Review

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The association of gut microbiome with recurrent pregnancy loss: A comprehensive review

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SUMMARY The steady-state gut microbiome not only promotes the metabolism and absorption of nutrients that are difficult to digest by the host itself, but also participates in systemic metabolism. Once the dynamic balance is disturbed, the gut microbiome may lead to a variety of diseases. Recurrent pregnancy loss (RPL) affects 1-2% of women of reproductive age, and its prevalence has increased in recent years. According to the literature review, the gut microbiome is a new potential driver of the pathophysiology of recurrent abortion, and the gut microbiome has emerged as a new candidate for clinical prevention and treatment of RPL. However, few studies have concentrated on the direct correlation between RPL and the gut microbiome, and the mechanisms by which the gut microbiome influences recurrent miscarriage need further investigation. In this review, the effects of the gut microbiome on RPL were discussed and found to be associated with inflammatory response, the disruption of insulin signaling pathway and the formation of insulin resistance, maintenance of immunological tolerance at the maternal-fetal interface due to the interference with the immune imbalance of Treg/Th17 cells, and obesity.

Keywords gut microbiome, recurrent pregnancy loss, inflammation, insulin resistance, immunity, obesity

1. Introduction

Recurrent pregnancy loss (RPL), whose definition has still remained controversial, was traditionally defined as multiple spontaneous abortions with the same spouse. The American Society for Reproductive Medicine (ASRM) defined two or more pregnancy failures and explicitly excluded biochemical pregnancies in 2020 (*1*). In addition, the European Society of Human Reproduction and Embryology (ESHRE) defined two or more pregnancy failures before 24 weeks of gestation, including biochemical pregnancies (*2*). RPL could affect 1-2% of women of reproductive age, and its prevalence has increased in recent years (*2*). Hence, RPL is a disease that impairs patients' physical and mental health and their families' stability. The etiology of RPL is complex, and the main recognized factors include chromosomal abnormalities, inflammation, insulin resistance (IR), immunity, obesity, *etc*.

In an individual, the gut microbiome is composed of more than 1,000 species of bacteria, including approximately 3.8×10^{13} bacterial cells and 3.3 million genes (*3*). Compared with human genes, the gut microbiota contains 150 times more genes than the human genes (*3,4*), which are essential for the survival of these organisms in the gut and confer different functions to various bacteria that play important roles in regulating metabolism, immunity, and inflammation. Over time, this dynamic population evolves with the host, including bacteria, fungi, parasites, archaebacteria, and viruses. Metagenomic analyses of volunteers and lean mice have shown that the microbiome was comprised of two main primary phyla, *Bacteroides* and *Firmicutes*, and *Bacteroidetes* can be commonly found in healthy hosts, making them superior to *Firmicutes* (*4*).

Gut microbiome can attach to the intestinal mucosal surface, forming a protective barrier and participating in the maintenance of homeostasis within the tissues to prevent the growth and invasion of pathogenic bacteria (*5*). The steady-state gut microbiome not only promotes the metabolism and absorption of nutrients that are difficult to digest by the host itself, but also participates in systemic metabolism (*6*). The intestinal barrier, primarily consisting of a mucus layer, an

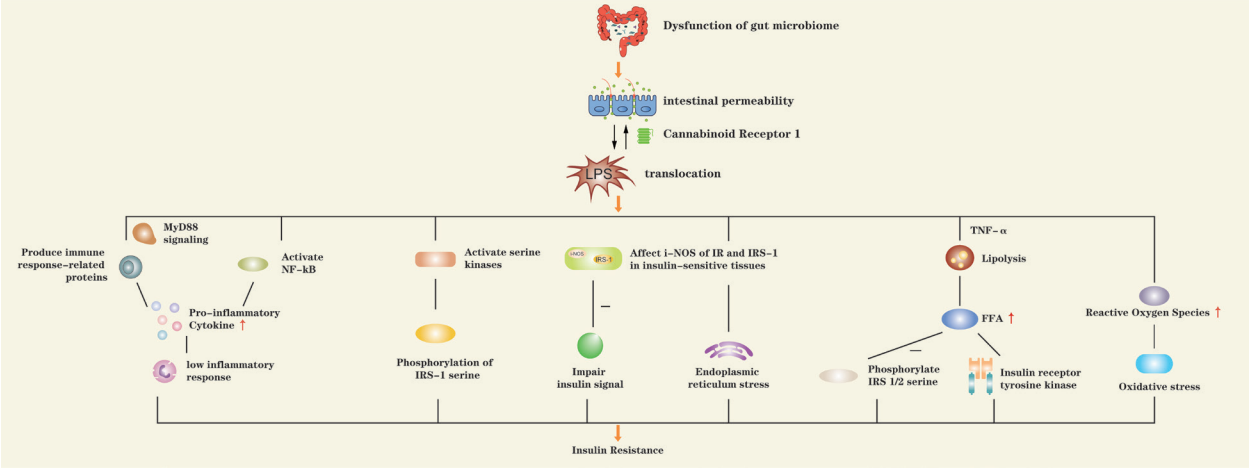

Figure 1. The possible mechanisms of RPL based on the effects of LPS by regulating gut microbiome. ↑: increase; ↓: decrease; -: inhibit.

epithelial barrier, and a gut vascular barrier, limits the transport of intestinal contents into systemic circulation by providing a physical and functional barrier. First, the tight junctions between the epithelial cells restrict the entry of bacteria and their products into the systemic circulation. Second, the mucin layer consists of the highly glycated protein mucin secreted by goblet cells, which does not allow bacteria to penetrate by firmly adhering to the epithelial cells (*5*). The thickness and composition of the mucus layer are mainly affected by the gut microbiome. Consequently, the interaction between the intestinal mucus layer and microorganisms is of great significance for maintaining healthy intestinal homeostasis. In addition, the gut microbiome participates in the establishment of mucosal immunity, and the glycine-rich structural domains on mucins can provide binding sites for certain microorganisms secreting specific lectins or glycosides for selective adhesion (*7*). In germ-free animals, there are fewer goblet cells, a thinner mucus layer, and a higher proportion of neutral mucin. Whereas the intestinal mucus layer of germ-free animals is restored rapidly after feeding on the bacterial products, which also promotes the maturation of the immune system through the mucosal system and protects the host from the attack of opportunistic pathogens (*8*). In general, the gut microbiome is in dynamic balance, while it is susceptible to the environmental factors, such as long-term dietary habits and medications. Once the dynamic balance is disturbed, the gut microbiome may lead to a variety of diseases.

According to the available literature, the gut microbiome is a new potential driver of the pathophysiology of recurrent abortion, and the gut microbiome has emerged as a new candidate for clinical prevention and treatment of RPL. However, few studies have concentrated on the direct correlation between RPL and the gut microbiome, and the mechanisms by which the gut microbiome influences recurrent miscarriage need further investigation. The present review aimed to discuss the relationship between RPL and gut microbiome.

2. Effects of gut microbiome on RPL

2.1. Insights into inflammation and gut microbial imbalance

Few studies have directly shown that gut microbes are associated with RPL, while there is strong evidence to support the correlation between the gut microbiome and the increased risk of inflammation, which in turn leads to IR. Dysfunction of intestinal microbiome involves abnormal production of cytokines, which may be at the risk of inflammation. There is significant evidence supporting that the increased risk of pro-inflammation is associated with a decrease in the diversity of the gut microbiome (*9*). In addition to the loss of diversity, Liu *et al*. reported a significant reduction in the dominant microbiota beneficial to human health and an increase in the proportion of *Firmicutes* in patients with abortion (*10*).

2.1.1. Lipopolysaccharide (LPS)

LPS, known as endotoxin, is considered one of the initiators of inflammatory responses and apoptosis. Although the development of inflammation may be caused by several factors, according to studies on mice, the gut microbiome is one of the important causes, and the LPS is one of the best-studied inflammation inducers (*5,11*). In general, the intestinal barrier prevents the entry of LPS from the gut into systemic circulation. The toxicity of LPS is present in the portion of Lipid A moiety, which allows LPS to bind to toll-like receptor-4 (TLR4), and then enter the systemic circulation. Intestinal alkaline phosphatase produced by epithelial cells catalyzes the dephosphorylation of Lipid A moiety to detoxify bacterial endotoxin, thereby preventing the translocation of LPS into the systemic circulation (*5*). However, changes in the ratio of *Bacteroides* to *Firmicutes* make LPS to translocate and move into the systemic circulation by the junctions (Figure 1).

There is a specific interaction between LPS and TLR4. LPS-binding protein binds to LPS and delivers it to the TLR4/myeloid differentiation protein-2 complex, which then induces the early activation of nuclear factor-kappa B (NF-κB), Interferon regulatory factor 3, and mitogen-activated protein kinase (MAPK) pathways, which can be mediated by the adapters myeloid differentiation primary response 88 (MyD88) and MyD88 adaptor-like. After the subsequent activation and phosphorylation of interleukin-1 receptor-associated kinase, tumor necrosis factor (TNF) receptor-associated factor 6 becomes activated (*12*), which gives rise to the expression of pro-inflammatory genes. Then, inflammatory cytokines lead to a low inflammatory response and IR (*13*). A contender for the MyD88-independent branch of signaling pathways induced by LPS is dsRNA-dependent protein kinase (PKR), which has been shown to be associated with MyD88 adaptor-like. The translocation of LPS also activates serine kinases, which induces serine phosphorylation of insulin receptor substrate 1 (IRS-1) and promotes IR formation. Moreover, LPS entering to the gut through a compromised intestinal barrier also affects S-nitrosation of IR and IRS-1 in insulin-sensitive tissues, leading to the impaired insulin signal (*14,15*). In addition, S-nitrosylation/S-nitrosylation has been shown to be a central phenomenon induced by endoplasmic reticulum stress, which could also be an important molecular mechanism of IR (*16*).

Changes in the gut microbiome can activate the endocannabinoid system. LPS can exacerbate the inflammatory response by binding to cannabinoid receptor 1 to increase intestinal permeability and promote its absorption into the blood (*17*). LPS can also promote lipolysis directly, or indirectly through TNF-α, significantly increasing the production of free fatty acids (FFAs). The increased levels of FFAs can phosphorylate IRS1/2 serine and inhibit the activity of insulin receptor tyrosine kinase, leading to IR (*18*). In addition, LPS promotes the production of reactive oxygen species (ROS) and induces oxidative stress, which in turn increases the risk of IR (*19*). Importantly, in mice supplemented with lipoic acid, oxidative stress was lower than in mice that did not receive lipoic acid (*20*).

2.1.2. Short-chain fatty acids (SCFAs)

Gut microbiome can metabolize dietary fiber and indigestible carbohydrates into SCFAs by producing specific enzymes (*6*). To our knowledge, SCFAs are immunomodulatory molecules that are involved in the regulation of cytokine production and T regulatory (Treg) cell expansion, activating or inhibiting of the inflammatory cascade (*21*).

With the progression of research, SCFAs can be detected in the blood circulation, suggesting a wide range of extra-gastrointestinal effects of gut microbiota-derived SCFAs. SCFAs include acetate, propionate, and butyrate (the highest level is 95%). In a study on rats, it was revealed that altering the gut microbiota was resulted in the increased acetate production, which activated the parasympathetic nervous system, thereby promoting the increased insulin secretion (*22*). Propionate is a substrate for hepatic gluconeogenesis. Several studies have shown that high levels of imidazole propionates produced by *Bacteroides* and *Prevotella* are involved in the immune activation and low inflammation (*23,24*). Besides, another study on mice showed that imidazole propionate inhibits insulin signaling at the level of IRS by activating P38 MAPKs and promoting the phosphorylation of P62 (*25*).

Another SCFA, butyrate, is the most important metabolite in colonocyte metabolism. Of note, butyrate is an anti-inflammatory metabolite that inhibits pathways, leading to the production of pro-inflammatory cytokines. A previous study has demonstrated that *Bifidobacterium bifidum* strain Bb could decrease *Prevotellaceae*, a major driver of inflammation, by modulating SCFAs, particularly butyrate (*26*). Related to this anti-inflammatory effect, butyrate induces extrathymic production of anti-inflammatory Treg cells, which in turn minimizes the risk of IR by improving insulin signaling (*21*). Besides, butyrate increases the expression levels of tight junction proteins, leading to reduced intestinal permeability (*27*) and maintenance of the intestinal barrier, thereby minimizing LPS mobility in the gut and reducing LPS-related effects.

2.1.3. Bile acids

Bile acids, metabolized by enzymes in gut microbiome, are essential for the maintenance of homeostatic metabolism, insulin sensitivity, and innate immunity. Gut microbes can convert primary bile acids to secondary bile acids *via* deconjugation, dihydroxylation, or dehydrogenation (*28*), and thus, gut microbiota can influence the synthesis of bile acids. Bile acids serve several functions, enabling lipids to be emulsified and absorbed by the body, as well as exerting their role as signaling ligands (*13*). Bile acids play an inflammatory role by activating the farnesoid X receptor signaling pathway in enterocytes (*29*). However, activation of Takeda G-protein receptor 5 by secondary bile acids activates glucagon-like peptide 1 (GLP1) secretion from L-cells in the intestine (*30*). Furthermore, GLP1 prevents IR (*31*). Meanwhile, bile acid metabolites are abundant in the intestinal tract of mammals and produce a large pool of bioactive molecules to control the differentiation of T helper 17 (Th17) cells and Treg cells (*32*). Hang *et al*. studied two different derivatives of lithocholic acid (LCA), 3-oxoLCA and isoalloLCA, and found that 3-oxoLCA was directly associated with retinoic acid γt binding that could inhibit the differentiations of Th cells, a key transcription factor in Th17 cell differentiation.

Nevertheless, isoalloLCA enhanced the differentiation of Treg cells by generating ROS (*32*).

2.1.4. Cytokines

Cytokines are signaling molecules that influence several processes in the host, such as immune regulation. As a pro-thrombotic cytokine (IL), IL-6 is involved in the differentiation process of Th17 cells, and it is closely associated with RPL (*33*). Compared with normal pregnancy, levels of Th17-related cytokines (*e.g*., TNF-α and IL-17) were significantly elevated in the decidua of RPL patients (*34*). Furthermore, a study found that miscarriage was caused by abnormally elevated IL-17 level at maternal-to-fetus interface, and administration of anti-IL-17 antibodies prevented unexplained recurrent miscarriages *(35*).

Gut microbiota can induce an imbalance in cytokine levels (*10*), and a systematic review found the abundance of *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, and *Prevotella* was negatively correlated with IL-6 expression level (*36*). However, the underlying molecular mechanisms have not yet been fully clarified.

Bacterial overgrowth in the intestine could lead to bacterial translocation, which could exacerbate the inflammation and disrupt tight junctions, thereby facilitating intestinal permeability (*37*). For instance, *Collinia* could increase intestinal permeability by decreasing the expression levels of tight junction proteins in epithelial cells and inducing IL17 expression level (*38*). *Lactobacillus* strains can modify production of cytokines in different cells. Aline *et al*. found that *Lactobacillus* strains could reduce concentrations of pro-inflammatory cytokines, such as IL-6 and TNF-α, and significantly increase concentrations of anti-inflammatory cytokines (*39*). Importantly, in patients with miscarriage, concentrations of IL-2, IL-17, TNF-α, and interferon-γ (IFN-γ) were upregulated, and microbial diversity and the relative abundance of *Prevotella_1*, *Prevotellaceae_UCG_003* and *Selenomonas_1* were significantly reduced (*10*).

In addition, the gut microbiome metabolites modulate immunity through cytokines. Liu *et al*. found that imidazolepropionic acid and 1,4-methylimidazoleacetic acid were associated with recurrent miscarriage by Th1/Th17-mediated immunity (*10*). Histone deacetylase inhibitory activity of butyrate induces changes in gene expression in dendritic cells, including inhibition of IL-6, which contributes to the differentiation of Treg cells (*40*). Butyrate regulates the immune response of macrophages by inhibiting the transcription of precursor macrophages, such as IL-6 (*41*), contributing to the maintenance of tolerance toward commensal bacteria.

TNF-α, a cytokine known as an inflammatory driver, activates various signaling pathways to develop metabolic disorders. Additionally, elevated incidence rates of metabolic disorders have been found to be associated with IR (*42*). To successfully establish a pregnancy, extravillous trophoblast cells must invade the placental bed. Harry A found that the increased level of TNF-α in women with miscarriage could inhibit extravillous trophoblast cells invasion by increasing trophoblast apoptosis, and/or altering production of active proteases (*43*). Importantly, TNF-α level was higher in mice with intestinal enrichment of *Staphylococcus aureus* and *Danieliae* than that in normal mice (*44*). However, Mechoud *et al*. demonstrated that *Lactobacillus* could reduce TNF-α production by interfering with the ability of NF-κB to bind to DNA targets (*45*).

2.2. Insights into IR and gut microbial imbalance

The gut microbiome dysbiosis may reshape intestinal barrier functions and host metabolic and signaling pathways, which are directly or indirectly related to the IR in type 2 diabetes. Independent of obesity, insulin resistance and associated hyperglycemia trigger a rapid, drastic, and reversible intestinal dysbiosis, which contributes at least partly to enhanced gut permeability.

IR includes a series of pathological and clinical manifestations caused by a reduced or lost response of the target organs and tissues to the biological effects of insulin. During pregnancy, physiological IR is due to the high secretion of hormones, such as cortisol and progesterone, antagonizing the effects of insulin. Maternal tissue reduces blood glucose intake to maintain stable blood glucose levels to meet the needs of fetal growth. However, pathological IR is an important factor in adverse pregnancy outcomes. Destruction of intestinal barrier would destroy the symbiotic relationship between the gut microbiome and their hosts, leading to low-grade inflammation and metabolic disturbances that impair the insulin signaling pathway, alter insulin sensitivity, and ultimately lead to IR. The gut microbiome dysbiosis may reshape intestinal barrier functions and host metabolic and signaling pathways, which are directly or indirectly related to the insulin resistance in type 2 diabetes. Independent of obesity, IR and associated hyperglycemia trigger a rapid, drastic, and reversible intestinal dysbiosis, contributing at least partly to the enhanced gut permeability (*46*). Moreover, gut microbiota dysbiosis may result in IR.

Over the past decades, it was revealed that IR is one of the factors influencing the susceptibility of patients with polycystic ovarian syndrome (PCOS) to recurrent miscarriages, while the risk of IR in RPL patients remains noticeable after excluding PCOS- and diabetes-related factors. In the newly published guidelines, abnormal insulin metabolism has been recognized as an independent risk factor for RPL (*47*).

There is a relationship between IR and PRL, while the mechanism of IR inducing abortion is complex. Hyperinsulinemia increases the expression level of plasminogen activator inhibitor-1, which can induce

a hypofibrinolytic state. This promotes vascular thrombosis reduces blood supply to the placenta, which in turn leads to trophoblast dysplasia and miscarriage (*48*). Insulin directly and indirectly stimulates the production and secretion of androgens by ovarian theca cells. First, high concentrations of insulin inpatients with IR can act directly on ovarian follicular membrane cells, accelerating the transformation of intracellular progesterone into 17α-hydroxyprogesterone and promoting the conversion of 17α-hydroxyprogesterone, androstenedione, and testosterone, ultimately resulting in hyperandrogenemia. Second, high levels of insulin can increase the luteinizing hormone (LH) pulse amplitude of pituitary gland by increasing the body's sensitivity to gonadotropin-releasing hormone, followed by worsening of hyperandrogenemia. In addition, IR reduces the production of sex hormone binding globulin in the liver, which increases free testosterone and functional hyper and rogenemia in the body. Furthermore, free androgens in serum can directly damage to ovum and embryo, and even lead to PRL in severe cases (*49*). Moreover, androgen receptor mediates the regulation of androgen in structure and function of uterine tissue, and a single nucleotide polymorphism of androgen receptor (G1733A) gene changes cytoskeletal tissue and cell cycle regulation, affecting decidualization of human endometrial stromal cells, which is detrimental to embryonic implantation and increases abortion rate (*50*). Hyperandrogenemia and IR may cause mitochondria-mediated damage, cause the imbalance of oxidative stress and antioxidant stress response of the uterus, and ultimately lead to abortion (*51*).

IR or hyperinsulinemia has various deleterious metabolic effects, such as increased serum level of hyperhomocysteinemia (HHcy). HHcy increases oxidative stress in the vascular endothelium and activates platelets by interfering with endometrial blood flow and vascular integrity, leading to implantation failure or abortion (*52*). Jakubowicz *et al*. found that hyperinsulinemia at the implantation situs could decrease the concentration of insulin-like growth factor binding protein-1 (*53*), promoting the adhesion process at the maternal-fetal interface. Thus, insulin is negatively associated with insulin-like growth factor binding protein-1 concentration, increasing the risk of miscarriage. In addition, hyperinsulinemia may cause dysregulation of prokineticin 1 expression level, which inhibits endometrial stromal cell migration and trophoblast invasion, and interferes with embryonic implantation, thereby leading to miscarriage (*54*).

2.3. Insights into immunity and gut microbial imbalance

The etiology of RPL is complex and approximately 50% of the complexity is related to immune factors (*34*). Intestinal microorganisms are closely associated with Treg/Th17 cellular immune homeostasis, and gut microorganisms and their metabolites may interfere with Treg/Th17 cellular immune balance. First, Treg/Th17 cellular immune imbalance may occur when gut microbial composition changes, leading to the increase of IL-17 secretion and a shift in Treg/Th17 homeostasis to Th17 cells (*55*). Liu *et al*. also found a significant decrease in the dominant microbiota beneficial to human health and an increase in the ratio of *Firmicutes* to *Bacteroides*. Further analysis showed that these changes in the gut microbiome were related to the increase in the levels of Th1- and Th17-related cytokines (*10*). Segmented filamentous bacteria could induce the differentiation of Th17 cells by stimulating ROS and promoting the production of serum amyloid A (*56*). *Bifidobacterium* was confirmed to induce differentiation of Th17 cells (*57*). However, *Bacteroides fragilis* could promote the differentiation of $CD4^+$ T lymphocytes into Treg cells and inhibit the differentiation of $CD4^+$ T lymphocytes into Th17 cells *via* the TLR signaling pathway on the surface of T lymphocytes (*57*). Second, each bacterium could also regulate the differentiation balance of Treg/Th17 cells by its metabolites. For instance, *Clostridium* clusters XIVa and IV could also induce Treg cell proliferation through SCFAs (*58*).

2.3.1. Autoimmune diseases (AIDs)

AIDs refer to the syndromes, in which the body produces high titers of autoantibodies and/or auto-reactive lymphocytes that attack the corresponding normal cells and tissues, resulting in tissue and organ dysfunction. AIDs are characterized by disruption of immune tolerance, and they are an important cause of pregnancy-associated complications, such as RPL. Christiansen *et al*. reported that the incidence of RPL was 1.7-5.3 times higher in pregnant women with AIDs, including lupus erythematosus and autoimmune thyroid disease (AITD) than in healthy controls (*59*).

It is broadly accepted that dysfunction of the gut microbiome can cause an overactive immune system, leading to AIDs, while few *in vivo* relevant studies were conducted. In a seminal experiment performed by Min Jin *et al*. (*9*), significant differences between the positive and negative groups were identified by investigating the gut microbiota-induced recurrent miscarriages associated with immune antibody positivity. The relative abundance of Bacteroidetes was the highest in the positive group. In contrast, *Bacteroides*, *Faecalibacterium*, *Erysipelotrichaceae_UCG-003*, and *Prevotella_9* had a high relative abundance in the negative group. The results of alpha diversity analysis showed that the community richness, community diversity, and phylogenetic diversity were higher in the positive group than those in the negative group. It is speculated that there may be some relationships between the gut microbiome and RPL with positive immune antibodies. However, the mechanism has not been extensively studied.

Autoimmune factors-induced RPL refers to the presence of various autoimmune antibodies in the maternal, which attack the maternal tissues and placenta, thereby causing abortion. Autoantibodies can be classified as non-organ-specific antibodies, such as antiphospholipid antibody (aPL), antinuclear antibody (ANA), and organ-specific antibodies (*e.g*., anti-thyroid antibody).

The antiphospholipid syndrome (APS) is an autoimmune disorder mediated by T cell-dependent aPL, and it is known as an RPL-related immunological diagnosis in the world. APL is the most common autoimmune antibody that causes RPL, which is composed of heterogeneous sets of antibodies that recognize plasma or cell surface antigens, including the antibodies against proteins related to coagulation, such as β2 glycoprotein I.

The pathogenic aPLs inhibit fibrinolytic and protein C pathways and promote thrombosis in arterial vessels and microcirculation in multiple target cell types, such as endothelial cells, platelets, and monocytes. One-third of the placental tissues in patients with APS is thrombosed, resulting in fetal ischemia and hypoxia (*60*). APLs affect prostaglandin production through decidual cells and various cytokines and increase secretion and expression levels of pro-inflammatory cytokines, such as TNF-α and IL-6. APL activates the innate immunity of trophoblast cells and inhibits the proliferative activity of trophoblast cells by binding pattern recognition receptors to the surface of trophoblast cells. This reduces the migration and invasion of trophoblast cells, and promotes the release of a large number of inflammatory factors, which accelerate apoptosis of trophoblast cells and interfere with uterine spiral artery recasting (*61*). Dietary changes can alter the gut microbiome and mortality of mice with high titers of β2 glycoprotein I antibodies, and APS manifestations are markedly reduced by dietary restriction. Importantly, probiotic bacterial strain could alter aPLs in non-autoimmune animals (*62*).

RPL may be attributed to the influence of ANA on the platelet activity, coagulation or anticoagulation mechanisms, and damage of vascular endothelial cells to induce thrombosis, thereby affecting placental angiogenesis and uterine hemodynamics (*63*). Gülden *et al*. administered a combination of four antibiotics into humanized mice to treat them with ANA and found an increase in the number of effector T cells in the gut, indicating how the microbiome could influence human immune cells (*64*).

2.3.2. Autoimmune thyroid disease

AITD is an organ-specific autoimmune disorder mediated by Th1 cells. A meta-analysis showed that miscarriage was associated with a positive thyroid antibody (DR: 3.73, 95% confidence interval (CI): 1.8-7.6) and RPL (odds ratio (OR): 2.3, 95% CI: 1.5-3.5) (*65*). At present, it is widely accepted that changes in the composition and structures of the intestinal microbiota are associated with the occurrence and development of AITD. Proteins of certain strains of *Yersinia*, *Bifidobacteria*, and *Lactobacillus* have similar amino acid sequences with thyroid-stimulating hormone receptor, thyroglobulin, and thyroid peroxidase. When dysregulation of intestinal microbiota causes damage to the intestinal barrier, they can be entered into the blood by the gap, and break the immune tolerance to induce the autoimmune response through molecular simulation and other mechanisms, thereby inducing AITD (66,67). In addition, *Phyllofilamentous*, *Prevotella*, *Eubacter rectum*, and other bacteria may induce or aggravate AITD by affecting Th17/Treg axis (*68,69*).

2.3.3. Maternal-fetal immunological disorder

As patients with recurrent abortion account for more than half of the population, the procedures are sometimes referred to as some of the unexplained recurrent miscarriages that might be explained by abnormalities in maternal-fetal immunology (*34*). Pregnancy is a unique immunological phenomenon, and maternal-fetal immune tolerance is the basis of embryonic implantation and pregnancy maintenance. Hence, a normal immune response is essential to maintain pregnancy in early-stage. The foundation is the development of immunological tolerance during pregnancy, which includes reduced activity of natural killer (NK) cells, Th2 type immune response, increased activity of Treg cells, and modest trophoblast infiltration. Any abnormalities may lead to imbalance of maternal-fetal immune tolerance (*70*). Once the immune tolerance is broken, the maternal immune system may reject the embryo, failing of embryonic implantation and abortion (*34*).

Changes in the number and species of the gut microbiome and the concentrations and activities of their metabolites are important factors, affecting systemic immune response and endocrine. By interfering with the immunological balance of Treg/Th17 cells at the maternal-fetal interface, their aberrant alterations may result in IR and induce RPL (*34,58*). When Treg and Th17 cells interact with each other and tend to balance, the decidua can not only resist external infection but also prevent the embryo from being rejected by the maternal immune system. As a result, an imbalance of Treg/Th17 cells is an important cause of maternal-fetal immune intolerance (*34,70*).

In general, IFN-γ is involved in supporting uterine spiral artery remodeling and decidualization, and NK cells are beneficial to pregnancy by secretion of IFN-γ. At the maternal-fetal interface, a large number of NK cells are accumulated, and 70% of decidual lymphocytes are $CD56^{bright}CD16^{-}$ NK cells. These cells can take part in endometrial vascular remodeling, trophoblast invasion,

and the maintenance of immunological tolerance at the maternal-fetal interface, according to research conducted on animals and humans (*71*). Therefore, abnormal number and activity of NK cells are closely related to URSA. Recently, Fu *et al*. demonstrated that the decidual NK cells could prevent the Th17 cells from secreting IFN-γ, which could cause a local inflammatory reaction, and lead to tolerance. Contrarily, in RPL patients or animal models, the number of decidual Th17 could increase, and the inhibition of NK cell-mediated response could be vanish, finally leading to the loss of tolerance (*71*). Additionally, healthy decidual NK cells release large amounts of the anti-inflammatory cytokine IL-10, which is crucial for inhibiting TH17 cells (*72*). Therefore, NK cells could maintain fetal development at the maternal-fetal interface by controlling the local inflammation.

Intestinal microbiota can activate NK cells (*73*) and stimulate the development of the intestinal mucosal immune system directly or indirectly by activating helper cells (*74*). For instance, it was revealed that *Lactobacillus Eosinophilus* could promote the secretion of IL-12 by dendritic cells, activating NK cells to promote the synthesis and secretion of IFN-γ (*75*). *Lactobacillus plantarum* could also promote synthesis of NK cells and secretion of IL-22 by recognizing TLR2, upregulating the expression levels of tight junction proteins in intestinal epithelial cells (*73*). In addition, *Roseburia intestinalis* was found to be positively associated with IL-22 and IL-6, improving insulin sensitivity (*76*). It was well documented that human leukocyte antigen class I molecules could regulate the function of NK cells. Importantly, changes in human leukocyte antigen structure could be associated with distinct variations in the gut microbiome (*77*).

2.4. Obesity can be an independent risk factor for RPL

In women of reproductive age, obesity is on the rise, and it has a detrimental effect on their capacity to conceive. Obesity causes RPL through heredity, inflammation, immune imbalance, decidua damage, endocrine disorder, pre-thrombotic state, *etc*. Obesity could be an independent risk factor for RPL. However, there is a significant difference in the gut microbiome diversity between healthy individuals and obese individuals, and the diversity is related to body mass index (BMI). Hence, the gut microbiome is associated with susceptibility to obesity (Figure 2). Schele *et al*. demonstrated that the gut microbiome reduced the expression level of proglucagon, which encoded the fat-suppressing peptide GLP1 in the brain stem and contributed to the increased fat mass (*78*). A previous study showed that *Bacteroides* to *Firmicutes* ratio was altered in obese individuals and this ratio could revert to normal weight loss, suggesting that the gut microbiome might play a key role in obesity (*79*).

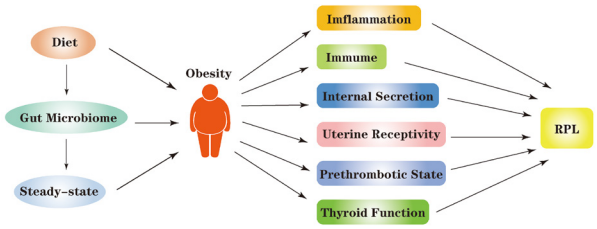

Figure 2. The possible mechanisms of the effects of diet on the gut microbiome and the relationship leading to the increased risk of the development of RPL.

2.4.1. Heredity

Clinically, RPL is highly associated with an abnormal chromosomal karyotype (*80*). Obese women were reported to be at a significantly higher risk of miscarriage, whereas at a lower risk of chromosomal abnormalities in stream products compared with women with normal BMI (*81*). Therefore, the majority of chromosomal abnormalities are irrelevant to obesity-induced RPL.

2.4.2. Obesity-induced gut microbiome promotes inflammation

The association between obesity and inflammation could be mediated by the inflammatory agents produced by gut microbiome, such as LPS and TNF-α. Wang *et al*. found that a high-fat diet (HFD) could result in an abundance of gram-negative bacteria in intestinal tracts of obese mice, which could cause damage to the mucosal barrier and increase intestinal permeability, releasing a significant quantity of LPS into the blood (*82*). Fei *et al*. used Enterobacteriaceae isolated from the gut microbiome of obese individuals to establish a genetic mouse model with only this microbiome, and it was revealed that the experimental group showed elevated LPS, developing obesity and IR, while their germ-free counterparts who were exposed to the same conditions exhibited no change (*83*). In accordance with Fei *et al*.'s findings (*83*), Just *et al*. concluded that germ-free mice would not become obese even if they were given an HFD (*84*).

Additional inflammatory products are produced by gut microbiome in obese patients than in normal individuals. Li *et al*. found that obese mice in the HFD group had significantly higher levels of LPS and TNF-α compared with those in the control group (*85*). Moreover, the relative abundance of *Firmicutes* and Proteobacteria increased, while the relative abundance of Bacteroidetes, Actinobacteria, and Verrucomicrophyla decreased, suggesting that the high levels of LPS and TNF-α could be related to the changes of intestinal microbiota. LPS stimulates the pro-inflammatory pathway and causes IR by activating TLR4 on adipocytes and upregulating NF-κB expression level (*86*). When the function of adipocytes is abnormal, it's usually macrophages in fat

tissue that may release IL-6 (*42*), downregulating the expression levels of glucose transporter-4 and IRS-1 in adipocytes. TNF-α binding to insulin receptors in liver cells and skeletal muscle cells can inhibit IRS phosphorylation and decrease its activity, which may reduce the efficiency of insulin signal transmission and lead to IR (*87*).

The gut microbiome in obese patients produces more inflammatory products. In contrast to the healthy control group, Zheng *et al*. demonstrated that obese HFD-fed mice had an intestinal microbiota that was dysregulated (88), and the bile acid content of their cecal contents had dramatically risen. Some scholars have shown that IL-18 and FFA levels were overexpressed in the endometrium of overweight patients with PCOS at the proliferative stage (*89*). Increased inflammatory substances cause reduction of ovarian blood, which may cause damage to granulosa and follicular membrane cells and lower progesterone production. The reduced progesterone production leads to attenuate endometrial secretory function, which is unconducive to embryonic implantation and growth, and it may manifest as infertility or abortion. Besides, an animal study showed that FFA could inhibit the synthesis of inflammatory kinases in pituitary gonadotropins and suppress activin-induced follicle-stimulating hormone β subunit expression level through Toll like receptor 2. The upregulated LH mRNA expression could inhibit FSH mRNA expression. In female HFD-fed mice, estrus cycle length was shortened and luteal count in the ovary was reduced, severely attenuating luteal function in early pregnancy because they did not experience the anticipated rise in LH and FSH levels before estrus (*90*).

2.4.3. Obesity-mediated alterations in immunity

The cultivation of embryos requires maternal immune tolerance. Abortion and unsuccessful embryonic implantation originate from the maternal immune system rejecting the embryo after the immunological tolerance has been compromised. Parker *et al*. found that the expression of uterine $CD8^+$ cells was reduced by nearly 40% in HFD-fed mice, suggesting that obesity could adversely affect maternal immunological adaptation (*91*). Thus, excessive fat may contribute to the development of AIDs. Long *et al*. reported that IL-18 was significantly overexpressed in the endometrium of overweight patients with PCOS at the proliferative stage (*89*), its overexpression increased IFN-γ production, and led to chronic low-grade inflammation. Parker *et al*. fed C57/BL6 mice with a high fat/sugar diet for 12 weeks to establish an obese mouse model, and mice were fed with a low fat/sugar diet as control. Progesterone and prolactin levels in plasma were measured at 7.5 days after copulation. The results showed that the percentage of NK cells and the expression level of IFN-γ were significantly reduced in obese mice. Besides, IFN-γ is involved in supporting uterine spiral artery remodeling and decidualization (*91*). Therefore, it could be hypothesized that obesity during the first trimester of pregnancy could inhibit angiogenesis by upregulating IL-18 expression level and downregulating IFN-γ expression level in NK cells.

2.4.4. Internal secretion

Leptin is an adipocytokine synthesized and secreted into the blood by lipocytes. It was demonstrated that HFD-fed mice had gut microbiota-associated diseases, which accelerated the leptin response (*78*). Leptin can not only activate adenosine-activated protein kinase (AMPK) signal transduction pathway, but also directly regulate lipid metabolism. It can also mediate phosphatidylinositol 3-kinase signaling pathway through binding to its receptor in hypothalamus, promoting insulin sensitivity. Leptin resistance can develop from a noticeable drop in the sensitivity of the leptin receptor caused by an increase in blood levels of the hormone (*92*). Leptin resistance may lead to metabolic disorders and promote IR (*93*). In addition to leptin, the dysbiosis of intestinal microbiome caused by an HFD may also lead to adiponectin resistance. Furthermore, adiponectin, an insulin-sensitizing hormone, promotes fatty acid oxidation and reduces triglyceride level in the muscle and liver of obese mice by activating the AMPK signaling pathway. Adiponectin resistance decreases fatty acid oxidation by inhibiting AMPK activity. Moreover, adiponectin resistance impairs phosphorylation of important insulin signaling proteins (Akt and AS160) in muscle. Ultimately, adiponectin resistance decreases insulin sensitivity and IR (*94*).

It is noteworthy that overweight individuals are at a lower risk of HHcy. A growing body of evidence demonstrated that the gut microbiota affects the blood homocysteine level of obese individuals, causing RPL. However, research has shown that folic acid generated by *Bifidobacterium longum* in the human intestines could lower the homocysteine level in individuals receiving hemodialysis (*95*). HHcy can damage endothelial cells by inhibiting the activity of glutathione oxidase and promoting the formation of peroxides. Further increase of the production of chemokines and cell adhesion molecules could promote platelet thrombosis, enhancing coagulation and preventing anticoagulation (*52*).

2.4.5. Uterine receptivity

Uterine receptivity is controlled by the activation of self-regulating feedback loops of decidual stromal cells in the subluminal epithelium. For pregnancy to succeed, the endometrium should engage with an embryo, and the embryo is implanted into the decidual matrix. However, obesity may cause decreased endometrial receptivity and poor endometrial decidualization during

implantation. Endometrial stromal cells in decidua after being transformed to the biosensor might cause decidual chemical harm to the PRL mechanism because they are extremely fatty to provide enough decidual response when they are implanted (*96*). Rhee *et al*. mated female mice after 12 weeks of HFD and high-sugar diet and found that the number of syncytia in decidua after fertilization was significantly lower than that in female mice with a normal diet. They performed diagnostic curettage under hysteroscopy in obese women with BMI $\geq$ 30 kg/m^2 and normal women with BMI < 25 kg/m^2. The expression levels of prolactin and insulin-like binding protein in the endometrial decidua in the obese group were lower than those in the normal weight group. These two factors are important markers for endometrial decidua (*97*). Yu *et al*. found that β-sitosterol might regulate the endometrial receptivity of patients with PCOS by changes in the structure and composition of the gut microbiome (*98*). Hence, changes in the gut microbiome can be associated with endometrial receptivity, and further relevant research should be conducted in the future.

2.4.6. Prethrombotic state (PTS)

Although there is strong evidence indicating the association of the gut microbiome with an elevated risk of obesity, which in turn increases the risk of the PTS, there is no clear, direct method in which the gut microbiota is associated with the PTS. The PTS, which has been identified as a significant contributor to RPL, is a pathological hypercoagulant condition and a pathological process of coagulation and anticoagulation system dysfunction in the body. Obesity is a risk factor for PTS and increases the expression levels of plasma plasminogen activator inhibitor-1 and tissue factor (TF). TF drives several aspects of metabolic disorders through the G-protein-coupled protein-activated receptor (PAR2, PAR1) signaling pathway. TF-PAR2 signaling pathway in adipocytes leads to diet-induced obesity by attenuating metabolism, while TF-PAR2 signaling pathway in hematopoietic and myeloid cells causes inflammation. As a result, this inflammatory effect could activate TF-induced clotting, and the two reinforce each other, leading to a vicious cycle (*99*).

3. Conclusions and future prospects

In recent years, a growing body of evidence demonstrated the association of the gut microbiota with a variety of diseases. Previous research suggested that the gut microbiome could respond to RPL, however, few studies have reported a direct correlation between RPL and intestinal microbes. Therefore, numerous physiological and pathological mechanisms need to be confirmed. In this review, the effects of the gut microbiome on RPL were discussed to be related to inflammatory response, the disruption of insulin signaling pathway and the formation of IR, maintenance of immunological tolerance at the maternal-fetal interface due to the interference with the immune imbalance of Treg/Th17 cells, and obesity. Exploring the effects of the gut microbiome on RPL is suggested *via* further research on the cytokines and signaling pathways involved in the pathogenesis of RPL caused by microorganisms, which may be beneficial for clinical prevention and treatment of RPL.

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