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The Influence of Vaginal, Endometrial, Cervical, Gut, and Semen Microbiota on Recurrent Pregnancy Loss: A Narrative Review

Jaworowicz Anna[1]*, Piasecka Joanna[2], Gołembiewski Bartosz[1], Mulski Grzegorz[3], Manicka Martyna[2], Baranowicz Michał[4], Mazurkiewicz Weronika[1], Borecka Zuzanna[5], Sobczak Agnieszka Marta[3], and Szymczak Alicja[2]

[1]MSWiA Hospital in Poznan, Dojazd 34, 60-631 Poznań, Poland

[2]Medical Center HCP, Poznan, Poland

[3]University Hospital in Poznan, Poland

[4]Poznan University of Medical Sciences

[5]Regional Hospital in Poznan, Poland

SUMMARY

Background: Recurrent Pregnancy Loss (RPL) is a multifactorial condition in which more than half of cases remain unexplained despite advances in genetic, anatomical, endocrine and immunological evaluation. Growing evidence indicates that host-associated microbial ecosystems across the female and male reproductive tracts, as well as the gastrointestinal tract, exert significant influence on implantation, early placentation and maternal-fetal immune tolerance. This narrative review synthesizes current data on the vaginal, cervical, endometrial, gut and seminal microbiota in the context of RPL.

Across the lower and upper reproductive tract, a Lactobacillus-dominant profile is consistently associated with reduced mucosal inflammation, preserved epithelial barrier integrity and a cytokine environment conducive to early gestational tolerance. Dysbiosis (characterized by Lactobacillus depletion, enrichment of anaerobes, increased microbial diversity or expansion of taxa linked to bacterial vaginosis) is repeatedly associated with heightened local inflammation, impaired endometrial receptivity, altered immune cell recruitment and suboptimal embryo-endometrium interactions. Endometrial dysbiosis additionally correlates with chronic endometritis and perturbed maternal-fetal immune adaptation. Gut microbiome alterations, particularly loss of short-chain fatty acid-producing taxa and metabolite disturbances influencing systemic immune tone, appear to promote pro-inflammatory cytokine profiles implicated in RPL pathogenesis. The seminal microbiome may also contribute through microbe-driven oxidative stress, increased sperm DNA fragmentation, altered seminal cytokine milieu and semen-mediated modulation of the female immune response at conception.

Although causal relationships remain incompletely defined, evidence supports a model in which multisite microbial-immune interactions influence early pregnancy stability. The heterogeneity and methodological limitations of available studies highlight the need for standardized multi-site profiling, longitudinal designs and mechanistic trials evaluating targeted microbiome-directed interventions. Understanding these microbial contributions may open future translational avenues for personalized diagnostic and therapeutic strategies in RPL.

Keywords: Abortion; Dysbiosis; Fertility; Microbiota; Pregnancy complications; Recurrent pregnancy loss

Address for correspondence:

Jaworowicz Anna

MSWiA Hospital in Poznan, Dojazd 34, 60-631 Poznań, Poland;

Tel No:+48 517 750 504

E-mail: annajaworowicz2000@gmail.com

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ABBREVIATION: RPL: Recurrent Pregnancy Loss; BV: Bacterial Vaginosis; IL: Interleukin; HPV: Human Papillomavirus; HSV: Herpes Simplex Virus; RCT: Randomized Controlled Trial; PCR: Polymerase Chain Reaction; EMMA: Endometrial Microbiome Metagenomic Analysis; RIF: Recurrent Implantation Failure; PRR: Pattern Recognition Receptor; SCFA: Short-Chain Fatty Acids; BMI: Body Mass Index; IFN: Interferon; URSA: Unexplained Recurrent Spontaneous Abortion; TNF: Tumor Necrosis Factor; FMT: Fecal Microbiota Transplantation; ROS: Reactive Oxygen Species

INTRODUCTION

Miscarriage is a remarkably common event, affecting approximately one in four pregnancies and accounting for an estimated 23 million losses worldwide each year. Globally, around 44 pregnancies end every minute [1,2]. Although definitions vary between countries, early miscarriage is most often defined as pregnancy loss before 12 weeks of gestation, whereas losses between 12 and 22 weeks are classified as late miscarriages. When gestational age is unknown, a fetal weight of 500 grams is generally used as the threshold distinguishing miscarriage from stillbirth [3–5]. Recurrent pregnancy loss (RPL), defined as two or more consecutive miscarriages, affects 1-2% of women of reproductive age [6].

The clinical presentation of miscarriage is heterogeneous, but the most typical warning signs include vaginal bleeding, cramping and lower abdominal pain, which may exceed the severity of menstrual discomfort. Beyond the physical manifestations, pregnancy loss frequently carries a substantial psychological burden and can be associated with post-traumatic stress disorder and depression [1].

Despite the high prevalence of miscarriage, its underlying causes remain incompletely understood [4,7]. Established contributors include genetic and epigenetic abnormalities [8,9], chromosomal anomalies [10], inaccurate embryo selection [11], abnormalities of uterine anatomy [12], hormonal and metabolic disorders [13], immune dysregulation [14,15] and socioeconomic and cultural factors [16,17]. Maternal age [18] and Black ethnicity [19,20] have also been identified as independent risk factors. Yet, even with advances in reproductive endocrinology, cytogenetics and reproductive immunology, the etiology of the majority of miscarriages remains unexplained. In recent years, attention has shifted toward emerging biological pathways, among which the human microbiome has gained

particular interest as a potential determinant of pregnancy maintenance or loss [21].

The microbiome represents a diverse ecosystem of microorganisms inhabiting the human body, including bacteria, archaea, viruses, fungi and protists. Its composition and relative abundance vary across localization and are shaped by age, hormonal and immune status, medication use (including antibiotics, pro- and prebiotics) and sociocultural factors (such as hygiene practices and sexual behavior). Microbiome plays important and increasingly recognized roles in modulating immune responses, metabolic homeostasis and disease susceptibility [22–32].

Pregnancy itself constitutes a separate physiological factor that uniquely modulates microbiome composition [33]. Advances in sequencing technologies have facilitated detailed investigation of whether disturbances in microbial ecosystems may contribute to pregnancy loss. Dysbiosis (defined as disruption of microbial composition and ecological balance) has been proposed to influence miscarriage risk through several mechanisms, including aberrant modulation of immune responses, excessive production of pro-inflammatory cytokines and impaired maternal-fetal immune tolerance [34]. Furthermore, microbiota from different anatomical sites may contribute through distinct pathways: vaginal and cervical communities influence ascending infection and local inflammatory signaling, endometrial microbiota affect endometrial receptivity and implantation, whereas gut microbiota regulate systemic immunity and metabolic status. Seminal microbiota may also play a role by affecting sperm function and contributing to inflammatory processes within the reproductive tract [35–39].

The aim of this narrative review is to synthesize current knowledge regarding the role of the vaginal, endometrial, cervical, gut and seminal microbiota in RPL. Understanding these relationships may support the development of targeted interventions to reduce miscarriage risk and ultimately enhance reproductive care.

METHODOLOGY

The literature review was conducted using the PubMed, Google Scholar and Wiley Online Library databases. The search covered articles published between 2020 and 2025 using a combination of the following keywords: microbiome, microbiota, miscarriage, recurrent pregnancy loss, early pregnancy loss, vagina, endometrium, cervix, gut and semen. The review included clinical studies, observational studies, systematic reviews and meta-analyses. Only full-text articles published in English were considered. Studies not related to pregnancy loss, animal studies and articles with insufficient methodological clarity were excluded. Reference lists of included publications were also screened to identify additional relevant studies.

RESULTS

Vaginal Microbiome And Recurrent Pregnancy Loss (RPL)

Vaginal Microbiome

The healthy reproductive-age vaginal ecosystem is typically dominated by Lactobacillus spp., which maintain a low pH through lactic acid production and generate antimicrobial factors that limit colonization by anaerobic and pathogenic taxa. This lactobacilli-dominant state is associated with reduced mucosal inflammation and is considered protective for reproductive outcomes. Variation exists between women: common community state types include L. crispatus-dominant, L. iners-dominant, mixed-Lactobacillus, and Lactobacillus-depleted (diverse, anaerobe-rich) communities [39,40].

Vaginal microbiome in pregnancy

During pregnancy, elevated estrogen levels promote glycogen accumulation within the vaginal epithelium, which in turn supports the dominance and stability of Lactobacillus spp. throughout gestation. Such adaptation is hypothesized to reduce the risk of ascending infection and support implantation and early placentation. However, this trajectory is not universal, some women exhibit transition toward higher diversity or dominance by less protective taxa (e.g., L. iners or BV-associated anaerobes), which may predispose to adverse gestational outcomes. Cohort-level analyses show that deviations from the pregnancy-associated lactobacilli-dominant profile are associated with increased risk of early pregnancy loss and other obstetric complications [40–44].

Vaginal dysbiosis and RPL

Studies converge on several recurring patterns in RPL/early miscarriage cohorts:

- Lactobacillus depletion (reduced L. crispatus, sometimes L. jensenii), increased α-diversity. Studies find that women with RPL more often harbour Lactobacillus-depleted, higher-diversity vaginal communities compared with women carrying viable pregnancies. These communities correlated with elevated pro-inflammatory cytokines (IL-1β, IL-6, IL-8) in cervicovaginal fluid, supporting a mechanistic link between local inflammation and pregnancy failure [39,40,45].
- Enrichment of BV-associated or opportunistic taxa: Gardnerella (certain clades), Atopobium vaginae, Prevotella spp., some Streptococcus/Fusobacterium elements in cervicovaginal samples, Ureaplasma, Chlamydia, Toxoplasma, HPV, HSV-1, HSV-2 [1,39,46].
- L. iners dominance as an unstable/transition state associated with higher risk of later shift to dysbiosis [40,47].

The precise risk-associated taxa vary between cohorts, reflecting geographic, sequencing-region (V1-V2 vs V3-V4) and population differences, hence emphasis should be on community states (Lactobacillus-dominant vs depleted) rather than single species as universal biomarkers [45].

Putative mechanisms of RPL in vaginal dysbiosis

The literature supports several, not mutually exclusive, pathways:

- Local inflammation and cytokine dysregulation. Lactobacillus-depleted communities are associated with increased levels of pro-inflammatory cytokines

in cervicovaginal fluid (IL-1β, IL-6, IL-8) that may promote a hostile milieu for implantation or early embryonic development [39].

- Barrier dysfunction and ascending infection. Dysbiotic vaginal communities can compromise mucosal defenses and favor ascension of opportunistic bacteria into the upper reproductive tract, potentially triggering endometrial inflammation or chorioamnionitis, both implicated in pregnancy loss. Cohort data show overlap between vaginal and endometrial dysbioses in some patients, supporting potential upward microbial migration [39,45].
- Immune-tolerance perturbation. Microbial patterns that drive innate immune activation may interfere with the establishment of maternal-fetal immune tolerance (e.g., shift in local Treg/Th17 balance), a recognized requirement for early pregnancy maintenance. Indirect evidence linking vaginal dysbiosis to altered immune cell phenotypes has been reported in RPL cohorts and broader reproductive immunology studies [39,48].
- Metabolic and microbial metabolite effects. Dysbiotic communities differ in metabolite production (e.g., reduced lactic acid, altered short-chain fatty acids or biogenic amines), which can alter pH, epithelial behaviour, and immune signalling, plausible contributors to implantation failure and early loss [41,47].

Therapeutic considerations of vaginal dysbiosis

Intervention data specific to RPL are sparse. Approaches studied in related obstetric contexts include:

- Antibiotics can treat BV but evidence for prevention of miscarriage is inconsistent. Indiscriminate antibiotic use may further perturb beneficial communities. Some studies in infertility context suggest microbiome modulation before embryo transfer can affect outcomes, but RCT-quality evidence is lacking [39,40].
- Probiotics (Lactobacillus strains) have been trialled for BV and to restore Lactobacillus dominance. Studies and mechanistic rationale support potential benefit, and authors of several RPL-focused microbiome papers propose targeted Lactobacillus supplementation as a candidate intervention, yet robust RPL-specific RCTs are missing [40,46].
- Microbiome transplantation is experimental and remains investigational; no high-quality data exist to recommend it in RPL [1].

Overall, while the vaginal microbiome is a plausible modifiable target, clinical intervention trials specifically designed to reduce RPL risk by microbiome modulation are still required [6,39].

Endometrial microbiome and recurrent pregnancy loss (RPL)

Endometrial microbiome

The endometrium, once considered sterile, is now recognized to host a low-biomass microbial community whose composition can vary between individuals and across the menstrual cycle. These resident organisms exist at much lower densities than vaginal communities but are hypothesised to play a role in endometrial receptivity, local immune regulation and tissue homeostasis. Recent high-throughput studies using 16S rRNA sequencing and metagenomic approaches have detected both Lactobacillus-dominated profiles and more diverse communities enriched with facultative or obligate anaerobes in different patients. Methodological rigor (sampling technique, reagent controls, decontamination pipelines) is critical when interpreting endometrial data due to the risk of exogenous contamination [38,49–51].

Endometrial microbiome in pregnancy

Compared with non-pregnant cycles, pregnancy is associated with changes in mucosal immunity and stromal physiology that are likely to alter the endometrial niche. Although human data across early gestation remain limited, available studies indicate that a Lactobacillus-favouring profile often correlates with successful implantation and pregnancy maintenance, whereas unstable or non-Lactobacillus-dominant communities have been observed more frequently in women with implantation failure or pregnancy loss. Several reports emphasise that the timing of sampling (mid-luteal preconception vs. early pregnancy) and technical factors substantially influence detected community patterns [38,52].

Endometrial dysbiosis and RPL

Although heterogeneity exists across studies, commonly observed patterns in RPL cohorts include:

- Reduced relative abundance of Lactobacillus spp. and lower dominance indices in endometrial samples [38,52].
- Enrichment of opportunistic taxa, such as Streptococcus, Fusobacterium, Gardnerella and other proteobacterial lineages. These taxa have been variably linked to inflammation or chronic endometritis [52,53].
- Increased instability of the microbial community, reflected by disrupted or weakened co-occurrence relationships between taxa [52].

It is important to emphasise that taxon-level findings differ with geography, sample handling, sequencing region, and bioinformatic pipelines. Therefore, emphasis on community state (stable Lactobacillus-dominant vs. diverse) is more reproducible than single-species biomarkers [38,49].

Putative mechanisms of RPL in endometrial dysbiosis

Multiple mechanistic pathways have been proposed, grounded in both clinical observations and translational immunology:

- Local inflammation and impaired receptivity. Non-Lactobacillus communities are associated with elevated expression of pro-inflammatory mediators and histologic markers of chronic endometritis, which can impair receptivity required for embryo implantation. Targeted studies have shown correlations between particular taxa and markers of mucosal inflammation in RPL patients [38,54].

- Disruption of immunotolerance. A balanced local microbiome may contribute to the induction and maintenance of regulatory immune pathways (e.g., Treg induction) essential for maternal-fetal tolerance. Dysbiosis may shift these balances toward pro-inflammatory Th subsets, increasing the likelihood of early pregnancy loss. Evidence is indirect but biologically plausible and consistent with cytokine and cell-phenotype data in affected cohorts [52,55,56].
- Direct microbial invasion and ascending infection. Endometrial colonisation by pathogenic organisms or higher bacterial loads could provoke tissue damage or subclinical infection that is incompatible with early pregnancy. Culture-based and PCR studies identifying classical endometritis pathogens in some RPL patients support this pathway for a subset of cases [54,56].
- Metabolic and signalling perturbations. Dysbiotic communities produce altered metabolite profiles (e.g., changes in short-chain fatty acids or biogenic amines) that can modify epithelial function and immune signalling [49,52].

Therapeutic considerations of endometrial dysbiosis

Intervention studies focused specifically on RPL are limited but informative:

- Antibiotic therapy targeting chronic endometritis (identified histologically or via PCR) has been associated with improved outcomes in older literature and in some contemporary cohorts. However, trial evidence specifically addressing microbiome-guided antibiotic strategies in RPL is still sparse [54,57].
- Microbiome-guided interventions (EMMA approach). Commercial and clinical programmes, that combine endometrial metagenomic profiling with targeted antibiotics followed by probiotic restoration, have reported improved implantation rates in RIF cohorts and anecdotal benefit in RPL subsets. Randomized controlled evidence is lacking and methodological concerns persist. A multicentre trial reported preliminary positive signals but requires independent replication [52,58].
- Probiotics and restoration strategies. Use of Lactobacillus-based probiotics to re-establish a low-pH, protective niche is biologically rational. Clinical data in RPL are currently limited, and optimal strains, dosing and administration routes remain to be defined [49].

Overall, current interventional evidence supports a targeted approach (diagnose and treat chronic endometritis) rather than blind broad-spectrum interventions. Prospective, adequately powered RCTs with pre-specified microbiome and immune endpoints are required to define efficacy and safety [54,58].

Cervical microbiome and Recurrent Pregnancy Loss (RPL)

Cervical microbiome

The cervix constitutes a crucial anatomical and immunological barrier between the lower genital tract and the uterine cavity. Its mucous layer and resident microbial communities contribute to defence against ascending infection and to local immune signalling that supports implantation and early gestation. Cervical microbial communities largely reflect, but are not identical to, vaginal ecosystems. In healthy reproductive-age women the cervical canal is commonly dominated by Lactobacillus spp., notably L. crispatus or L. iners. The cervix harbours both planktonic and mucus-associated bacteria. Its unique micro-environment (mucin, local immune effectors) shapes community structure and functional outputs (pH buffering, antimicrobial peptides). Sampling site (surface mucus *vs.* deeper epithelium) and technique substantially influence detected profiles [37,59–61].

Cervical microbiome in pregnancy

Pregnancy is associated with immunological and hormonal changes that often stabilise Lactobacillus dominance in lower genital tract niches, including the cervix, particularly in early gestation. This is thought to reduce ascending infection risk and maintain a favorable milieu for implantation and placentation. Nevertheless, some women show cervicovaginal dysbiosis during early pregnancy. The trajectory of cervical community dynamics during conception and early pregnancy is not uniform and appears influenced by host factors (hormones, sexual activity, previous deliveries) and exogenous exposures (antibiotics, vaginal procedures) [6,60].

Cervical dysbiosis in RPL

Direct, cervix-focused data are limited but accumulating. Across recent cohorts, recurring patterns include:

- Reduced Lactobacillus dominance, notably decreased L. crispatus and relative enrichment of L. iners, which cost an unstable state [37,60–62].
- Increased abundance of Non-Lactobacillus communities such as Gardnerella, Atopobium, Mycoplasma, and other anaerobes [37,60].
- Emergence of unusual taxa (e.g., Cutibacterium, Anaerobacillus) in cervical sites was reported as predictive of euploid miscarriage, suggesting the cervical community may contain prognostic information beyond the vaginal sample alone [37].

Given substantial heterogeneity between studies, comparisons based on overall community state (dominant Lactobacillus versus depleted or diverse communities) appear more clinically meaningful than reliance on individual genera as universal biomarkers [61].

Putative mechanisms of RPL in cervical dysbiosis

Four mechanistic pathways are supported by clinical and translational evidence:

- Impaired barrier function and ascending infection. The cervix acts as a physical and immunological gate. Dysbiotic cervical communities may weaken mucus properties and permit upward migration of potentially pathogenic bacteria into the uterine cavity, promoting endometrial inflammation or subclinical infection that compromises implantation or early placentation. Observational data showing overlap between cervical and endometrial dysbioses in some patients support this route [37,60].

- Local innate immune activation (PRR signalling). There is association between cervical microbial patterns and expression of pattern-recognition receptors (Toll-like receptors, NOD-like receptors) and downstream inflammatory mediators. Heightened PRR signalling in the cervix could translate into a pro-inflammatory uterine environment incompatible with embryo tolerance [63].
- Cytokine milieu and immune cell trafficking. Cervical dysbiosis correlates with elevated local pro-inflammatory cytokines (e.g., IL-1□, IL-6) and altered chemokine gradients. Such changes may modify maternal immune cell recruitment and the local balance of regulatory vs effector T cells critical for early gestation [37,60].
- Microbial metabolites and mucosal environment. Shifts away from lactic acid-producing Lactobacillus toward anaerobes change pH and metabolite profiles (biogenic amines, SCFA), affecting epithelial function and possibly promoting proteolytic activities that alter cervical mucus consistency and integrity. These biochemical changes can facilitate microbial ascension or local tissue responses deleterious to pregnancy [6,60].

Therapeutic considerations of cervical dysbiosis

Direct intervention trials targeting the cervical microbiome in RPL are scarce. Relevant translational considerations:

- Screening and diagnostic value. Cervical sampling is attractive (less invasive than endometrial sampling) and may provide prognostic information. Some groups propose integrating cervical microbiome profiling into RPL work-up, but standardized protocols and prospective validation are required [37,61].
- Antibiotics and targeted therapy. If specific pathogens or chronic cervicitis are documented, targeted antibiotic therapy may be indicated. However, empirical antibiotic use without microbial confirmation risks further dysbiosis [62].
- Probiotics and local restoration strategies. Reinstating Lactobacillus dominance (topical or oral probiotics) is conceptually promising but lacks robust RPL-specific trial evidence. Intervention studies in related fields provide a rationale but cannot be extrapolated without dedicated trials [6].
- Immune-modulatory approaches. If cervical dysbiosis is linked to heightened PRR signalling and local inflammation, adjunctive anti-inflammatory or immunomodulatory strategies could be considered experimentally, but clinical evidence is not established [63].

Gut microbiome and recurrent pregnancy loss (RPL)

Gut microbiome

The gut microbiome is the largest and metabolically most active microbial ecosystem in the human body. Through its metabolites (short-chain fatty acids, bile-acid derivatives, tryptophan catabolites, etc.) and by shaping systemic immune tone, it can modulate distant mucosal immune systems, including those relevant to reproduction. Given that immune tolerance and finely tuned inflammatory signalling are essential for implantation and early placentation, perturbations of gut microbial composition or function offer plausible routes by which systemic microbiota could influence pregnancy maintenance. In healthy non-pregnant adults the gut community is normally diverse and dominated by Firmicutes and Bacteroidetes phyla, with functional roles in SCFA production, bile-acid transformation and immune education [64,65].

Gut dysbiosis in RPL

Human studies are limited but convergent on a few themes:

- Reduced abundance of putative anti-inflammatory, SCFA-producing taxa (e.g., Faecalibacterium, Anaerostipes, Roseburia). Loss of these taxa may reduce systemic anti-inflammatory tone [36,66].
- Altered representation of Prevotella groups. Some studies report shifts in Prevotella spp. (either increases or decreases depending on the cohort), which may reflect diet-driven community structures with immunomodulatory consequences such as elevated systemic Th1/Th17 cytokines [36].
- Altered fecal metabolite profiles that correlate with immune activation. Particular fecal metabolites, e.g., imidazolepropionic acid, other microbial-derived compounds, were associated with increased IL-17/IFN-γ and with recurrent miscarriage, suggesting metabolite-mediated immune modulation [36,67].
- Subgroups with immunologic phenotypes. Some data suggest that gut dysbiosis may be particularly relevant in RPL subgroups with immune abnormalities (e.g., positive antiphospholipid or antinuclear antibodies). In such patients altered gut diversity appeared more pronounced [68].
- Changes in bile-acid-transforming bacteria and metabolite profiles. Altered bile-acid metabolites and SCFA signals are linked to differences in circulating immune cell subsets in URSA, suggesting functional shifts rather than taxon-only changes [67].

Most existing human studies are modest in size, often cross-sectional, and vary methodologically. Therefore associations are hypothesis-generating rather than definitively causal. Variability of taxa across studies argues for emphasis on functional readouts (metabolites, immune correlates) in addition to taxonomic descriptions [36,67].

Putative mechanisms of RPL in gut dysbiosis

The mechanistic hypotheses supported by the human multi-omic data are:

- Systemic immune priming toward pro-inflammatory phenotypes. Gut dysbiosis correlates with elevations in Th1/Th17-related cytokines (IL-2, IL-17A/F, IFN-γ,

TNF-α) in miscarriage cohorts. These systemic signals can impair maternal-fetal tolerance and promote rejection-like pathways at the implantation site [36].

- Loss of anti-inflammatory metabolites (SCFAs, secondary bile acids). Depletion of butyrate-producing bacteria reduces circulating SCFAs, molecules known to support Treg induction and epithelial barrier integrity. Such reductions may lower systemic tolerogenic capacity [67].
- Metabolite-mediated modulation of coagulation. Some microbial metabolites can influence host metabolism and coagulation systems (possible relevance for implantation and placental vascularization), though direct human evidence in RPL is preliminary [36].
- Molecular mimicry and antigenic cross-reactivity (speculative). Gut microbes might generate antigens that cross-react with placental and embryonic targets in genetically predisposed individuals, potentially triggering immune responses. This remains hypothetical and requires mechanistic work [68].

Therapeutic considerations of gut dysbiosis

There are no established gut-targeted therapies proven to prevent RPL. However, mechanistic and small interventional data suggest possible directions:

- Probiotics, pro-, prebiotics and diet. Oral probiotics (Bifidobacterium, Lactobacillus strains) may beneficially modulate gut metabolites and systemic immunity. Evidence specific to RPL is lacking and based mainly on small studies or extrapolation. Dietary interventions (high-fiber, Mediterranean-style diets) that enrich SCFA production are biologically plausible but untested in RPL trials [67,69].
- Metabolite-directed approaches. If specific metabolite deficits (e.g., butyrate) are causal, targeted supplementation or microbiota-directed foods could be explored. Currently this is conceptual [36].
- Fecal microbiota transplantation (FMT) has been used experimentally in other diseases. For RPL it remains purely investigational and ethically complex; no clinical trials support FMT for pregnancy loss prevention. Animal models suggest gut composition can affect pregnancy outcome, supporting further research but not clinical use yet [70].

Present evidence is insufficient to recommend gut-targeted treatments for preventing RPL. The strongest near-term translational path is rigorous prospective characterization followed by carefully designed interventional trials in selected subgroups [36,67].

Semen microbiome and recurrent pregnancy loss (RPL)

Semen microbiome

Semen is not sterile. Modern sequencing shows that ejaculated fluid carries a low-to-moderate biomass microbial community that varies between men. Some men present Lactobacillus-dominant profiles, others show communities enriched in anaerobes or skin or urethral taxa (e.g., Corynebacterium, Staphylococcus, Prevotella, Gardnerella, Actinomyces, Varibaculum). Male reproductive tract ecology is shaped primarily by host factors (age, sexual behaviour, urogenital infections, hygiene, antibiotics, metabolic status). Because semen delivers sperm and seminal plasma into the female reproductive tract at conception, seminal microbes (or their products) may influence sperm function, embryo quality and the maternal immune response. Consequently, paternal microbiome factors represent a biologically plausible contributor to adverse pregnancy outcomes including RPL [35,71,72].

Semen dysbiosis in RPL

Direct, large prospective studies explicitly linking seminal microbiome composition to RPL incidence are still rare. However, converging lines of original evidence are relevant:

- Associations with sperm DNA damage and reactive oxygen species. Multiple studies demonstrate that certain seminal microbial profiles are associated with increased semen ROS and higher sperm DNA fragmentation indices. Because elevated sperm DNA fragmentation and oxidative sperm damage are recognized paternal factors associated with implantation failure and miscarriage, these microbial associations provide an indirect but plausible route linking seminal microbiome to RPL [35,73].
- Seminal taxa associated with poor sperm function. Samples enriched in particular taxa (e.g., Varibaculum, Prevotella, some Proteobacteria and Actinobacteria lineages) correlate with abnormal motility, morphology and indices of sperm chromatin integrity, what can be linked with RPL [71,74].
- Relative overrepresentation of Lactobacillus iners in some high-DNA-fragmentation groups. Unstable state linked to inflammation [35,73].
- Altered seminal metabolome (e.g., perturbations in butanoate and acetyl-CoA related metabolites) that correlate with DNA damage metrics in semen, suggesting functionally relevant metabolic dysregulation rather than mere taxonomic change [73,75].
- Possible impact on embryo quality and female tract after coitus. Emerging evidence from IVF and embryo culture studies suggests that semen-associated bacteria or seminal plasma factors can influence early embryo development and the female genital tract environment. Hence seminal dysbiosis could indirectly affect implantation success and early pregnancy stability. Animal and translational studies also support that paternal factors carried in semen can modify maternal immune programming [72,76].

Taxa implicated vary among populations and methods. Direct causal human evidence that a given seminal microbial signature causes RPL is not yet definitive, but multiple reproducible associations between seminal microbiome, sperm DNA damage and miscarriage risk support a plausible mechanistic pathway [35,73,74,77].

Putative mechanisms of RPL in semen dysbiosis

Mechanistic pathways supported by the literature (human and translational) include:

1. Oxidative stress and sperm DNA fragmentation. Bacteria or bacterially driven leukocytospermia can elicit local inflammation and ROS production in semen. ROS cause sperm DNA breaks, chromatin instability and fragmentation. Elevated sperm DNA fragmentation is associated with impaired embryo development and higher miscarriage rates [35,73,78].
2. Seminal plasma-mediated modulation of the maternal immune response. Seminal plasma contains immunomodulatory factors and microbial components. Aberrant seminal composition could skew the maternal immune response at conception (e.g., promote pro-inflammatory activation rather than tolerogenic adaptation), compromising implantation or early placentation [72,76].
3. Direct microbial transfer and ascending effects. Bacteria introduced into the female tract at coitus can transiently change vaginal communities. If seminal microbes ascend or provoke local inflammation, endometrial receptivity may be disturbed. Data showing partner microbiome complementarity and post-coital shifts in female communities support this mechanism [35,72].

Therapeutic considerations of semen dysbiosis

At present, no definitive interventional trial demonstrates that modifying the seminal microbiome prevents RPL. Nonetheless:

- Treating urogenital infections (when a pathogen is identified) or leukocytospermia may improve semen parameters. Clinical guidelines acknowledge treatment of symptomatic infections but evidence for improved live-birth rates is limited [35].
- Antioxidant therapy (to reduce oxidative damage) is used empirically in men with high sperm DNA fragmentation. If seminal dysbiosis exerts effects via ROS, antioxidants might be beneficial, but trials are heterogenous and not microbiome-targeted [78].
- Microbiome-directed strategies: probiotics, antibiotics targeted by sequencing and lifestyle changes, are conceptually plausible. A few pilot studies examine probiotics regimens for male infertility but robust RCT evidence for RPL prevention is absent [35,77].

Assessing male factors (including sperm DNA fragmentation and urogenital infection) remains standard in RPL work-up. Seminal microbiome profiling is promising as an adjunctive research tool but not yet standardised for routine clinical decision-making.

CONCLUSION

Current evidence indicates that reproductive outcomes in couples experiencing RPL are closely linked to the compositional stability, metabolic activity and immunomodulatory potential of multiple host-associated microbiomes. Although each anatomical niche maintains its own ecological characteristics, several unifying themes emerge. Across the lower and upper female reproductive tract, a Lactobacillus-dominant profile appears consistently associated with reduced mucosal inflammation, tighter epithelial barrier integrity, lower pathogen abundance and a cytokine milieu compatible with early gestational tolerance. Dysbiosis of the vagina, cervix or endometrium (characterized by reduced lactobacilli, expansion of anaerobes, increased microbial diversity or enrichment of taxa associated with bacterial vaginosis) correlates with heightened local inflammation, disrupted endometrial receptivity, altered immune cell recruitment and impaired embryo-endometrium crosstalk. These perturbations converge mechanistically toward defective implantation and early placental maladaptation, increasing the likelihood of RPL.

The gut microbiome contributes indirectly through systemic metabolic and immunological pathways. Gut dysbiosis, particularly reduced short-chain fatty acid production and overrepresentation of pro-inflammatory taxa, affects trophoblast function, systemic cytokine balance and intestinal permeability, promoting inflammation that can propagate to the reproductive tract. Emerging data suggest that gut-reproductive tract microbial axis alterations may lower implantation potential and increase early gestational instability.

The seminal microbiome represents a parallel but often overlooked pathway. Altered seminal microbial communities can influence sperm motility, DNA integrity, oxidative stress load and seminal cytokine concentrations. These changes affect both male fertility parameters and the immune environment encountered by the female reproductive tract after intercourse, with downstream effects on implantation and placentation. Seminal dysbiosis may therefore act as a contributory factor to RPL through both male-intrinsic and partner-mediated mechanisms.

Collectively, available studies indicate that RPL is not driven solely by isolated microbial disturbances but rather by multisite microbial-immune interactions involving both partners. Dysbiosis across any of these microbial ecosystems can disrupt reproductive tolerance, while concurrent disturbances across niches likely have additive or synergistic effects. Although causal pathways are not fully established, mechanistic links through inflammation, oxidative stress, impaired receptivity, altered sperm function and disrupted maternal-fetal immune adaptation are repeatedly observed.

Given the heterogeneity of findings, limited longitudinal data and modest sample sizes in many studies, the field requires standardized methodologies, simultaneous multi-site microbiome profiling, rigorous control for confounders, and mechanistic investigations integrating host immune and metabolic responses. Nonetheless, current evidence supports the concept that targeted modulation of microbial ecosystems (once causality and biomarkers are better defined) may offer a novel avenue for prevention or treatment of RPL.

CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this article.

REFERENCES

1. **Saadaoui M, Singh P, Ortashi O, et al.** Role of the vaginal microbiome in miscarriage: Exploring the relationship. *Front Cell Infect Microbiol*. 2023 (13); 13:1232825.
2. **Quenby S, Gallos ID, Dhillon-Smith RK, et al.** Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. *Lancet*. 2021 (397): [1658-1667].
3. **Al□Memar M, Bobdiwala S, Fourie H, et al.** The association between vaginal bacterial composition and miscarriage: A nested case–control study. *BJOG Int J Obstet Gynaecol*. 2020 (127): [264-274].
4. **Larsen EC, Christiansen OB, Kolte AM, et al.** New insights into mechanisms behind miscarriage. *BMC med*. 2013 (11): 154.
5. **Magnus MC, Wilcox AJ, Morken NH, et al.** Role of maternal age and pregnancy history in risk of miscarriage: Prospective register based study. *bmj*. 2019 (364): 1869.
6. **Garmendia JV, De Sanctis CV, Hajdúch M, et al.** Microbiota and Recurrent Pregnancy Loss (RPL); more than a simple connection. *microorg*. 2024 (12): 1641.
7. **Lathi RB, Hazard FK, Heerema-McKenney A, et al.** First trimester miscarriage evaluation. In *Semin Reprod Med* 2011 (29): [463-469].
8. **Branch DW, Gibson M, Silver RM.** Clinical practice. Recurrent miscarriage. *N Engl J Med*. 2010 (363): [1740-1747].
9. **Daher S, Mattar R, Gueuvoghlanian□Silva BY, et al.** Genetic polymorphisms and recurrent spontaneous abortions: An overview of current knowledge. *Am J Reprod Immunol*. 2012 (67): [341-347].
10. **Hyde KJ, Schust DJ.** Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med*. 2015 (5): a023119.
11. **Kiecka A, Macura B, Szczepanik M.** Can Lactobacillus spp. be a factor reducing the risk of miscarriage?. *Pol J Microbiol*. 2021 (70): 431.
12. **Chan YY, Jayaprakasan K, Zamora J, et al.** The prevalence of congenital uterine anomalies in unselected and high-risk populations: A systematic review. *Hum Reprod Update*. 2011 (17): [761-771].
13. **Cocksedge KA, Saravelos SH, Metwally M, et al.** How common is polycystic ovary syndrome in recurrent miscarriage?. *Reprod Biomed Online*. 2009 (19): [572-576].
14. **Holers VM, Girardi G, Mo L, et al.** Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med*. 2002 (195): [211-220].
15. **Calleja-Agius J, Jauniaux E, Pizzey AR, et al.** Investigation of systemic inflammatory response in first trimester pregnancy failure. *Hum Reprod*. 2012 (27): [349-357].
16. **Yazdkhasti M, Pourreza A, Pirak A, et al.** Unintended pregnancy and its adverse social and economic consequences on health system: A narrative review article. *Iran J Public Health*. 2015 (44): 12.
17. **Zheng D, Li C, Wu T, et al.** Factors associated with spontaneous abortion: A cross-sectional study of Chinese populations. *Reprod Health*. 2017 (14): 33.
18. **Andersen AM, Wohlfahrt J, Christens P, et al.** Maternal age and fetal loss: Population based register linkage study. *Bmj*. 2000 (320): [1708-1712].
19. **Mukherjee S, Velez Edwards DR, Baird DD, et al.** Risk of miscarriage among black women and white women in a US prospective cohort study. *Am J Epidemiol*. 2013 (177): [1271-1278].
20. **Quenby S, Gallos ID, Dhillon-Smith RK, et al.** Miscarriage matters: The epidemiological, physical, psychological, and economic costs of early pregnancy loss. *Lancet*. 2021 (397): [1658-1667].
21. **Vomstein K, Krog MC, Wrønding T, et al.** The microbiome in recurrent pregnancy loss–a scoping review. *J Reprod Immunol*. 2024 (163): 104251.
22. **Agostinis C, Mangogna A, Bossi F, et al.** Uterine immunity and microbiota: A shifting paradigm. *Front Immunol.* 2019 (10): 2387.
23. **Gao H, Liu Q, Wang X, et al.** Deciphering the role of female reproductive tract microbiome in reproductive health: A review. *Front Cell Infect Microbiol*. 2024 (14):1351540.
24. **Gao X, Louwers YV, Laven JS, et al.** Clinical relevance of vaginal and endometrial microbiome investigation in women with repeated implantation failure and recurrent pregnancy loss. *Int J Mol Sci*. 2024 (25): 622.
25. **Lee SK, Kim CJ, Kim DJ, et al.** Immune cells in the female reproductive tract. *Immune Netw*. 2015 (15): 16.
26. **Gilbert JA, Blaser MJ, Caporaso JG, et al.** Current understanding of the human microbiome. *Nat Med*. 2018 (24): [392-400].
27. **Lloyd-Price J, Abu-Ali G, Huttenhower C.** The healthy human microbiome. *Genome Med*. 2016 (8): 51.
28. **Kroon SJ, Ravel J, Huston WM.** Cervicovaginal microbiota, women's health, and reproductive outcomes. *Fertil Steril*. 2018 (110): [327-36].
29. **Noyes N, Cho KC, Ravel J, et al.** Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PloS one*. 2018 (13):e0191625.
30. **Dall'Asta M, Laghi L, Morselli S, et al.** Pre-pregnancy diet and vaginal environment in caucasian pregnant women: An exploratory study. *Front Mol Biosci*. 2021 (8): 702370.
31. **Kervinen K, Kalliala I, Glazer-Livson S, et al.** Vaginal microbiota in pregnancy: Role in induction of labor and seeding the neonate's microbiota?. *J Biosci*. 2019 (44): 116.
32. **Brooks JP, Edwards DJ, Blithe DL, et al.** Effects of combined oral contraceptives, depot medroxyprogesterone acetate and the levonorgestrel-releasing intrauterine system on the vaginal microbiome. *Contraception*. 2017 (95): [405-413].
33. **Freitas AC, Chaban B, Bocking A, et al.** The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci Rep*. 2017 (7): 9212.
34. **Brown RG, Marchesi JR, Lee YS, et al.** Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. *BMC med*. 2018 (16): 9.
35. **Garcia-Segura S, Del Rey J, Closa L, et al.** Seminal microbiota of idiopathic infertile patients and its relationship with sperm DNA integrity. *Front Cell Dev Biol*. 2022 (10): 937157.
36. **Liu Y, Chen H, Feng L, et al.** Interactions between gut microbiota and metabolites modulate cytokine network imbalances in women with unexplained miscarriage. *NPJ Biofilms Microbiomes*. 2021 (7): 24.
37. **Mori R, Hayakawa T, Hirayama M, et al.** Cervicovaginal microbiome in patients with recurrent pregnancy loss. *J Reprod Immunol*. 2023 (157): 103944.
38. **Odendaal J, Black N, Bennett PR, et al.** The endometrial microbiota and early pregnancy loss. *Hum Reprod*. 2024 (39): [638-646].
39. **Grewal K, Lee YS, Smith A, et al.** Chromosomally normal miscarriage is associated with vaginal dysbiosis and local inflammation. *BMC med*. 2022 (20): 38.
40. **Zhao F, Chen Y, Gao J, et al.** Characterization of vaginal microbiota in women with recurrent spontaneous abortion that can be modified by drug treatment. *Front Cell Infect Microbiol*. 2021 (11): 680643.
41. **Guang Y, Shen X, Tan Y, et al.** Systematic analysis of microbiota in pregnant Chinese women and its association with miscarriage. *Ann Transl Med*. 2022 (10): 1099.
42. **Aagaard K, Riehle K, Ma J, et al.** A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PloS one*. 2012 (7): e36466.
43. **MacIntyre DA, Chandiramani M, Lee YS, et al.** The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep*. 2015 (5): 8988.
44. **Spear GT, French AL, Gilbert D, et al.** Human □-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis*. 2014 (210): [1019-1028].
45. **Polifke A, von Schwedler A, Gulba R, et al.** Differential characteristics of vaginal versus endometrial microbiota in IVF patients. *Sci Rep*. 2024 (14): 30508.

46. **Jiao X, Zhang L, Du D, et al.** Alteration of vaginal microbiota in patients with recurrent miscarriage. *J Obstet Gynaecol*. 2022 42): [248-255].

47. **Shen LP, Cai BJ, Guan JX, et al.** Comprehensive analysis of vaginal microbiota, metabolites, and inflammatory factors in preterm and term pregnancies. *Front Microbiol*. 2025 (16): 1689494.

48. **Lin M, Xu H, Qiu J.** Inflammation in recurrent miscarriage—a comprehensive perspective from uterine microenvironment and immune cell imbalance to therapeutic strategies. *Ginekol Pol*. 2024 (95): [266-275].

49. **Han AR.** Endometrial microbiome in reproductive failure: The possibility of metagenomic analysis. *Clin Exp Reprod Med*. 2024 (52): 107.

50. **Baker JM, Chase DM, Herbst-Kralovetz MM.** Uterine microbiota: Residents, tourists, or invaders?. Front Immunol. 2018 (9): 208.

51. **Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, et al.** A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. *microbiome*. 2017 (5): 48.

52. **Zhang B, Lin S, Wang S, et al.** Investigation of the Endometrial Microbiome in Recurrent Pregnancy Loss Individuals: Microbial Imbalance and Network Fragility. *Int J Women's Health*. 2025: [2853-2868].

53. **Bai S, Xu G, Mo H, et al.** Investigating into microbiota in the uterine cavity of the unexplained recurrent pregnancy loss patients in early pregnancy. *Placenta*. 2024 (152): [1-8].

54. **Klimaszyk K, Wirstlein P, Bednarek-Rajewska K, et al.** Endometrial factors and pregnancy loss frequency in recurrent pregnancy loss patients: Comparing RT-PCR microbiology, microbial cultures, and immunohistochemistry of endometrium biopsy. *J Appl Genet*. 2025 May;66(2):459-68.

55. **Al-Nasiry S, Ambrosino E, Schlaepfer M, et al.** The interplay between reproductive tract microbiota and immunological system in human reproduction. *Front Immunol*. 2020 (11): 378.

56. **Iwami N, Kawamata M, Ozawa N, et al.** Efficacy of Endometrial Microbiome Metagenomic Analysis and Analysis of Infectious Chronic Endometritis in In Vitro Fertilisation Outcome in Women with Recurrent Implantation Failure. 2020

57. **Iwami N, Komiya S, Asada Y, et al.** P-384 Efficacy of endometrial microbiome metagenomic analysis with recurrent implantation failure and recurrent pregnancy loss: Multicenter study in Japan. *Hum Reprod*. 2022 (37).

58. **Sheiner E.** Advances in Preterm Delivery. *MDPI-Multidisciplinary Digital Publishing Institute*. 2022.

59. **Gryaznova M, Kozarenko O, Smirnova Y, et al.** Cervical and vaginal microbiomes in early miscarriages and ongoing pregnancy with and without dydrogesterone usage. *Int J Mol Sci.* 2023 (24): 13836.

60. **Lebedeva OP, Popov VN, Syromyatnikov MY, et al.** Female reproductive tract microbiome and early miscarriages. *Apmis*. 2023 (131): [61-76].

61. **Peuranpää P, Holster T, Saqib S, et al.** Female reproductive tract microbiota and recurrent pregnancy loss: A nested case-control study. *Reprod Biomed Online*. 2022 (45): [1021-1031].

62. **Gryaznova MV, Kozarenko ON, Ivannikova VM, et al.** Pattern Recognition Receptors and Cervical Microbiome in Patients with Early Miscarriages. *Int J Inflamm*. 2024 (2024): 5320926.

63. **Yang H, Guo R, Li S, et al.** Systematic analysis of gut microbiota in pregnant women and its correlations with individual heterogeneity. *NPJ Biofilms Microbiomes*. 2020 (6): 32.

64. **Li M, Zhang G, Cui L, et al.** Dynamic changes in gut microbiota during pregnancy among Chinese women and influencing factors: A prospective cohort study. *Front Microbiol*. 2023 (14): 1114228.

65. **Chen Y, Chen X, Chen P, et al.** Alteration of the gut microbiota in missed abortion. *Indian J Microbiol*. 2023 (63): [106-119].

66. **Li Z, Zheng Y, Zhang M, et al.** Gut microbiota-derived metabolites associate with circulating immune cell subsets in unexplained recurrent spontaneous abortion. *Heliyon*. 2024 (10).

67. **Garmendia JV, De Sanctis CV, Hajdúch M, et al.** Microbiota and Recurrent Pregnancy Loss (RPL); more than a simple connection. *Microorg*. 2024 (12): 1641.

68. **Patki A, Kunjimoideen K, Sawankar S, et al.** Expert Opinion on the Use of Probiotics in Recurrent Pregnancy Loss. *Cureus*. 2025 (17).

69. **Jia B, Tang L, Liu H, et al.** Potential roles of the interactions between gut microbiota and metabolites in LPS-Induced Intrauterine Inflammation (IUI) and associated Preterm Birth (PTB). *J Transl Med*. 2024 (22): 7.

70. **Osadchiy V, Belarmino A, Kianian R, et al.** Semen microbiota are dramatically altered in men with abnormal sperm parameters. *Sci Rep*. 2024 (14): 1068.

71. **Corral-Vazquez C, Blanco J, Sarrate Z, et al.** Unraveling the intricacies of the seminal microbiome and its impact on human fertility. *Biol*. 2024 (13): 150.

72. **He J, Ma M, Xu Z, et al.** Association between semen microbiome disorder and sperm DNA damage. *Microbiol Spectr.* 2024 (12): e00759-24.

73. **Mowla S, Farahani L, Tharakan T, et al.** Characterisation and comparison of semen microbiota and bacterial load in men with infertility, recurrent miscarriage, or proven fertility. *Elife*. 2025 (13): RP96090.

74. **Baud D, Zuber A, Peric A, et al.** Impact of semen microbiota on the composition of seminal plasma. *Microbiol Spectr*. 2024 (12): [e02911-23].

75. **Kilama J, Dahlen CR, Reynolds LP, et al.** Contribution of the seminal microbiome to paternal programming. *Biol Reprod*. 2024 (111): [242-268].

76. **Vajpeyee M, Tiwari S, Yadav LB.** Characterization of seminal microbiome associated with semen parameters using next-generation sequencing. *Middle East Fertil Soc J.* 2024 (29): 22.

77. **Krog MC, Nielsen JR, Slot A, et al.** Prospective reproductive outcomes according to sperm parameters, including DNA fragmentation, in recurrent pregnancy loss. *Reprod Biomed Online*. 2024 (49): 103773.

78. **Kaltsas A, Zikopoulos A, Kojovic V, et al.** Paternal contributions to recurrent pregnancy loss: Mechanisms, biomarkers, and therapeutic approaches. *Med*. 2024 (60): 1920.