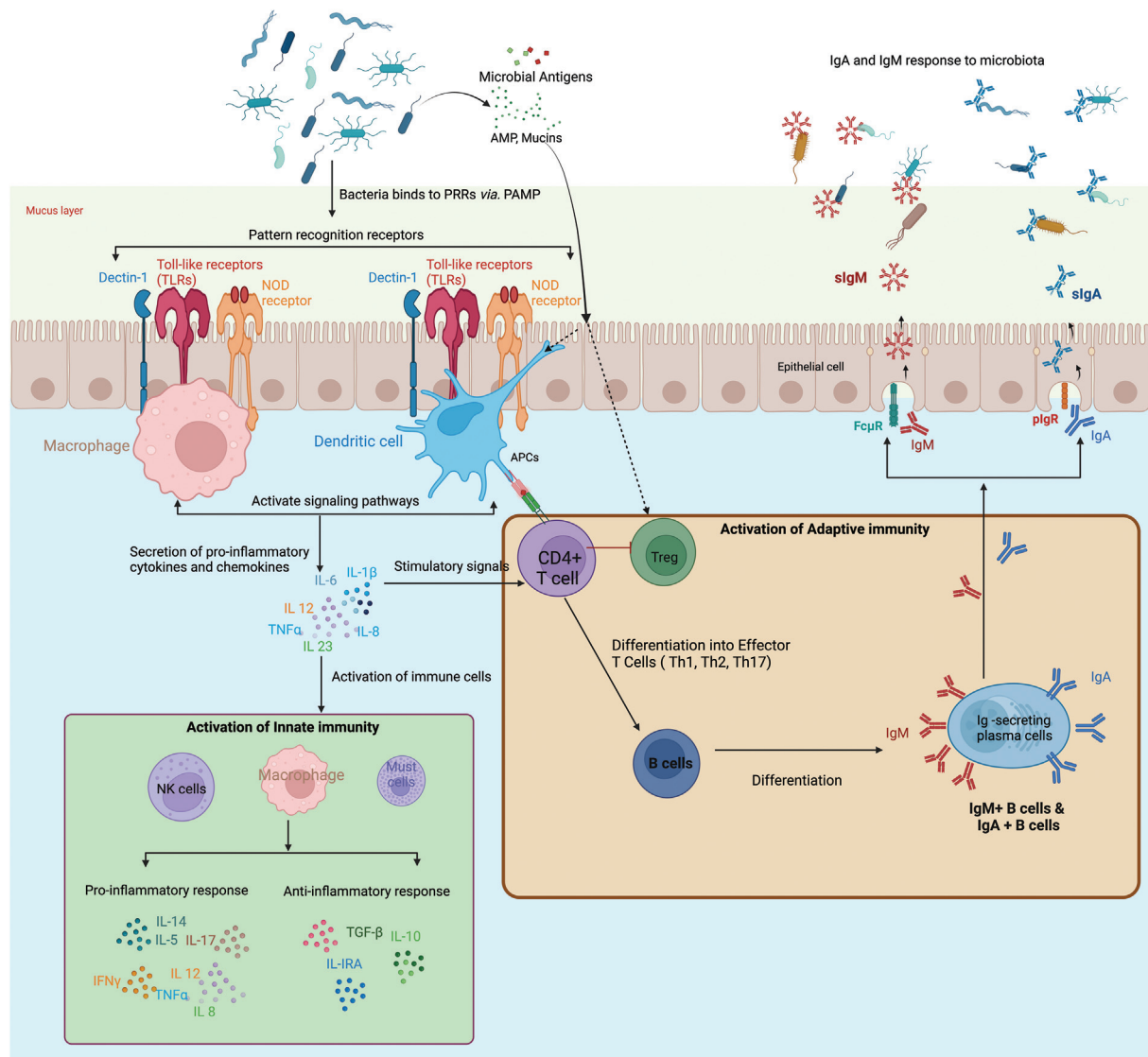
affect immunity and bacterial growth, altering the bacterial composition and susceptibility to infection in FRT.<sup>89,119</sup> Recent research has highlighted the interrelation between microbiomes and adaptive immune systems. Antibodies such as IgA and IgM play a vital role in RT health.<sup>111,120</sup> IgA prevents adhesion of pathogens to epithelial cells, whereas IgM removes them.<sup>121,122</sup> Together, these interactions maintain reproductive health and require further research to understand them fully.

Immunity plays an integral role in reproductive health, especially during gestation, involving complex immune reactions. The orchestrated collaboration between innate and adaptive immunity is vital for maintaining immune homeostasis and defending against potential threats (see ►Fig. 2). This collaboration encompasses various components, such as IgA antibodies, decidual natural killer (dNK) cells, decidual macrophages (DM), DCs, and adaptive immune cells.<sup>99,123–126</sup> Immune responses vary during implantation, placental support, and labor.<sup>99,127</sup> Trophoblast–bacteria interactions activate interferon- $\beta$  (IFN- $\beta$ ) and interferon-stimulating genes (ISG), helping maintain immune homeostasis.<sup>128</sup> Tregs provide immunity and tolerance; however, their exact role remains unknown.<sup>127</sup> The FRT has distinct anatomical layers. The URT is lined with a single-layered columnar epithelium, serving as a vital barrier between the external and internal environments. In contrast, the LRT is characterized by a stratified squamous, non-keratinized epithelium, offering enhanced protection.<sup>89,117</sup> Estrogen plays an essential role in modulating antimicrobial molecules and cytokine production for fertility, including sperm

survival.<sup>102,129</sup> Commensal bacteria influence immune responses through immune cells and mechanisms that vary according to menstrual cycle phase.<sup>126</sup> Tissue-resident NK and Treg cells maintain immunity and tolerance. They are vital for angiogenesis, trophoblast migration, and immune tolerance.<sup>102,126</sup> Commensal bacteria found in the endometrium have been shown to regulate immune responses via NK cells, T-cell subsets, and cytokines.<sup>130–132</sup> The levels of pro-inflammatory cytokines and antimicrobial peptides increase during hormonal fluctuations.<sup>122,129</sup> Cytokine production plays a critical role in response to BV.<sup>133</sup> TLRs in mucosal cells detect molecular patterns associated with pathogens (PAMPs).<sup>100,134</sup> However, prolonged cytokine production can weaken the epithelial barrier and increase susceptibility to infection.<sup>105</sup>

### Interaction between Immunoglobulin A and Microbiota

The interplay between IgA and microbiota is a complex and finely tuned process within the human body, having significant implications for health and disease.<sup>135</sup> As the predominant class of antibodies found in mucosal tissues, IgA plays an integral role in maintaining relationships with various microbial communities on mucosal surfaces such as those found within the digestive, respiratory, and also in RT.<sup>91,104,136</sup> Additionally, IgA antibodies modulate immune defense, pathogen defense, and maintenance of mucosal homeostasis.<sup>136,137</sup> Mucosal immunity encompasses both tolerance for benign microbes and antigens as well as robust protection against pathogens. Mucosal IgA acts as a guardian with several essential functions, including improving microbial diversity,



**Fig. 3** Interactions between microbiota in the reproductive and immune systems. The figure illustrates the mechanisms that govern the generation of IgA and IgM within the reproductive system and how these immunoglobulins, in turn, influence the microbiota. Microbiome-derived factors interact with pattern recognition receptors (PRR), including TLR, Dectin-1, and NOD ligands, while also affecting local and systemic immunity through metabolites. PRRs can directly bind to specific pathogen-associated molecular patterns (PAMPs) on the bacterial surface. Following this interaction, PRRs initiate intracellular signaling through macrophages and dendritic cells, inducing the production of cytokines and chemokines. These molecules, in turn, activate innate immunity through various innate immune cells, producing both pro- and anti-inflammatory responses for microbiota maintenance. The activation of CD4+ T cells by bacteria is crucial in initiating adaptive immune responses. This process involves the presentation of bacterial antigens to CD4+ T cells by antigen-presenting cells (APCs). CD4+ T cells, recognizing the antigens, are co-stimulated by cytokines produced by dendritic cells. Activated CD4 T cells differentiate into various effector cell types, such as regulatory T cells (Tregs), follicular helper T cells (Tfh), and T-helper 17 cells (Th17). Depending on the cytokine microenvironment, cytokines participate in antigen recognition and immune response regulation. These interactions facilitate B cell differentiation into plasma cells, which produce IgA and IgM, thereby contributing to the production of secretory IgA (sIgA) and secretory IgM (sIgM) via pIgR and FcγR receptors, consequently maintaining microbiota homeostasis. In addition, antimicrobial peptides (AMPs) and mucins play multifaceted roles in immunity, including direct antimicrobial defense, modulation of immune responses, influence on the generation of immunoglobulins (IgA and IgM), and contribution to the maintenance of immune tolerance and homeostasis. This figure was adapted from studies on immune interactions of the gut microbiota and was created using BioRender.com (2023). Source: <https://app.Biorender.com>.

countering the effects of toxins and viruses, impeding the colonization of pathogenic bacteria, removing undesirable particles, and facilitating antigen sampling.<sup>138</sup>

Furthermore, the interaction between IgA and the microbiota has far-reaching consequences for immune modulation, facilitating immune exclusion, pathogen defense, and maintenance of mucosal homeostasis. Humans have five classes of

immunoglobulins: IgM, IgG, IgA, IgD, and IgE. IgA is the primary antibody class within mucosal tissues, with two subtypes, IgA1 and IgA2, identified by their unique glycosylation patterns.<sup>136</sup> IgA1 is a widely distributed variant found in the bloodstream, and its functional role remains relatively unknown. On the contrary, IgA2 is secreted mainly in mucosal regions.<sup>138</sup> Previous research has suggested that IgA1 and IgA2 antibodies

broadly react with commensal bacteria found in the small intestine. Remarkably, these antibodies exhibited impressive cross-reactivity by binding to multiple targets simultaneously, even at the clonal level. However, it remains unclear about the selection process and specificity of antibodies independent from microbiota.<sup>139,140</sup> B cells producing IgA2 have become widely recognized for their resistance to bacteria-produced proteases compared with IgA1.<sup>141</sup>

IgA plays an essential role in coating and encasing the commensal microbiota within the digestive tract, known as immune inclusion. Furthermore, immune exclusion is integral to the protection against enteric pathogens by blocking access to the intestinal epithelium.<sup>121,142</sup> IgA deficiency can arise due to B cell dysfunction, an imbalance in cytokines (e.g., IL-4, IL-6, IL-7, IL-10, IL-21, and TGF- $\beta$ ), and compromised survival of plasma cells producing IgA antibodies. To compensate for IgA deficiency, IgM and/or IgG production often increases to regulate intestinal immunity and prevent infection.<sup>143,144</sup> IgA interacts with multiple receptors on immune cells and serves various effector functions. When IgA binds to the polymeric immunoglobulin receptor (pIgR), it initiates the production of secretory IgA (sIgA) (see ► **Fig. 3**). sIgA is highly glycosylated, resistant to degradation, and effectively inhibits pathogen adhesion.<sup>145</sup>

In particular, commensal-specific antibodies predominantly target microbial capsular polysaccharides and lipopolysaccharides through T cell-independent mechanisms, whereas T cell-dependent high-affinity IgA binding sites are used against more immunogenic and potentially harmful commensals.<sup>91,141</sup> IgA exerts its influence on microbiota by selectively coating antigens with antibodies. Furthermore, its interaction with immune cell Fc receptors such as Fc fragment of IgA receptor (Fc $\alpha$ RI) has regulatory effects on their response mechanisms. The expression of Fc $\alpha$ RI in myeloid cells results in a range of cellular responses when cross-linked with IgA immune complexes.<sup>137</sup> Opportunistic bacteria such as *Staphylococcus aureus* and *Streptococcus* have developed strategies to avoid IgA-mediated elimination by interfering with Fc $\alpha$ RI binding.<sup>137,146</sup> Dysregulation of the IgA–Fc $\alpha$ RI interaction contributes to autoimmune and inflammatory conditions. The reciprocal relationship between microbiota and IgA levels adds another layer of complexity, suggesting a potential feedback loop in which microbiota influences IgA production. Simultaneously, IgA can alter the composition of the microbiota, suggesting dynamic crosstalk between immune responses and microbial colonization. Hence, comprehending this interplay holds therapeutic promise for microbiota-related diseases.

Women with *L. crispatus*-dominated vaginal microbiota had higher IgA-coated bacterial levels than those without *L. crispatus*. *L. crispatus* predominates the healthy vaginal microbiota, whereas IgG dominates the vaginal mucosa, and its levels fluctuate throughout the reproductive cycle.<sup>91</sup> Furthermore, cervical immunoglobulin levels of IgA and IgG showed significant variations during ovulation. Factors including elevated estradiol and hemoglobin levels, menstrual cycle phases, advanced age, oral contraceptive use, and

pregnancy have all been associated with increased levels of IgA and IgG antibodies.<sup>147</sup>

In contrast, previous studies have demonstrated that lower estradiol levels during ovulation are strongly correlated with reduced cervical immunoglobulin levels.<sup>147</sup> However, little is known about the role of IgA, IgM, and IgG antibodies in FRT, with an inadequate understanding of their interactions with the microbiota. Furthermore, the composition of reproductive microbiota bound to IgA or IgM remains unknown. Unleashing IgA for therapeutic use requires in-depth knowledge of its complex interactions with microbiota. Addressing these complex interactions could open doors for therapies targeting inflammation, infectious agents, and even cancer, by modulating multiple roles.

### Interaction between Immunoglobulin M and Microbiota

The interaction between immunoglobulin M (IgM) antibodies and human microbiota is fundamental to the innate immune response. IgM is an ancient and highly conserved immunoglobulin isotype found in all species from zebra fish to humans. It serves multiple functions, including acting as the first line of defense against infections, supporting immune cell functions, and regulating tissue homeostasis.<sup>148,149</sup> IgM is distinct from other immunoglobulins because of its ability to exist in both natural (nIgM) and antigen-induced forms, such as membrane-bound IgM-type B cell receptors (BCR) or secreted IgM.<sup>150</sup> nIgM is continuously produced by B1 cells and exhibits polyreactivity, which allows it to interact with autoantigens and pathogens. Its functions include removing apoptotic cells and debris, promoting B cell survival, forming lymphoid tissue architecture, and initiating immune responses.<sup>151–153</sup> Pathogen-induced IgM is produced by both B-1 and conventional B-2 cells in response to pathogens, strengthening early passive IgM-mediated defenses and regulating IgG production. Secreted IgM (sIgM) interacts with cells through various receptors and is essential for neutralizing microbes, T cell recognition, B cell isotype switching, and antigen transport to lymphoid tissues.<sup>148,150,154</sup>

The IgM pentameric structure made up of five interconnected IgM monomers by the J-chain has a higher valency than other immunoglobulins, leading to superior agglutination efficacy, up to 10,000 times that of IgG. This structure allows IgM to bind effectively to multiple antigenic sites, which is known as avidity binding. IgM operates systemically and in the mucosal environment, thereby providing versatility. Unlike IgA, which works mainly on mucosal surfaces, IgM circulates systemically, allowing it to perform immune surveillance and strengthen defenses against threats.<sup>138,153,155</sup> In addition to the broad binding capabilities of circulating polyreactive nIgM, other immune IgM clones also display specific antigen affinity.<sup>153</sup> nIgM antibodies react with the conserved epitopes found in both microbes and self-antigens. Natural IgM production begins with self-antigen interactions.<sup>156</sup> IgM plays a key role in immune surveillance, recognition of threats, and the early

detection of harmful microorganisms.<sup>154</sup> IgM is vital for the diversity and stability of microbes in mucosal tissues, particularly in the intestines and respiratory tract, where plasma cells secreting IgM interact with microbiota.<sup>157,158</sup>

IgM antibodies bind to the Fc fragment of IgM receptor (FcμR) and pIgR, facilitating the transport of soluble secretory IgM across the mucosal epithelium (see **Fig. 3**). This prevents pathogens from attaching to epithelial cells and destroys the pathogens.<sup>159–162</sup> For example, IgA and IgM from HIV-1-positive women inhibit HIV-1 transport across the mucosal surfaces of the cervical–vaginal tissues.<sup>161,163</sup> IgM receptor FcμR internalizes IgM-coated microorganisms similar to pIgR, which binds to IgM and IgA. FcμR is the only IgM-specific receptor in mammals that exclusively binds to IgM. The mechanisms underlying the regulation of sIgM, including its development, control, and function, are not yet fully understood. Previous studies have suggested that FcμR facilitates direct interaction of sIgM with B and T cells.<sup>159</sup> Selective and secreted IgM deficiencies are prone to viral, bacterial, and protozoal infections, and have a higher risk of allergies, inflammation, and autoimmune disorders.<sup>156</sup> The protective functions of IgG and IgM antibodies often involve opsonization and complement recruitment, whereas activation of the classical complement pathway of IgA is limited.<sup>159,164</sup>

IgM tends to localize in the endometrial epithelia, unlike IgG, suggesting active transport of serum-derived polymeric IgM through the endometrial glands.<sup>146</sup> Estrogen increases total IgG and IgM production in the human peripheral blood mononuclear cells (PBMCs) of healthy individuals.<sup>165</sup> In contrast, women infected with *Neisseria gonorrhoeae* exhibited a much higher IgM response.<sup>166</sup> B cells found within the FRT are spread throughout but are concentrated primarily within the vagina, ectocervix, endocervix, and fallopian tubes, although small numbers also produce IgA, IgG, and IgM antibodies that exist primarily within these areas, but may also appear within the endometrium and ovary tissues. The endocervix, fallopian tubes, uterus, and ectocervical glands contain single-layer epithelial cells that express pIgR, which allows the selective transport of sIgA.<sup>167</sup> By contrast, the vagina and ectocervix, which contain multilayered cells lacking pIgR, do not transport pIgA or IgM. However, vaginal subepithelial connective tissue contains plasma cells that are positive for IgA and J chains, suggesting that locally produced immunoglobulins are transported from the blood to the cervical fluid.<sup>168</sup> Cervical shortening, a precursor of spontaneous preterm birth, is associated with *Lactobacillus iners* and elevated levels of IgM, C3b, C5, C5a, and IL-6.<sup>169</sup> The endocervix contains IgA+ and IgM+ plasma cells that regulate humoral immune response.<sup>170</sup> In cases of IgA deficiency, compensatory IgM responses targeting commensal bacteria arise. This phenomenon has been observed in both mice and humans, highlighting the adaptive nature of the immune system.<sup>144,157,171–173</sup>

Mucosal antibody responses are heightened in the context of inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis. This hyperactivity results from a complex interplay among host genetics, environmental factors, and microbiota composition.<sup>174</sup> Plasma IgM+ and IgG+ cells accumulate in the inflamed intestine, possibly

worsening inflammation; however, their role in pathology is not fully understood.<sup>175,176</sup> The interaction of IgM with microbiota extends beyond its classical role in systemic immunity. IgM actively shapes the microbial landscape and contribute to immune surveillance, diversity maintenance, and pathogen protection. Understanding these interactions may lead to new treatments for microbiota-related health issues. Further research may uncover IgM and microbial interactions in reproductive health.

## Future Directions and Advanced Technologies

The field of microbiota–immunome interactions in reproductive health is rapidly evolving and several future directions and therapeutic implications are emerging. These advances will enhance our understanding of the complex interactions between the microbiota, the immune system, and reproductive disorders and to create targeted interventions. This section highlights key areas for future research as well as possible diagnostic and therapeutic implications. However, host–microbiota interactions are dynamic, and their mechanisms remain poorly understood. Evidence has shown that reproductive microbiome dynamics play a pivotal role in reproductive health. It is critical to gain an in-depth understanding of their function and impact on host immunity responses. The precise mechanisms by which host antibodies recognize and respond to bacteria remain unclear. Understanding this interaction between the microbiota and immune system remains integral to understanding these interactions.

The current methods used to construct personalized profiles of IgM, IgA, and IgG antibodies that bind to endogenous microbiota require technological improvement before the knowledge can be used to provide tailored treatments more efficiently for reproductive diseases. Antibody-based assays have long been indispensable in the diagnosis of infectious diseases. IgM, IgG, IgA, and IgE antibodies offer additional information that aids in the diagnosis and understanding of the immunity against reinfection. These techniques, including Western blotting, immunofluorescence assays, and enzyme-linked immunosorbent assays, form the backbone of clinical diagnostics and research laboratories. However, limitations in throughput and multiplexing have necessitated the development of high-throughput multiplexable assays, including bead- and chip-based assays. These advanced assays have addressed some of these limitations. However, they can be inefficient because they are slow, incapable of simultaneously analyzing multiple pathogens, and require the expression and purification of target antigens, which may not be achievable for certain pathogen proteins.<sup>177,178</sup>

The introduction of next-generation sequencing (NGS) has provided an innovative opportunity with far-reaching results for microbiome–immunome studies. NGS holds great promise for revolutionizing diagnostics by directly detecting microbial nucleic acids and deepening our understanding of host–microbe relationships. However, the limitations of DNA-based microbiome studies, as 16S and metagenome shotgun sequencing, pose significant challenges to its



implementation and standardization, particularly in capturing the dynamics of live microbial functions. To bridge this gap, ongoing technological advancements are indispensable to unravel the intricate complexities of microbiota-immunome interactions in reproductive health. Innovative, cutting-edge methodologies, such as mFLOW-Seq and PhIP-Seq, offer powerful ways of studying the RT microbiota and immune cell dynamics at high resolution. These technologies enable high-throughput comprehensive profiling of microbial communities and antibody profiles, offering invaluable insights into new antibody detection modalities and helping researchers to discover biomarkers for various diseases.

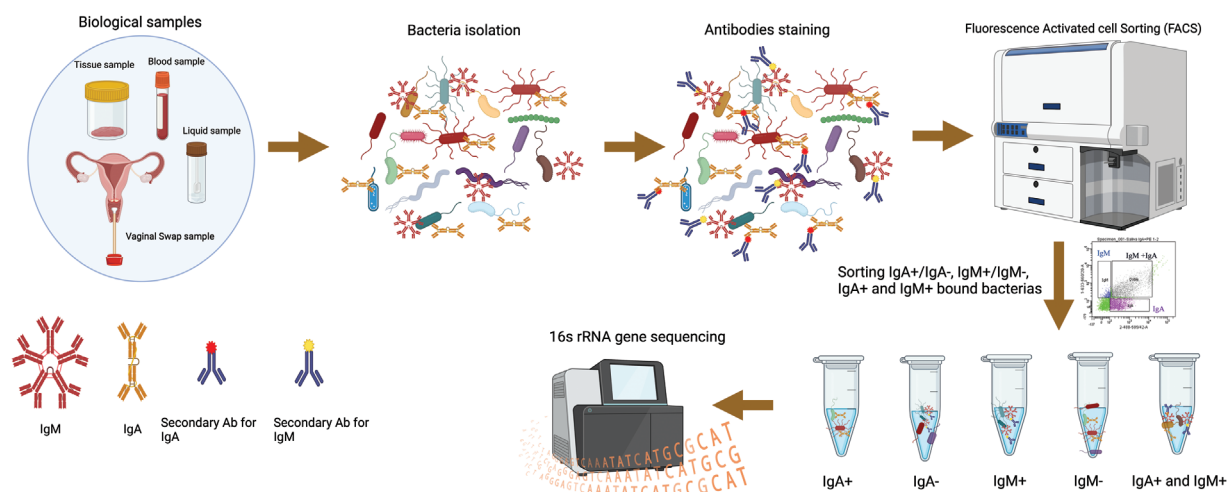
Furthermore, the technologies mentioned above empower investigations into physical characteristics, interactions, and functional roles, shedding light on the complex network of interactions between the microbiota and immune system in the context of reproductive health. As this field continues to evolve, it holds promise for the advancement of disease diagnostics and treatment, and ultimately contributes to improved reproductive health outcomes.

### Microbial Flow Cytometry coupled to NGS (mFLOW-Seq)

Flow cytometry, known for its high-throughput cell analysis capabilities, is a versatile tool for examining various cell types in research and clinical settings. Microbial flow cytometry (mFLOW) offers additional benefits in investigating microbes found in both environmental and clinical samples. It facilitates the analysis of physical properties and metabolic states associated with microbes, including size, shape, and granularity, using fluorescence markers. The utility of mFLOW-Seq has been expanded by incorporating host antibodies to assess humoral immune responses to specific microbes or microbial groups. This innovative approach

uses FACS and NGS to sort and detect microbes that contain specific antibodies (IgA, IgM, and IgG) from complex communities. Fusing microbial flow cytometry with NGS (mFLOW-Seq) provides an effective means of deciphering intricate interactions between the immune system and endogenous microbial communities. This technology offers novel insights into commensal–host interactions in mucosal and systemic environments. mFLOW-Seq provides quantitative information on microbial communities. This approach discriminates microbial subcommunities based on the immunoglobulin-bound microbiota, cell size, and DNA content. mFLOW-Seq has enabled researchers to isolate microbiota targeted by systemic antibodies across various samples (see ▶Fig. 4).

Initially, the bacteria extracted from the samples are stained with fluorescently labeled secondary antibodies specific for IgA, IgG, or IgM. Subsequently, fluorescence-activated cell sorting (FACS) segregates bacteria into distinct populations based on their binding characteristics: IgA+, IgG+, IgM+, IgA–, IgG–, and IgM–. These separate populations are subjected to 16S rRNA gene sequencing to identify microbes that are enriched in each fraction. 16S rRNA gene sequencing is a standard method for microbiome analysis. It targets specific regions of bacterial 16S ribosomal RNA (rRNA) genes with nine hypervariable regions (V1–V9) with significant sequence diversity among bacterial species. Microbiome studies often incorporate multiple regions like V3–V4 to improve bacterial taxon identification, offering insights into bacterial and archaeal community composition at the genus level. In contrast, metagenome sequencing targets all genomic DNA in a sample, enabling the study of bacteria, archaea, DNA viruses, and other microorganisms in complex microbial communities. It offers a comprehensive view, allowing for species-level identification and broader



**Fig. 4.** Schematic of mFLOW-Seq for identifying microbiota targeted by host antibodies in biological samples. The process involves several key steps. Isolation of bacteria: bacteria are extracted from the biological samples. Incubation with antibodies: The isolated bacteria are then incubated with fluorescently labeled secondary antibodies, specifically anti-IgA, anti-IgM, and anti-IgG. FACS: employed to segregate the bacterial populations into two groups: IgA +/–, IgM +/–, and IgG +/– according to antibodies bound to bacteria. Microbiota analysis: the sorted bacterial populations are subjected to 16S rRNA gene/metagenomic sequencing. This sequencing helps identify which microbes are enriched in the Ig fraction, revealing microbiota and immunome interactions. This figure was created using BioRender.com (2023). Source: <https://app.Biorender.com>.

detection of microorganisms.<sup>179,180</sup> This process revealed that IgA, IgG, IgM, and unbound fractions selectively bind to specific microbes, providing valuable information on antibody–microbe interactions<sup>181–185</sup> (see ►Fig. 4). This method excludes lysed bacteria and its fragments and concentrates solely on intact bacteria, thus offering both high sensitivity and a broad dynamic range. Remarkably, it operates effectively with minimal amounts of immunoglobulin, which makes it suitable for scenarios with low bacterial target densities, allowing for the analysis of immune responses, even against challenging bacterial species.

The previously established mFLOW-Seq technique has helped to quantify antibody binding specifically to live bacterial surface epitopes. mFLOW has been applied to fecal, intestinal, and various bodily fluids, such as serum, plasma, intestinal/bronchial/vaginal lavage, saliva, and breast milk bacterial samples.<sup>186–189</sup> Previous studies using mFLOW-Seq in pediatric selective immunoglobulin A (IgA) deficiency (SIgAD) patients found that mucosal and systemic antibodies target the same commensal microbes. The absence of secretory IgA results in abnormal systemic exposures and immune responses to commensal microbes, increasing the risk of immune dysregulation and symptomatic disease. IgA prevents microbial translocation and related immune dysregulation, leading to milder symptoms in SIgAD patients.<sup>190</sup> Furthermore, a study underscores commensal bacteria's impact on serum IgA levels, emphasizing T cell-dependent systemic responses. It links microbiota composition to serum IgA concentrations and identifies protective roles against sepsis when using mFLOW-Seq for microbiota analysis.<sup>191,192</sup>

Although NGS, a groundbreaking method for exploring microbial communities, has a limitation in distinguishing between live and deceased cells, as it extracts DNA from all materials indiscriminately.<sup>52</sup> In contrast, mFLOW-Seq holds promise in this regard by utilizing FACS to differentiate between live and dead microbial members before NGS sequencing.<sup>193</sup> Several studies have used high-throughput FACS combined with 16S rRNA sequencing to investigate the interactions between immunoglobulin IgA, IgM, IgG, and microbiota.<sup>143,172,191,194–197</sup> However, a significant gap remains in the field of reproductive immuno-microbiota analysis, with only one study using the mFLOW-Seq method for vaginal swab analysis of IgA and IgG bound microbiota populations.<sup>91</sup> This underscores the need for further exploration in this specific area.

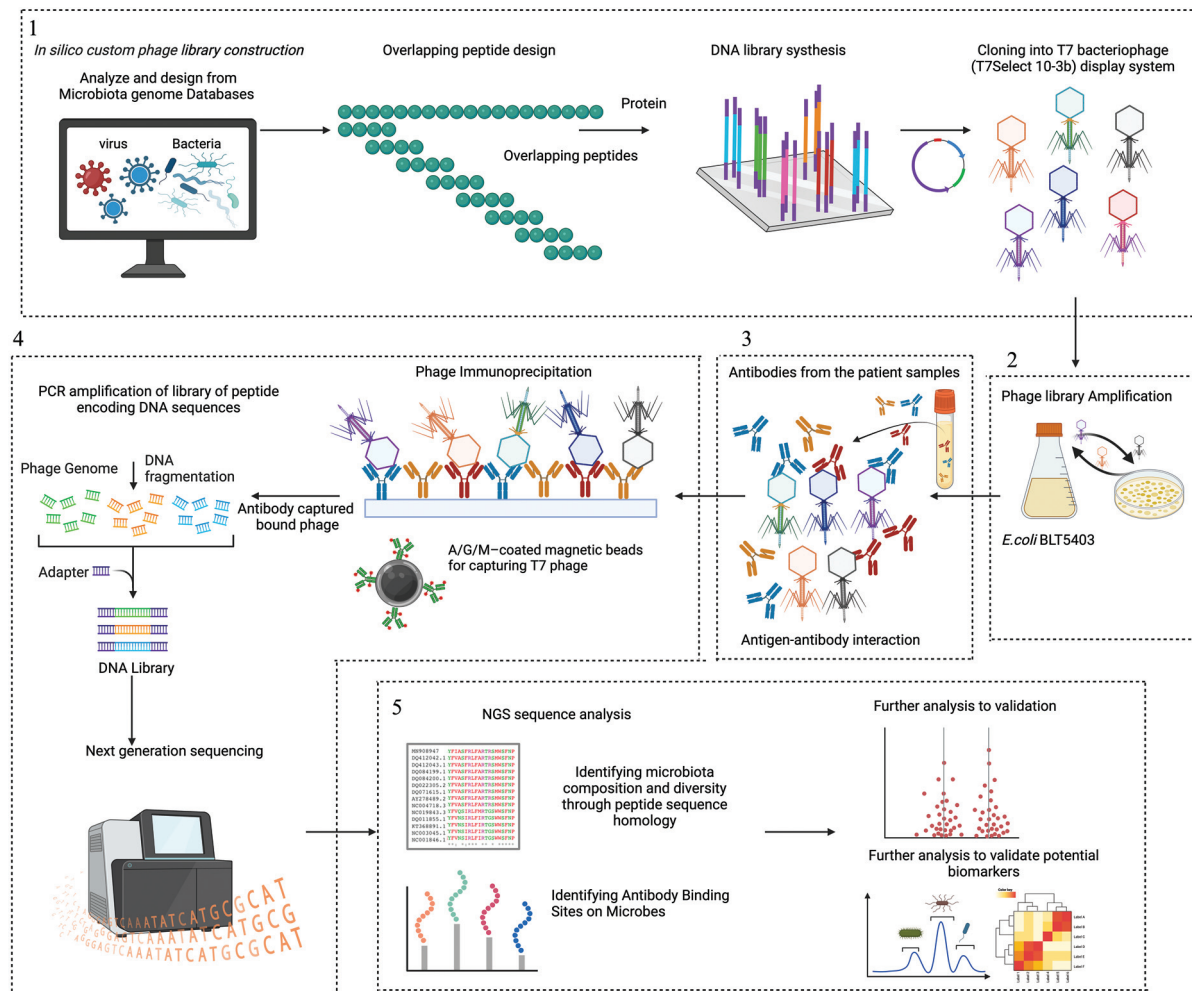
Therefore, this technique enables monitoring microbial community changes over time due to host environmental changes and disease progression. Further research is required to investigate whether antibodies binding to endogenous bacteria can predict the risk of infection (i.e., facilitating or preventing the transition from commensals to pathogens). In the move toward clinical use of mFLOW-Seq, further research is vital to fully grasp the significance of microbe-specific serum levels of IgA, IgM, and IgG in immunoglobulin replacement therapies. Immunoglobulin replacement therapy delivers IgA, IgM, and IgG to individuals with immunodeficiencies. Tailoring these antibody levels to

specific pathogens is crucial in improving therapy effectiveness, minimizing over-treatment risks, and optimizing treatment for individual immunodeficiencies. Understanding the potential of microbe-specific serum levels of IgA, IgM, and IgG in immunoglobulin replacement therapies is crucial. Although antibody responses typically take several days to develop, limiting their utility during the early phases of infection, it may be beneficial to test whether specific antibody preparations can bind to endogenous microbiota to prevent invasive infections. It can potentially revolutionize our understanding of host–microbiota relationships in various health contexts, including reproductive health. These findings may pave the way for innovative diagnostic and therapeutic approaches. Still, further research and clinical investigations are required to fully unlock the potential of mFLOW-Seq in microbiome-related research and clinical applications for personalized reproductive healthcare.

### Phage ImmunoPrecipitation Sequencing (PhIP-Seq)

PhIP-Seq has emerged as a powerful seroepidemiology tool that offers a promising avenue for investigating various aspects of human health including reproductive health. This cutting-edge technology combines T7 phage display with NGS, enabling the exploration of complex interactions between human microbiota and the immune system. PhIP-Seq is a versatile method that simultaneously profiles the antibody responses to various antigens. In the context of reproductive health, where the dynamic interaction between the microbiota and host immune system is of immense importance, this opens a unique avenue for investigation. In addition, their ability to detect antibody responses to new or less-explored RT pathogens is indispensable. PhIP-Seq, a relatively recent antibody detection technology, offers the ability to simultaneously investigate numerous antigens, thereby facilitating identification of microbial gene epitopes that interact with the host immune system. This technology sheds light on the intricate interactions between the microbiome and host immune system, uncovering the specific epitopes that drive these complex relationships. It also has the potential to identify biomarkers for various diseases, including those of an immune origin. The opportunity to investigate the diversity and distribution of microbiota within different organs and to understand how distinct antibody classes respond to pathogens is of significant value. Furthermore, understanding how the reproductive microbiome influences host immunity and the development of gynecological diseases offers promising avenues for research.

PhIP-Seq technology, based on phage-displayed synthetic oligo libraries representing the microbiome, offers a comprehensive approach to investigate the dynamics of the microbiome and its intricate association with host antibody responses. This versatile technology accommodates various sample and antibody isotypes including IgM, IgG, IgA, IgD, and IgE. This procedure can be adapted to construct libraries spanning different biological domains. Briefly, the protocol involves the design of peptides that tile across the proteins. Subsequently, these peptides are synthesized into



**Fig. 5.** Overview of PhIP-Seq methodology. The PhIP-Seq methodology involves four key steps. (1) Phage library construction: Downloading or designing a protein database and utilizing bioinformatics tools to generate overlapping peptide sequences from microbiome database proteins. Synthesis of an oligonucleotide library encoding the peptide sequences. PCR-amplify the oligonucleotide library with adapters for cloning into the T7Select 10–3b mid-copy phage display system. (2) Propagation of phage library: The phage library amplified using *Escherichia coli* BLT 5403 to ensure library diversity and sufficient phage clones for subsequent experiments and sequencing. (3) Patient antibody–antigen interaction: Patient samples containing antibodies are incubated with the amplified phage library for specific interactions. (4) Phage immunoprecipitation and data analysis: Patient samples and their antigens are used in phage immunoprecipitation reactions to capture antibodies and their bound phages on beads coated with proteins A, M, and G. DNA recovered from the immunoprecipitated phage. IgA, IgM, and IgG contents in each sample quantified using ELISA for antibody input normalization. Amplify the library of peptide-encoding DNA sequences directly from the immunoprecipitate. PCR-amplified DNA, sample-specific barcodes, and sequencing adapters for NGS. Pool-barcoded amplicons for sequencing using NGS. (5) Data analysis and validation: Multiplexed data are aligned to reference sequences to create a count matrix. Statistical analysis identifies peptide enrichments, facilitating project-specific investigations, such as identifying common autoantigens, validating microbe-specific epitopes, and identifying potential biomarkers. This methodology provides a comprehensive approach for analyzing antibody epitopes and their association with microbiota. Figure adapted from BioRender.com (2023). Source: <https://app.Biorender.com>.

overlapping oligo libraries and cloned into T7 bacteriophage display vectors. This streamlined approach is versatile and provides a straightforward method for crafting customized libraries. Typically, these libraries rely on protein reference sequences from public databases, with overlapping peptides ranging from 56 to 90 amino acids (see ▶Fig. 5). This inclusivity allows the incorporation of various known strains and infectious pathogens, making it applicable to reproductive health scenarios. Furthermore, the ability of PhIP-Seq to propagate phage libraries provides a consistent source of antigenic material to study immune responses.<sup>198–200</sup> Phage display and immunoprecipitation are employed to assess

antibody binding to all peptides in parallel, and DNA sequencing is used to determine the relative abundance of the immunoprecipitated microbial population.<sup>201</sup> The article by Mohan et al provides a comprehensive protocol for constructing a PhIP-Seq peptide library designed for the in-depth analysis of serum antibodies.<sup>202</sup>

PhIP-Seq libraries have been meticulously designed to span many biological domains, including human proteome, allergens, protein toxins, microbiota, viruses, and gut bacteria. In 2011, Larman et al introduced a groundbreaking method that combined PhIP-Seq with a synthetic human proteome library. This innovative approach aims to identify autoantibodies and

unravel the autoantigens linked to autoimmune diseases, particularly in individuals with paraneoplastic syndromes, type 1 diabetes, multiple sclerosis, and rheumatoid arthritis.<sup>201,203,204</sup> Subsequent studies with human virome and human proteome phage display libraries have focused on dissecting antiviral antibody profiles,<sup>205</sup> unraveling immune responses in membrane nephropathy,<sup>206</sup> antibody responses to environmental toxins and virulence factors,<sup>207</sup> and focusing on specific viral families such as anelloviruses.<sup>208</sup> In another groundbreaking study, PhIP-Seq was used to construct a human gut–bacterial microbiome library, examining serum antibody responses in 997 healthy individuals. Surprisingly, these responses are diverse and exceptionally stable over time, thereby introducing the concept of an “immunological fingerprint.”<sup>209</sup> Further research involving 1,003 nonallergic individuals explored the response of IgG antibodies to food and environmental antigens. They used an extensive library of bacterial and viral peptides displayed on phages covering various food and environmental allergens from multiple databases.<sup>210</sup> Previous studies have combined the human gut microbiome with an allergic phage library to examine human antibody repertoires in 1,443 individuals. These studies considered genetic, environmental, and intrinsic factors; assessed antibody responses in people with IBD such as Crohn’s disease and ulcerative colitis; and compared them with a control group.<sup>211,212</sup>

However, despite these advantages, PhIP-Seq has several limitations. It cannot identify autoantibodies that recognize post-translationally modified proteins (i.e., glycosylation isoforms); lack information on post-translational modifications, like sugar moieties; and misses the three-dimensional structure of antigens. It is also limited to linear epitopes and excludes non-protein immunogenic molecules such as lipids and glycans.<sup>211</sup> Research exploring the interactions between human microbiota and the immune system in reproductive health remains limited. There is a compelling opportunity to leverage PhIP-Seq to develop comprehensive microbiome libraries that are specific to RT. This would allow an in-depth study of the dynamic interactions between the microbiota and host immune system. This development has the potential for widespread commercial applications, particularly in the development of diagnostic tools and therapies related to microbiome–host interactions, and facilitates the RT probiotic strain studies. Personalized antibody profiles and assessment of antibody responses against microbiome antigens can be utilized for risk assessment, pathogen detection, and disease monitoring, thus presenting extensive health-care and commercial potential. PhIP-Seq has immense potential to improve our understanding of reproductive health by providing a sophisticated means of scrutinizing human microbiota and immune interactions. Its adaptability, high-throughput capabilities, and precise data analysis make it an invaluable asset for reproductive health research. Given the profound impact of microbial communities and immune responses on reproductive health, PhIP-Seq has emerged as a cutting-edge tool to elucidate this intricate interplay. Together with other molecular techniques, harnessing the advantages of PhIP-Seq in reproductive

health investigations promises breakthroughs in diagnosing, preventing, and managing reproductive health issues.

## Conclusions

Microbiota–immunome interactions in reproductive health represent a complex and promising field of research with significant potential. This review covers various relevant sections that provide insights into these relationships, technological developments, diagnostic and therapeutic possibilities. The field of reproductive microbiota, particularly of that of upper RTs, requires extensive exploration to reveal their composition and effects on women’s reproductive health. Moreover, the current knowledge regarding the microbiome in specific locations within the FRT, such as the ovaries, endometrium, and placenta, is limited and primarily obtained from women with preexisting conditions. Further research is essential to understand the microbiome in healthy nonpregnant and pregnant women. As a result, distinguishing between the normal microbiome and their potential causative agents of infections remains a challenge. To address this gap, further investigations should be conducted regarding the controversial endometrial/uterine, ovaries, fallopian tube, and placental resident microbiome. It is crucial to implement strictly controlled studies on live microbial cells to avoid contamination during sample processing, ensuring the reliability of results.

At the same time, the human microbiota has an immense effect on the immune system, shaping the adaptive and innate responses that are essential to overall health. Investigating how IgA and IgM regulate these responses is a continuous pursuit. Differentiating between healthy and pathological microbiota–immunome interactions would be critical. Acquiring knowledge on how antibodies identify and respond to bacteria is crucial for gaining insights into microbiota–immunome interactions. Still, manipulation of antibodies for diagnostic and therapeutic use requires further investigation. Standardized methods are essential to understand their roles in clinical disorders and provide the foundation for large-scale personalized medicine and diagnostic trials. Technologies such as mFLOW-Seq and PhIP-Seq provide exceptional insights into microbiota–immune interactions, including the epitopes that drive these interactions in FRT and larger in the entire organism. Current insights into microbiota–immunome interactions come from DNA-based microbiome studies, which may not fully capture the functional aspects of live microbes and their dynamics. To address this critical gap, future research should shift from microbiome to microbiota studies and use techniques such as mFLOW-Seq and PhIP-Seq, capable of assessing live microbes, for a more comprehensive understanding and more robust conclusions.

This comprehensive review highlights the potential to expand our knowledge of the composition and diversity of the microbiota within RT, which offers promise for personalized medicine for reproductive disorders and improved outcomes. These insights are helpful in predicting and preventing infectious diseases and autoimmune disorders that affect reproductive health. Innovative technologies such as



mFLOW-Seq and PhIP-Seq are promising for revolutionizing disease diagnosis, monitoring, and offering truly personalized medical interventions. Technological innovations go beyond reproductive health and have become invaluable tools for studying microbiota-immunome interactions in various reproductive contexts. This undertaking provides the basis for new diagnostic and therapeutic avenues for reproductive healthcare.

#### Authors' Contribution

All authors helped to conceive the topic for the review. P.L. prepared the initial draft of the manuscript; all authors edited the manuscript and confirmed the last version of the manuscript.

#### Conflict of Interest

All authors declare that they have no competing interests.

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