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Microbiome, probiotics and lifestyle modulation in women's health across the lifespan: endocrine, immune, and microbial interconnections

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Abstract

Hormonal fluctuations throughout a woman's life, from puberty to menopause, profoundly influence the composition of the vaginal and gut microbiota, with major implications for reproductive, metabolic, and psychological health. Recent findings indicate that lifestyle factors, such as diet, stress, sleep, and physical activity, modulate microbial communities and hormonal balance through an integrated microbiome–immune–endocrine axis. This review examines the evolution of the vaginal microbiota, the effects of hormonal changes on microbial balance, and evidence for probiotic and lifestyle-based interventions in conditions such as bacterial vaginosis, endometriosis, polycystic ovary syndrome, pregnancy complications, and menopausal disorders. Particular attention is given to the estrobolome, gut–brain communication, and metabolic regulators as mediators of systemic effects.

Introduction

Changes in vaginal microbiota composition over the lifespan are closely aligned with hormonal changes that happen in key periods of a woman's life. However, emerging evidence highlights that these microbial shifts are not only hormonally driven but also shaped by lifestyle exposures that alter endocrine and immune homeostasis. Sedentary behavior, stress, and unbalanced nutrition contribute to systemic inflammation and hormonal

dysregulation, favoring microbial instability. Conversely, healthy habits and dietary fiber promote eubiosis and estrogen homeostasis via microbial metabolites such as Short-Chain Fatty Acids (SCFAs).

This integrative concept, often referred to as the Microbiome–Immune–Endocrine (MIE) axis, describes the bidirectional communication between microbial metabolites, hormonal networks, and immune mediators that collectively sustain reproductive and metabolic health.[1–2] While probiotics are known to help maintain or restore the balance of the vaginal microbiota[2], there is increasing attention given to the alignment of probiotic supplementation to promote not only gynecological and reproductive health, but also broader aspects of physical and mental health. This approach is in line with recent perspectives that define women's health as "biological conditions and general health conditions that often affect women uniquely, differently or disproportionately". [3–4] The recent increase in interest for women's health has been sparked by the growing awareness of the limitations inherent to the generalization of results from trials conducted exclusively in men participants, and the resulting lack of knowledge about the differences and specificities of health outcomes in women[4]. Indeed, due to the exclusion of women from clinical trials between the late 1970s and early 1990s,[5] women's health conditions and the overall impact of female biology on health have been largely overlooked.[6–7] However, there is growing momentum to address these disparities and help bridge existing gaps in knowledge.[3,7] Meanwhile, there is growing awareness of the limitations and adverse effects of common treatment and management strategies used in women's health conditions (e.g., antibiotics, hormone replacement therapy) that motivate the development and optimization of novel and alternative solutions. This paper reviews changes in the vaginal microbiota across the lifespan, in health and in disease, and explores microbiome-based intervention to promote health in a holistic manner. The focus is on the vaginal microbiota of cisgender women, but we acknowledge the importance of microbiota research in gender-affirming care for transgender and gender-diverse populations.[8] Pivotal periods of the lifespan are reviewed, with a specific focus on vaginal microbiota modulations, their relationships with hormonal changes and fluctuations, and the ways in which probiotics can support the balance of this ecosystem. New approaches for the identification of vaginal imbalance, and for the maintenance or restoration of balance are discussed. Links between promoting vaginal homeostasis and benefits for various health aspects are highlighted throughout.

This article is conceived as a narrative review. The literature was selected through targeted searches of PubMed and Scopus, with emphasis on recent reviews, clinical studies, and mechanistic investigations addressing the vaginal and gut microbiota, endocrine regulation, immune interactions, and lifestyle-related factors across the woman lifespan.

The human vaginal microbiome and its development

The vaginal microbiota of reproductive age women is characterized by a high proportion of *Lactobacillus* spp. This unique feature is not observed in non-human primates or other mammals, where this genus generally constitutes less than 1% of the vaginal microbiota.[9–11] This distinctive composition results in a low vaginal pH, typically below 4.5, due to the production of lactic acid from glycogen breakdown by *Lactobacillus* species and their own or host-derived glycogen-degrading enzymes.[12–14] The unique symbiotic evolution with lactobacilli leading to acidity of the vaginal microbiota in humans has been mainly hypothesized to have evolved as an adaptation to disease and obstetrical risks, due to the strong antimicrobial activity. [2] Other hypotheses explaining the unique symbiosis with fermentative lactobacilli in the human vagina include the distinct human consumption of starch-rich diets, particularly since the advent of agriculture.[10]

The vaginal microbiota of young girls before menarche is characterized by a neutral or alkaline pH [15] and a microbial community
where *Prevotella*, *Porphyromonas*, *Ezakiella*, *Peptoniphilus*, *Campylobacter*, *Anaerococcus*, *Dialister* and *Haem ophilus* genera predominate, while Lactic Acid Bacteria (LAB) – including lactobacilli – are found in low proportions.[15–16] The onset of puberty and the associated rise in estrogen levels lead to an increase in vaginal epithelial glycogen levels, and significant shifts in the composition of the vaginal microbiota.[16] The acidification of the vaginal niche, driven by glycogen metabolism, creates an optimal environment for the growth of LAB.[12–14] Shifts from childhood microbiota to an adult-type profile occur early in puberty, with some girls already showing microbiota typical of women of reproductive age one year before menarche. [17] However, at that age, predominance of lactobacilli is not always associated with a pH decrease, which could be due to low bacterial loads.[17] By the end of puberty and during reproductive age, women's vaginal microbiota is typically dominated by one *Lactobacillus* species, although other anaerobic bacteria, including *Fannyhessea* (formerly *Atopobium*) sp*., Prevotella* spp., and facultative anaerobes such as *Gardnerella* spp. and *Streptococcus* spp., may be present.[18–19]

In young girls, the incidence of Urinary Tract Infections (UTI) and recurrent UTI has been associated with the presence of opportunistic bacteria and pathogens from the vaginal tract.[20–21] Given that antibiotics can disrupt vaginal homeostasis and are losing effectiveness due to rising resistance, [22] a deeper understanding of microbiome composition and interactions could open the door to targeted vaginal probiotic solutions as effective alternatives for reducing risks and managing UTIs.[20] Beyond puberty-related hormonal shifts, microbiota composition is also influenced by nutrition and stress, which can modify estrogen metabolism through gut–liver recirculation. Disruption of this systemic link may predispose to dysbiosis and inflammatory susceptibility.[1] The interaction between the vaginal and gut microbiota through the estrobolome and immune mediators represents a critical determinant of reproductive resilience, linking lifestyle, endocrine regulation, and microbial balance.

Balance and imbalance of the vaginal microbiota in women of reproductive age

In 2011, Ravel *et al*. [18] categorised the vaginal microbiota of women of reproductive age into five typical compositional state, also known as Community State Types (CST). In four of these state types, the vaginal microbiota was dominated by *Lactobacillus* species: *Lactobacillus crispatus* (CST I), *Lactobacillus gasseri* (CST II), *Lactobacillus iners* (CST III) or *Lactobacillus jensenii* (CST V). CST IV was characterized by the presence of various facultative and obligate anaerobes [18,21]. Since then, an increasing number of CSTs have been described, as well as other approaches to characterize the vaginal microbiome, such as models of co-occurring taxa and subcommunities via topics modeling [23]. Vaginal communities dominated by *Lactobacillus* species are thought to represent stable states of the vaginal microbiota because they are associated with lower risks of urogenital infections and better reproductive health [18,21]. Lactobacilli are believed to protect the vaginal ecosystem through the production of lactic acid and other antibacterial metabolites [21]. However, vaginal communities dominated by *L. iners* do not provide the same level of protection and have not systematically been associated with better health outcomes [21]. They are also considered less stable and more prone to transition to dysbiotic and unstable states such as CST IV. [18–19,21]

Vaginal dysbiosis is associated with the risk of developing Bacterial Vaginosis (BV) which is considered as the most common vaginal condition in women of reproductive age. [24–25] During an episode of BV, the vaginal microbiome is characterized by a marked reduction in lactobacilli, and an increase in the relative abundance of various facultative and obligate anaerobes.[24–25] *Gardnerella vaginalis* was traditionally considered the primary etiological agent for BV, but its detection in asymptomatic individuals and its recent taxonomic division into four species has led to a reconsideration of its role.[25] While some controversies remain on the pathogenicity of some strains and species of *Gardnerella*, documented virulence factors include sialidase (which facilitates adherence and biofilm formation), ability to form a biofilm on squamous vaginal epithelial cells (clue cells), secretion of vaginolysin and secretion of siderophores.[25] *Gardnerella* spp. also exert several effects on mucosal immunity that can result in inflammation.[25] Given its central role in BV, and despite its complex and sometimes commensal behavior, it is not surprising that many research groups are investigating the antagonistic activity of *Lactobacillus* species against *Gardnerella* spp., studying its ability to promote vaginal health. For example, recent studies on the vaginal microbiome in South African women led to the isolation of local *L. crispatus* strains with significantly greater inhibitory activity against regional *Prevotella* spp. and *Gardnerella* spp. isolates than the commonly used reference type strain.[26] Beyond individual studies, several systematic reviews and meta-analyses[27–28] have evaluated the efficacy of probiotic interventions in BV management, showing modest but consistent benefits in restoring *Lactobacillus* spp. dominance and reducing recurrence rates, though strain specificity, dosing, and study heterogeneity remain key limitations.

Emerging evidence from preclinical and observational studies suggests that disruptions in the vaginal microbiota or the presence of specific bacterial taxa may contribute to increase risks of development, progression, and

potentially metastasis of cervical, endometrial, and ovarian cancers (as reviewed by Brusselaers *et al.*).[29] Most of the current data are correlative, and causal relationships remain to be firmly established through longitudinal and interventional studies. In parallel, growing attention has been given to the gut microbiota, particularly its role in modulating systemic estrogen levels via the estrobolome, which can be defined as the ensemble of microorganisms and genes that influence estrogen metabolization.[30–32] This has led to increasing evidence linking gut dysbiosis to risks of breast cancer development, progression, and metastasis,[33] along with emerging proposals for microbiome-based interventions to enhance standard treatment outcomes.[33–34] For gynecological cancers, additional factors such as Human Papillomavirus (HPV) infection and vaccination status also play a critical role in disease risk and progression. HPV infection is the most common viral cause of cervical cancer, which is the fourth most prevalent cancer in women globally.[29,35] Women whose vaginal microbiota are not dominated by lactobacilli show both increased susceptibility to HPV infection and impaired viral clearance.[21,29] In a recent clinical study,[35] intravaginal administration of a *L. crispatus* strain, isolated from the vagina, was associated with restoration of a *Lactobacillus* spp. dominated microbiota, a 1 log reduction in HPV viral load, a 1.3-fold increased HPV clearance rates, as well as decreased local inflammation. While these findings are promising, the study had a relatively small sample size, requiring further large-scale and placebo-controlled trials to confirm efficacy and long-term safety.

A potential disruptor of the vaginal microbiota is the male genital tract microbiota, whose role in microbiota composition shifts following unprotected vaginal intercourse has been overlooked. Colonization of the male genital tract, especially seminal and penile, is increasingly studied.[36] Compared to the vaginal microbiota, male genital tract microbiota is highly diverse and may act as a reservoir of bacteria negatively associated with women's health.[37]

Recent data also associate lifestyle-induced dysbiosis with low estrogen availability and elevated pro-inflammatory cytokines. Chronic stress and poor diet can amplify these effects by disrupting cortisol and insulin signaling, illustrating the cross-talk between the Hypothalamic–Pituitary–Adrenal **(**HPA) axis and the vaginal ecosystem.[1]

The microbiome–immune–endocrine axis

Butyrate, acetate, and propionate, the main SCFAs produced by gut bacteria, modulate gene expression through epigenetic mechanisms[38] and may indirectly regulate the hypothalamic–pituitary–gonadal axis by influencing Gonadotropin-Releasing Hormone (GnRH) signaling and the downstream secretion[39] of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH).

The estrobolome, a subset of the gut microbiota expressing β-glucuronidase and β-glucosidase enzymes, modulates estrogen recirculation. Its imbalance may lead to hypo- or hyperestrogenism, favoring endometriosis, Polycystic Ovary Syndrome **(**PCOS), or menopausal symptoms.[1]

Furthermore, the HPA axis links psychological stress to gut and vaginal microbial composition: elevated cortisol suppresses GnRH and estrogen synthesis, while probiotics and physical activity can restore endocrine and microbial equilibrium.[27–28]

Endocrine dysfunctions and microbiota interactions

PCOS, hypothyroidism, and insulin resistance represent paradigms of MIE axis dysregulation.

In PCOS, reduced *Lactobacillus* spp. and *Bifidobacterium* spp. levels and enrichment in *Escherichia* spp., *Shigella* spp., and *Candida albicans* lead to chronic low-grade inflammation and hyperandrogenism.[1] In hypothyroidism, microbial dysbiosis exacerbates autoimmune inflammation, altering gonadotropicn release and endometrial function. Probiotic support has been proposed as an adjunctive therapy to improve metabolic and reproductive[40] outcomes.

The menstrual cycle, its link with the vaginal microbiota, and the estrobolome

The composition of the vaginal microbiota varies under natural physiological changes associated with the reproductive cycle.[25,41–4] During the menstrual cycle, fluctuating levels of circulating progesterone and estrogens drive shifts in the microbial vaginal landscape.[42–4]Elevated estrogen levels during the proliferative and the secretory phases stimulate the production of cervical mucus and the synthesis of glycogen in the vaginal epithelium,[43] which is mainly metabolized by *Lactobacillus* spp. into lactic acid. This chemical barrier, along with other competitive microbial mechanisms (e.g.*,* secretion of bacteriocins and biosurfactants) inhibits the proliferation of many potential pathogenic microorganisms.[44] Interestingly, during both the follicular (approximately days 5–14 after menstruation) and luteal phases (after ovulation, approximately days 15–28) of the menstrual cycle, the relative abundance of dominant bacterial taxa in vaginal microbial communities, primarily *Lactobacillus* species, remains relatively stable, although the ratio between species may vary.[2,42,44] A recent study involving 160 healthy young Danish women, demonstrated a correlation between elevated estradiol levels and an increased presence of *L. crispatus* during the follicular phase, suggesting that hormonal mechanisms may play a role in the maintenance and abundance of this species in the vagina.[45] Similarly, *L.*

iners has been reported to be more abundant during the luteal phase. These findings are further supported by the large-scale Isala citizen science study, which analyzed vaginal microbiomes from 3,345 Belgian women and found that *L. crispatus* was positively associated with estradiol levels, while *L. iners* was more prevalent during the luteal phase.[23] As indicated above, the role of *L. iners* remains controversial, with some studies indicating that its dominance over *L. crispatus* may signal a transition from a stable to a less optimal vaginal microbiota profile.[21,46]

Toward the end of the luteal phase, estrogen and progesterone levels drop, triggering endometrial shedding and significant fluctuations in vaginal microbiome composition, with a shift toward a more diverse and non-*Lactobacillus* dominated profile, resembling vaginal dysbiosis.[2,43–45] During menstruation, blood coats the vaginal epithelium increasing the mucosal pH to values near neutrality, reducing the antimicrobial effect of lactic acid and allowing the overgrowth of otherwise low-abundant microorganisms.[42] The iron-rich environment further promotes the proliferation of certain species. For instance, several isolates of *G. vaginalis* can synthesize siderophores. These iron-chelating compounds, considered virulence factors, confer a competitive advantage for the survival and growth of siderophore-producing species.[25] Indeed, the late secretory phase shows an increase in expression of siderophore biosynthesis pathways, which are generally absent during the proliferative phase.[44] Overall, these modifications in the vaginal environments result in a more diverse, non-lactobacilli dominated vaginal microbiota during menstruation. However, in most women, this imbalance is transient, with *Lactobacillus* spp. dominance typically restored by mid-cycle.[42,45]

Fluctuating estrogen levels are thus essential for the regulation of menstrual cycle, tissue homeostasis, fertility and modulation of the vaginal microbiota.[47] In the gastrointestinal tract, specific microorganisms that express β-glucuronidase and β-glucosidase are responsible for hormone regulation, particularly through the deconjugation of estrogens. These microorganisms and their genes (the estrobolome) may thus influence estrogen metabolization,[30–32] enabling its partial reabsorption via enterohepatic circulation and subsequent transport to distal sites, including the female genital tract. Alterations in the estrobolome may disrupt hormonal balance and negatively affect the female genital tract microbiota, which could potentially contribute to the pathophysiology of conditions such as endometriosis or even PCOS.

Endometriosis is an estrogen-dependent, inflammatory condition that is estimated to affect around 10% of women of reproductive age, that is approximately 190 million women worldwide.[47–49] The pathobiology of endometriosis is complex and involves inflammatory, hormonal, metabolic, and neurological factors.[47–49] It is characterized by the presence of endometrial-like tissue outside of the uterus and typically manifests as chronic pelvic pain, dysmenorrhea (menstrual pain), dyspareunia (pain during sexual intercourse), dysuria (pain during urination), dyschezia (pain during defecation), heavy menstrual bleeding, and constipation, although some women are asymptomatic.[47–49] Similar to other chronic pain syndromes, many women with endometriosis report suffering from fatigue and depression.[47] Diagnosis is made based on the observation of endometrial lesions at

surgery (generally laparoscopy). The average delay from symptom onset to diagnosis ranges between 5 and 12 years, and most women with endometriosis will have been evaluated by at least three clinicians before receiving a diagnosis.[49] Endometriosis is associated with infertility and increased risks of various diseases and conditions, including ovarian and breast cancer, cardiovascular disease, rheumatoid arthritis, and Irritable Bowel Syndrome (IBS).[47–49] In the absence of non-invasive, curative treatments, various dietary approaches have been investigated, yielding modest benefits or mixed results.[50–51] The Microbiota-Gut-Brain Axis (MGBA) has been identified as a potential target for the improvement of pain symptoms, but this suggestion has received limited attention to date.[47–48] Itoh *et al*.[52]recruited 66 women with endometriosis who were randomly assigned to receive a postbiotic supplement (a heat-inactivated *L. gasseri* strain) or placebo consumed orally once daily for 12 weeks. Compared to the placebo, the postbiotic supplement was associated with improvements in dysmenorrhea and pelvic pain, both in terms of pain intensity and impact over daily functioning. While this study had a small sample and presented other limitations, including in the definition of outcomes and instruments used, studies of preclinical models of endometriosis from the same group using the same heat-inactivated *L. gasseri* strain have yielded coherent and promising results.[53] PCOS is a common endocrine disorder that is estimated to affect between 8% and 13% of women of reproductive age.[54] Diagnosis can be complex, as it does not rely on a single test, and symptoms like irregular menstruation may overlap with normal pubertal development. Owing to its complexity and multifactorial nature, PCOS is accompanied by various reproductive, metabolic, and psychological impairments. Relatedly, comorbidities and complications of PCOS are numerous and include increased risk of obesity and associated metabolic (e.g., insulin resistance) and cardiovascular (e.g., hypertension) disorders, endometrial cancer, and mood disorders.[54] Recently, links between gut dysbiosis and PCOS have been established, opening new avenues for the management of symptoms.[55–57] While results vary across studies, many report a reduction in both α- and β- diversity, characterized by an overrepresentation of genera such as *Escherichia* and *Shigella* that alter the production of Short-Chain Fatty Acids (SCFAs), and a concomitant loss of immunomodulatory and nutrient-assimilating taxa such as lactobacilli and bifidobacteria. Systematic reviews with meta-analysis have suggested some potential for specific probiotics and synbiotics to beneficially modulate metabolic and inflammatory markers of PCOS.[58–59] However, the overall quality of evidence remains moderate to low, with considerable heterogeneity across studies, small sample sizes, and variability in probiotic strains and dosages. Therefore, while initial findings are encouraging, more rigorous and large-scale clinical trials are needed to confirm these effects and establish clinical recommendations.

Reproductive health, fertility and pregnancy

Pregnancy is generally associated with a higher abundance of *Lactobacillus* species in the vaginal microbiome, contributing to a low-diversity, protective microbial environment. This equilibrium is influenced not only by

hormonal changes but also by maternal nutrition and stress exposure, which affect the placental microbiota and immune tolerance. The review by Barraza-Ortega *et al*.[1] emphasizes how infections with *Gardnerella vaginalis*, *Ureaplasma urealyticum*, or *Mycoplasma genitalium* can disturb the MIE axis, promoting inflammation, premature rupture of membranes, and preterm birth. At the same time, beneficial species such as *L. rhamnosus* and *L. reuteri* can modulate local cytokine patterns, reduce Group B *Streptococcus* colonization, and improve implantation success during assisted reproduction. Psychological stress and overactivation of the Corticotropin-Releasing Hormone (CRH), adrenocorticotropic hormone Adrenocorticotropic Hormone (ACTH) and cortisol are increasingly recognized as risk factors for miscarriage and impaired placental function; probiotics may attenuate these effects through gut–brain–immune regulation.[1] This shift is thought to be driven by elevated estrogen levels during gestation, which increase glycogen availability and enhances colonization by lactobacilli.[60] In support of this, the InSPIRe study, a cohort study involving 749 pregnant women, demonstrated that *L. crispatus* dominance was associated with reduced microbial diversity and a lower risk of preterm birth, highlighting the protective role of this species during pregnancy.[61] Conversely, dysbiosis of the vaginal microbiota has been negatively associated with obstetrical outcomes.[61] Pregnant women with a low abundance of lactobacilli have a higher risk of preterm delivery (the major cause of neonatal death) compared to those with vaginal microbiota dominated by *L. crispatus.*[61] In addition, dysbiotic vaginal microbiota is also associated with an increased risk of preterm premature rupture of fetal membranes, thus exposing newborns to neonatal complications.[61] A large retrospective cohort study including over 12,000 mother-infant dyads found that BV exposure during pregnancy not only led to a significant increase in the risk of preterm birth, but was also associated with increased risks of respiratory distress or use of assisted ventilation at birth, neonatal sepsis, and Neonatal Intensive Care Unit (NICU) admission in infants born full-term.[62] The negative impact of vaginal lactobacilli depletion also extends to the field of assisted reproductive technology, as patients with BV undergoing *In Vitro* Fertilization (IVF) procedures exhibit lower clinical pregnancy rates, higher early pregnancy loss rates[63] and implantation failure.[64] Furthermore, a decrease of lactobacilli and an increase in the relative abundance of BV-associated bacteria like *G. vaginalis* have been associated with recurrent pregnancy loss[65] and a decrease of live birth in patients undergoing IVF.[66] As the female genital tract forms a continuum of microbial communities, the vaginal microbiota can influence the composition of the microbiota of the upper genital tract.[44] There is therefore an increasing interest in the impact of endometrial microbiota on fertility, given the endometrium's central role in receiving the fertilized oocyte and supporting the development of the embryo. However, while these associations are compelling, caution is warranted in using *Lactobacillus* spp. dominance as a prognostic marker or in applying probiotics to improve IVF outcomes, as current evidence remains limited. Further well-controlled studies are needed to establish causality and efficacy in the context of IVF. The vaginal microbiota plays a major role in newborns' health by providing the initial bacterial inoculum that helps initiate healthy microbiome development.[67] Infants born by cesarean section (C-section), who do not go through the birth canal and are

therefore not exposed to the mother's vaginal microbiota, show different gut microbiota characteristics compared to infants born vaginally.[68] Altered microbiome establishment in infants born by C-section has been associated with increased risk of allergic, autoimmune, and metabolic conditions. Maternal intake of probiotic supplements during pregnancy and lactation has been proposed as an effective strategy to promote the development of a healthy gut microbiome, characterized at least partially by high abundance of Bifidobacteria spp., especially in infants born by C-section.[68]

With the ever-growing knowledge and understanding of the MGBA and evidence supporting probiotic supplementation for mothers and infants, there is increasing interest in using microbiome-based intervention to help support mental health during pregnancy and after childbirth.[69–70] Perinatal depression is defined as an episode of major depressive disorder that has its onset during pregnancy or during the first four weeks after giving birth.[71] It is a prevalent condition that affects about one in ten women and that is distinct from the short period of tearfulness and irritability colloquially known as "baby blues", which affects up to 70% of women and generally resolves within two weeks.[72] Anxiety in the perinatal period is also common, and tends to be comorbid with depression.[73] Slykerman *et al*.[70] assessed the secondary outcome of perinatal depression and anxiety symptoms in 423 women who received *Lacticaseibacillus rhamnosus* supplementation during pregnancy and after childbirth in a trial originally designed to study eczema in the offspring at 12 months of age. Using modified versions of the Edinburgh Postnatal Depression Scale (EPDS) and the State Trait Anxiety Inventory (STAI) to measure symptoms, they found that women in the probiotics group had significantly lower depression and anxiety scores than women in the placebo group after childbirth. Vicariotto *et al*.[69] recruited 200 new mothers who were randomly assigned to receive a probiotic supplement containing *Limosilactobacillus reuteri* and *Bifidobacterium breve* combined with a multivitamin, or a multivitamin alone during the first trimester of their newborn's life. The intervention was initiated in the first three days after delivery, and outcomes focused on the mother's mood (EPDS) and breastfeeding quality (Breastfeeding Self-Efficacy Scale – Short Form, BSES-SF). Compared to women receiving multivitamin supplementation alone, those additionally supplemented with probiotics exhibited significantly better mood scores and breastfeeding quality. More large-scale studies focusing on the mother's mental health as a primary outcome and using validated tools are needed to further explore the use of probiotics to support mental wellbeing in the perinatal period.

Menopause

As women leave reproductive age and enter in perimenopause, a drastic decline in estrogen levels induces significant changes in both the vaginal microbiome and the vulvovaginal physiology. Recent trials show that strains such as *Lacticaseibacillus rhamnosus* and *Levilactobacillus brevis* with high β-glucuronidase activity may restore estrogen availability and alleviate genitourinary syndrome of menopause. Moreover, lifestyle

interventions, particularly Mediterranean diet patterns, enhance gut microbial diversity and support estrogen metabolism, complementing probiotic therapy.[1] Vaginal atrophy, a key feature of menopause, is characterized by thinning of the vaginal epithelium, reduced collagen synthesis, and decreased mucosal hydration. These physiological changes contribute to vaginal dryness, irritation, and discomfort.[41] Lower glycogen levels result in a reduced *Lactobacillus* population, leading to an increase in vaginal pH and a higher microbial diversity, with an overrepresentation of genera such as *Prevotella*, *Bacillus* and *Porphyromonas*, among others.[41,74] This disruption of the vaginal microbiome has been associated with the severity of symptoms of the genitourinary syndrome of menopause (GSM), including vaginal dryness, vulvovaginal atrophy, and dyspareunia.[75] This is supported by studies showing that *Lactobacillus* spp. are negatively associated with GSM symptoms, while bacteria belonging to *Escherichia*, *Shigella*, *Anaerococcus* and *Enterococcus* genera correlate positively with those ymptoms.[41,76] Estrogen-based Hormone Replacement Therapy (HRT) helps restore the vaginal microbiota by promoting *Lactobacillus* spp. recolonization, potentially improving the quality of life in postmenopausal women.[41] The use of specific probiotic strains with enzymatic potential to metabolize estrogens and restore *Lactobacillus* spp. dominance in the vaginal microbiota could serve as an alternative to alleviate menopausal symptoms, without the adverse effects associated with HRT, which include headaches, breast pain, vaginal bleeding or spotting, nausea, diarrhea, mood changes, leg cramps, skin problems (rash, itching, acne) and hair loss.[41] However, more research is needed to investigate the mechanisms and potential efficacy of this specific application of probiotics. Honda *et al*.[77] have carried out *in vitro* screening in 84 LAB and bifidobacterial strains for β-glucuronidase activity, an enzyme that deconjugates the inactive glucuronide form of estrogens, thus potentially restoring active hormone which can enter the bloodstream. They identified one strain of *Levilactobacillus brevis* and three strains of *Lacticaseibacillus rhamnosus* with an increased deconjugation ability. In a subsequent pilot clinical trial, they showed that the strain with the highest β-glucuronidase activity, a *L. brevis* strain, was effective at maintaining serum estrogen levels in 54 healthy peri- and postmenopausal women, and elevating estradiol and estrone levels compared with a placebo over 12 weeks of intervention. While these findings are promising, the small sample size and exploratory nature of the study warrant caution. Larger, well-powered trials are needed to confirm efficacy, assess long-term safety, and determine clinical relevance for symptom management or risk reduction.

As for the restoration of microbiota balance, a prospective observational study of 50 postmenopausal women aged 45 to 65 found that four weeks of oral supplementation with a blend of *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* subsp. *lactis*, and *Lacticaseibacillus rhamnosus* led to a significant improvement of the Vaginal Health Index, reduction of biomarkers of inflammation, and increase in the abundance of Lactobacilli spp.[78] However, the absence of a control group and the short duration of the intervention and follow-up limit the strength of the conclusions. The clinical relevance of the observed changes remains to be confirmed in larger, controlled trials. Similarly, a randomized, double-blind, placebo-controlled

clinical trial including 85 women with perimenopausal symptoms showed that 12-week oral intake of *Lactobacillus acidophilus* led to improvements of specific symptoms of menopause, including vaginal dryness, fatigue and headaches.[79] While these findings are encouraging, the effect sizes were modest and relied on self-reported symptom relief, which may introduce bias. Moreover, these studies used oral administration of probiotics, and it remains uncertain whether the strains used are capable of reliably colonizing the vaginal niche. Other studies have demonstrated the effects of probiotics on symptoms of menopause via vaginal administration. For example, Mueck *et al*.[80] reviewed eight studies using vaginal application of *L. acidophilus* with ultra-low-dose estriol in over 600 peri- and postmenopausal women and concluded that this combination could be an effective approach for the management of vaginal atrophy owing to its ability to restore vaginal microbiota homeostasis. However, most trials were small, lacked placebo controls, and had limited follow-up, highlighting the need for larger, well-designed randomized studies.

Menopause is also associated with an increase in overall weight and abdominal adiposity, which is a key risk factor for cardiovascular diseases.[34] Recent evidence suggests that interventions that can modulate the composition of the gut microbiota could help mitigate the impact of metabolic cardiovascular risk factors. A metagenomic study conducted in 53 postmenopausal women with obesity identified negative associations between specific bacterial taxa and insulin resistance (*Bacteroides faecis, Dorea longicatena, Intestinibacter bartlettii*) and dyslipidemia (*Akkermansia muciniphila*, *Bacteroides cellulosilyticus*, *Bacteroides nordii*, *Bacteroides pectinophilus*, *Odoribacter splanchnicus* and *Roseburia inulinivorans*). These associations remained significant after adjustment for confounding factors including age, body fat, and diet,[34] thus supporting the potential of probiotic strains for the management of metabolic disorders in postmenopausal women. However, more studies establishing their safety and providing preclinical evidence of their efficacy are needed before they can be tested in clinical trials.

Standard treatment for breast cancer, including chemotherapy and estrogen deprivation therapy, may worsen vaginal dysbiosis in postmenopausal women, and thus aggravate the genitourinary syndrome of menopause characterized by dyspareunia, dysuria, and recurrent infections.[81] As an example of how probiotics might reduce the risk of these effects, Marschalek *et al*.[81] tested the effects of a probiotic blend containing *L. crispatus*, *Lc. rhamnosus*, *L. jensenii* and *L. gasseri* taken orally twice daily for two weeks in 27 postmenopausal women undergoing chemotherapy for breast cancer. Women in the probiotics group experienced a shift in Nugent score (used to diagnose BV in clinical settings) towards normal microbiota levels, whereas those in the placebo group showed a significant deterioration. The study had a very small sample, but it was useful in showing the potential benefits of probiotics taken orally on markers of vaginal balance and on the role of probiotics for the wellbeing of women undergoing chemotherapy.

Late perimenopausal and postmenopausal women are more at risk of osteoporosis and loss of Bone Mass Density (BMD).[82] A 2024 meta-analysis including 12 randomized controlled trials (1,183 postmenopausal

women) supported the efficacy of probiotics to help restore BMD in the lumbar spine and hip in this population.[82] High heterogeneity between included studies and other methodological limitations (e.g., concomitant use of other supplements, including calcium and vitamin D) limits the strength of these conclusions. As for women with osteopenia, for example, Vanitchanont *et al*.[82] tested a group of 40 postmenopausal women and found that a multispecies probiotic formula improved serum concentrations of the bone resorption marker C-terminal telopeptide of type I collagen (CTX) in women consuming probiotics when compared to the placebo group. While findings like these are promising, the role of probiotics and other microbiome-based supplements in promoting bone health continues to be explored, as highlighted in recent reviews.[34,82]

Perspectives and new technologies for preclinical and clinical investigations

Preclinical research

As mentioned above, the human vaginal microbiota is unique and no other animals, not even our closest primate relatives, have the vaginal ecosystem dominated by bacteria belonging to the *Lactobacillus* genus, and therefore an acidic vaginal pH.[10] In this context, the use of animal models to study vaginal colonization by human isolates may not be completely physiologically relevant. Therefore, *in vitro* cultures of vaginal cells have been explored, initially using traditional 2D cell monolayers, but with significant limitations because they fail to replicate the complex morphology of vaginal tissue. Recently, 3D approaches have been investigated, including vagina-on-a-chip settings,[83] murine vaginal organoids,[84] and air-liquid interface cultures.[85] These models provide an optimal balance between *in vitro* and *in vivo* conditions, retaining the advantages of cell culture, being easily controlled, affordable, and reproducible, while displaying a structural complexity similar to *in vivo* tissue. These characteristics hold great potential for experiments involving bacterial colonization with strains found in the human vagina, allowing us to decipher the intricate connections between vaginal strains and the host[86] by creating artificial vaginal communities. In addition, these experimental models have the potential to expedite research on vaginal probiotics, with the ability to test growth and colonization characteristics of lactobacilli, as well as on phage therapy (a targeted approach using bacterial viruses), an emerging therapeutic alternative to antibiotic use. These findings are primarily based on observational evidence and should be interpreted as hypothesis-generating.

Clinical research

While the studies reviewed in the present paper highlight the opportunities offered by microbiome-based supplements to promote women's health, the development of a solid base of clinical evidence for the use of probiotics in both well-established and novel applications remains a challenge. One important consideration

concerns the regulatory aspects that surround the vaginal administration of probiotics.[87] While probiotics consumed orally fall under the category of food supplements, probiotics administered vaginally are typically regulated as drugs (including Live Biotherapeutic Products) or cosmetics, depending on the product and jurisdiction.[87] However, in Canada, probiotics administered vaginally are a Natural Health Product but can only be approved through the non-traditional registration procedure. In South Africa, probiotics are considered as health supplements, no matter the administration route.[88] This may explain why many clinical trials testing the effects of probiotics for the management of BV, for example, have used orally administered supplements comprising common probiotics, including lactobacilli, which are "Generally Regarded as Safe" (GRAS)[87] or have a Qualified Presumption of Safety (QPS) status in Europe. The administration of probiotic supplements directly to the target niche may, in principle, lead to the replacement of BV-related pathogens by probiotics, while avoiding the need to select strains based on their capacity to survive in the gastrointestinal tract.[87] The comparison of two studies on the management of BV that used the same strain with different administration methods showed a slightly better efficacy for vaginal administration compared to oral administration.[29,87]

In addition to identifying and defining relevant outcomes that are measured with sensitive and validated instruments and biomarkers, the studies need to consider a host lifestyle and personal factors that can exert substantial influence over outcomes (e.g., diet, stress, hygiene, sexual activity, use of contraceptives).[21] While these factors contribute to the significant complexity associated with clinical trials in the field of women's health, recent innovations in study design and data collection approaches help tackle this challenge. The use of connected objects, electronic data capture devices and Electronic Patient-Reported Outcomes tools allow continuous tracking of a multitude of measurements. Data from each participant can also be enriched using already collected data. For example, in France, the health data hub facilitates access to, and exploitation of these real-life data for clinical research. The reduction of cost and time required per analysis coupled with the possibility to collect samples at home using self-collected sample kits stored at room temperature has also favored large-scale studies using omics technologies, and in particular metagenomics allowing a fine characterization of the vaginal ecosystem.[23] In parallel, citizen science initiatives have emerged as a powerful complement to traditional clinical research. Projects like Isala[23] have demonstrated how engaging women directly in microbiome research can not only accelerate data collection at scale, but also foster pubic awareness and trust. These initiatives empower participants to contribute to science while generating valuable real-world data that reflect the diversity of women's lived experiences. Lastly, the rapid democratization of Artificial Intelligence (AI) tools has helped leverage large amounts of information collected during clinical trials, making it possible to cluster participants into subgroups of respondents (opening the way to personalized therapies), enhance and reuse existing data (using tools such as AnonyMine®), and even produce simulated data (via synthetic control arms). This new generation of AI-powered clinical trials with a holistic approach should open new opportunities for personalized solutions to address women's health issues. Even if the Food and Drug

Administration (FDA) has advised more than 30 years ago that clinical trial participants should be representative of the patient population (both women and men),[5] women have been and continue to be underrepresented in clinical trials, often due to concerns about hormonal fluctuations and pregnancy-related risks.[6–7] Necessary precautions surrounding the inclusion of pregnant or lactating women create challenges to conduct studies and collect sufficient data on safety and effectiveness. There is growing consensus that, in order to effectively address the complexity of hormonal changes and fluctuations and their interactions with the microbiome, as well as the multifactorial nature of many women's health issues, clinical trials should be designed in collaboration with experts in endocrinology, but also immunology, and microbiology (Figure 1). Recently, the FDA has recommended the inclusion of premenopausal women in breast cancer clinical trials, and has provided guidelines for the inclusion of pregnant women in clinical trials when scientifically and ethically indicated.[89]

Lifestyle, diet, and exercise as microbial modulators

As discussed in the section on the MIE axis, lifestyle factors exert their effects primarily through modulation of microbial metabolites and endocrine–immune signaling. The review by Barraza-Ortega *et al.*[1] provides extensive evidence that lifestyle habits critically shape the MIE axis. High-fiber and plant-based diets increase beneficial genera (*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*) that produce anti-inflammatory SCFAs, improving insulin sensitivity and ovarian function. In contrast, high-fat or high-sugar diets favor dysbiosis, oxidative stress, and hormonal imbalance. Regular moderate exercise increases microbial diversity, reduces cortisol, and normalizes LH/FSH ratios, especially in PCOS patients. Adequate sleep and stress management further stabilize the gut–brain axis and hormonal profiles. Together, these non-pharmacological interventions act synergistically with probiotics to maintain women's health throughout the lifespan (Figure 2). While lifestyle interventions alone exert significant effects on microbial and endocrine balance, probiotic supplementation has been investigated as a complementary strategy. Diet profoundly influences the diversity and function of the gut microbiome.

The Mediterranean diet, characterized by high consumption of fruits, vegetables, whole grains, legumes, nuts, and olive oil, is associated with improved ovulatory function and lower risk of anovulatory infertility.[90] Increased vegetable protein intake and reduced glycemic load were protective factors.

Moderate, regular exercise improves gut microbiota diversity, increases SCFA production, strengthens gut barrier integrity, and reduces inflammation. Strength training has particularly beneficial effects: it elevates LH levels, increases FSH sensitivity, supports ovulation, and stabilizes the menstrual cycle. In women with PCOS, it decreases circulating androgens and improves the LH/FSH ratio, creating a more favorable endocrine

environment for fertility. Strength training activates molecular pathways,[91] such as phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), AMP-activated Protein Kinase (AMPK), that enhance insulin-independent glucose uptake, essential in women with insulin resistance. These mechanisms enhance insulin sensitivity and maintain balanced hormonal profiles. Additionally, strength training modulates the HPA axis, lowers basal stress, and restores Hypothalamic-Pituitary-Gonadal (HPG) axis function, supporting follicular maturation and embryo implantation.

The combination of a fiber-rich diet and strength training increases intestinal microbial diversity, promoting butyrate-producing bacteria with anti-inflammatory properties. These bacteria influence the secretion of intestinal hormones that interact with the endocrine axis, affecting estrogen bioavailability and systemic hormonal balance. A diverse, plant-based diet remains essential for promoting intestinal microbiome diversity and supporting endocrine health. Prebiotic fibers (inulin, resistant starch, arabinoxylans) found in oats, legumes, onions, and chicory root selectively support beneficial bacteria like[92] *F. prausnitzii*, *A. muciniphila*, and *Roseburia* spp., fostering gut barrier function and insulin sensitivity.

Probiotic supplementation with strains such as *L. acidophilus*, *L. casei*, and *B. bifidum* has shown benefits for women with gestational diabetes, improving fasting blood glucose, insulin resistance markers, and lipid profiles after six weeks. Fermented foods (yogurt, kefir, kimchi, miso) provide live microbes and postbiotic metabolites that support immune regulation and microbial diversity during pregnancy. The gut-brain axis is a bidirectional communication system connecting the central nervous system, enteric nervous system, and gut microbiome. Chronic stress disrupts this axis by activating the HPA axis and elevating cortisol,[91] which decreases gut microbial diversity, particularly beneficial species like *Lactobacillus* and *Bifidobacterium*, while promoting pathogenic bacteria overgrowth (*Clostridium* spp., *Escherichia coli*).

These changes affect neurotransmitter production, such as serotonin, γ-Aminobutyric Acid (GABA), dopamine, which are partly synthesized in the gut and influence stress responses. Chronic stress contributes to hypothalamic functional anovulation, decreases estradiol and progesterone levels, and inhibits pulsatile release of GnRH, LH, and FSH, interfering with ovulation and fertility. Elevated cortisol can also suppress Thyrotropin (TSH) secretion and reduce thyroid hormone conversion, potentially causing subclinical hypothyroidism and further disrupting reproductive hormonal balance.

Exposure to pesticides and Endocrine-Disrupting Chemicals (EDCs) like Phthalates and Bisphenol A (BPA) significantly contributes to oxidative stress and reproductive dysfunction. These toxicants increase reactive oxygen species, deplete antioxidants, and are linked to hormonal disruption, recurrent miscarriage, and unexplained infertility.[93] Both pesticides and EDCs perturb the gut microbiota, reducing diversity and shifting bacterial composition, with downstream effects on host metabolism, inflammation, and immunity. These

dysbiotic changes interfere with endocrine signaling via the endobolome (microbiome-encoded pathways that metabolize steroid hormones and xenobiotics). Genetic polymorphisms in detoxification enzymes may increase individual susceptibility to oxidative stress-related reproductive damage.

Chronic use of antibiotics, oral contraceptives, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), and proton pump inhibitors significantly alters gut microbiome composition and diversity, often causing dysbiosis. Antibiotics decrease microbial diversity and reduce SCFA production,[93] essential for controlling inflammation and peripheral hormonal signaling. NSAIDs disrupt gut microbial balance, affecting bacterial diversity and metabolic activity. Oral contraceptives can induce changes in the estrobolome, influencing enterohepatic recirculation of hormones and increasing the risk of hormonal imbalances.

Sleep deprivation disrupts circadian rhythms, directly affecting both the endocrine system and gut microbiome. Sleep disruption reduces key bacteria[94] (*Roseburia* spp., *Lactobacillus* spp.) while increasing cortisol and decreasing melatonin, a hormone involved in ovarian regulation and reproductive hormonal axis synchronization. Chronic sleep disorders interfere with metabolic hormones, raising ghrelin and lowering leptin, which triggers hunger,[95] increases caloric intake, and impairs insulin sensitivity, contributing to glucose intolerance and metabolic dysfunction. Psychological distress during pregnancy, including stress, anxiety, or depressive symptoms, can disrupt endocrine-immune interactions and HPA axis function, and has been linked to preterm birth and low birthweight. Increased maternal and placental CRH, together with inflammatory signaling, may influence fetal development and precipitate labor.

Maternal psychological stress is associated with gut microbiota perturbations, such as lower diversity, increased intestinal permeability, and heightened systemic inflammation, which may impact fetal development through maternal-fetal gut-brain pathways. These findings support the importance of considering psychological health alongside sleep, diet, and physical activity in preconception and antenatal care.

Omics technologies and personalized approaches

Current applications of omics and AI in women's health are primarily exploratory and hypothesis-generating, while their translational and predictive potential remains an active area of investigation. Recent advances in omics technologies have enabled precise understanding of the human microbiome and its connection with the endocrine system and reproductive health.[96] Personalized therapeutic approaches have developed that aim to directly modify the host's microbial ecosystem to influence hormonal, immunological, and metabolic processes.

Personalized medicine is emerging as a powerful tool to address hormonal dysfunctions from an integrative perspective. Each individual has a unique microbial signature that influences their response to nutrients, medications, and hormonal factors.[97] This has driven the development of personalized diets based on fecal microbiota analysis, where specific imbalances are identified and dietary plans are designed to favor beneficial bacteria growth (*F. prausnitzii, A. muciniphila*) associated with insulin sensitivity and effective immune function.[98] These interventions enhance microbial composition and induce epigenetic changes that regulate genes related to inflammation and fertility. However, evidence remains heterogeneous, and findings are often based on small or exploratory studies.

Unlike generic probiotic supplementation, personalized approaches use specific strains tailored to an individual's gut environment. For instance, *L. crispatus and L. jensenii* have been extensively studied in women with recurrent vaginal infections or urogenital microbial imbalances, demonstrating improvements in vaginal epithelial integrity and pH reduction, creating an optimal environment for fertilization and implantation.[99] While traditionally employed to treat *Clostridium difficile* infections, fecal microbiota transplantation shows potential to address severe dysbiosis associated with infertility, endometriosis, or metabolic syndrome.

Integrating AI with microbiome data enables development of predictive algorithms that identify risks of hormonal dysfunction (e.g., PCOS), infertility/Assisted Reproductive Technology (ART) outcomes, and pregnancy complications (e.g., preterm birth, gestational diabetes mellitus). These algorithms incorporate variables such as diet, hormone levels, inflammatory biomarkers, and microbial composition, facilitating personalized intervention programs. AI-based approaches now include nutrigenetic/nutrigenomic data (SNPs, polygenic risk) alongside gut microbiome features to develop multi-omic predictors for diet and health. This combination enables highly personalized dietary advice, including targeted prebiotic interventions, aimed at promoting beneficial bacterial taxa based on each individual's genetic and microbial profile.

Postbiotics (bioactive metabolites produced by microorganisms)[97] are gaining interest due to their capacity to modulate pathways including interleukins, cortisol, estrogens, and SCFAs without requiring direct bacterial colonization.

Future perspectives and conclusions

Hormonal variations that occur across the female lifespan profoundly influence the structure and dynamics of the vaginal microbiota. The integration of microbial, endocrine, and lifestyle dimensions marks a paradigm shift in women’s health research. Probiotics, diet, and behavioral strategies constitute complementary tools for restoring

microbial and hormonal homeostasis. As discussed in this review, such interventions may lower the incidence of urinary tract infections during puberty, alleviate discomfort associated with the menstrual cycle, enhance reproductive and pregnancy outcomes, and mitigate menopausal symptoms, ultimately contributing to an improved overall quality of life.

Nevertheless, although accumulating studies suggest these benefits, much of the current evidence remains preliminary, heterogeneous, or dependent on specific contexts and strains. A more comprehensive understanding of the bidirectional relationship between hormonal activity and the vaginal microbial ecosystem could unveil innovative strategies for managing conditions that are unique to, or disproportionately affect, women. In this perspective, microbiome-centered approaches, including targeted probiotic supplementation and personalized modulation of vaginal flora, are likely to play an increasingly significant role, driven by the rapid progress of molecular, omics-based, and clinical research technologies. Future studies should adopt multi-omics and personalized frameworks to map microbiota–hormone–lifestyle interactions, enabling tailored interventions for reproductive, metabolic, and mental wellbeing.

References

1. Barraza-Ortega E, Gómez-Gil B, García-Gasca T, et al. The impact of lifestyle on reproductive health: microbial complexity, hormonal dysfunction, and pregnancy outcomes. Int J Mol Sci 2025;26:8574.
2. Tachedjian G, Aldunate M, Bradshaw CS, et al. The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. Res Microbiol 2017;168:782–92.
3. Falk P, Bishen S. Closing the women's health gap: a $1 trillion opportunity to improve lives and economies. World Economic Forum. 2024. Available from: https://www.weforum.org/stories/2024/01/women-healthcare-gap/?gad_source=1&gad_campaignid=22228224717&gbraid=0AAAAAoVy5F6ET23g5VWPjJPxEpj4Er0gN&gclid=Cj0KCQjwiJvQBhCYARIsAMjts3J8L0yITresJCTrEWhOztUs6HaxYPzPEG56jVt5yWvtTHICFaIz2HgaAvDmEALw_wcB
4. National Institutes of Health. *Women's Health.* https://www.nichd.nih.gov/health/topics/womenshealth (accessed February 19, 2025).
5. U.S. Food and Drug Administration. Department of Health and Human Services. *Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs.* July 22, 1993.
6. Pinnow E, Sharma P, Parekh A, et al. Increasing participation of women in early-phase clinical trials approved by the FDA. Womens Health Issues 2009;19:89–93.

7. Sosinsky AZ, Rich-Edwards JW, Wiley A, et al. Enrollment of female participants in US phase 1–3 clinical trials between 2016 and 2019. Contemp Clin Trials 2022;115:106718.
8. Krakowsky Y, Potter E, Hallarn J, et al. The effect of gender-affirming medical care on the vaginal and neovaginal microbiomes of transgender and gender-diverse people. Front Cell Infect Microbiol 2022;11:769950.
9. Chen Z, Yeoh YK, Hui M, et al. Diversity of macaque microbiota compared to human counterparts. Sci Rep 2018;8:15573.
10. Miller EA, Beasley DE, Dunn RR, et al. *Lactobacilli* dominance and vaginal pH: Why is the human vaginal microbiome unique? Front Microbiol 2016;7:1936.
11. Miller EA, Livermore JA, Alberts SC, et al. Ovarian cycling and reproductive state shape the vaginal microbiota in wild baboons. Microbiome *2017;5:*8.
12. Mirmonsef P, Hotton AL, Gilbert D, et al. Free glycogen in vaginal fluids is associated with *Lactobacillus*colonization and low vaginal pH. PLoS ONE 2014;9:e102467.
13. Spear GT, French AL, Gilbert D, et al. Human α-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by *Lactobacillus.* J Infect Dis 2014;210:1019–28.
14. Jenkins DJ, Woolston BM, Hood-Pishchany MI, et al. Bacterial amylases enable glycogen degradation by the vaginal microbiome. Nat Microbiol 2023;8:1641–52.
15. Kwon MS, Lee HK. Host and microbiome interplay shapes the vaginal microenvironment. Front Immunol. 2022;13:919728.
16. Frederiksen H, Johannsen TH, Andersen SE, et al. Sex-specific estrogen levels and reference intervals from infancy to late adulthood determined by LC-MS/MS. J Clin Endocrinol Metab 2019;105:754–68.
17. Hickey RJ, Zhou X, Settles ML, et al. Vaginal microbiota of adolescent girls prior to menarche resembles that of reproductive-age women. mBio 2015;6:e00097-15.
18. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 2011;108:4680–4687.
19. Petrova MI, Reid G, Vaneechoutte M, Lebeer S. *Lactobacillus iners*: friend or foe? Trends Microbiol 2017;25:182–91.
20. Saperston KN, Shapiro DJ, Hersh AL, Copp HL. A comparison of inpatient versus outpatient resistance patterns of pediatric urinary tract infection pathogens. Pediatrics 2014;133:e123–8.
21. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol 2012;66:371–89.
22. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T 2015;40:277–83.
23. De Clercq M, Van Hoorde K, Fiddelaers J, et al. The Isala citizen science project on the vaginal microbiome of 3,345 Belgian women. Nat Microbiol 2023;8:1378–91.

24. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol 2013;209:505–23.
25. Muzny CA, Taylor CM, Swords WE, et al. An updated conceptual model on the pathogenesis of bacterial vaginosis. J Infect Dis 2019;220:1399–405.
26. Nel Van Der Veer C, et al. Comparative activity of *L. crispatus* strains against *Gardnerella* and *Prevotella*. Microbiome 2022;10:51.
27. Huang H, Song L, Zhao W, et al. Probiotics for the treatment of bacterial vaginosis: a systematic review and meta-analysis. Front Cell Infect Microbiol 2022;12:827636.
28. Senok AC, Verstraelen H, Temmerman M, Botta GA. Probiotics for the treatment of bacterial vaginosis. Cochrane Database Syst Rev 2009;4:CD006289.
29. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and gynecological cancers: a review. Front Cell Infect Microbiol 2019;9:290.
30. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen–gut microbiome axis: physiological and clinical implications. Maturitas 2017;103:45–53.
31. Kwa M, Plottel CS, Blaser MJ, Adams S. The intestinal microbiome and estrogen receptor–positive female breast cancer. J Natl Cancer Inst 2016;108:djw029.
32. Fuhrman BJ, Feigelson HS, Flores R, et al. Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. J Clin Endocrinol Metab 2014;99:4632–40.
33. Meng S, Chen B, Yang J, et al. Gut microbiota and breast cancer: correlations, mechanisms and potential clinical implications. Front Oncol 2021;11:706003.
34. Zhu J, Liao M, Yao Z, et al. The role of gut microbiota in postmenopausal osteoporosis. Front Cell Infect Microbiol 2022;12:819541.
35. Mitra A, MacIntyre DA, Marchesi JR, et al. The vaginal microbiota, human papillomavirus infection, and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome 2016;4:58.
36. Liu CM, Hungate BA, Tobian AA, et al. Penile microbiota and female partner bacterial vaginosis in Rakai, Uganda. mBio 2015;6:e00589–15.
37. Onywera H, Williamson AL, Mbulawa ZZA, et al. The penile microbiota in uncircumcised and circumcised men: relationships with HIV and HPV infections. Front Public Health 2020;8:7.
38. Clarke G, Stilling RM, Kennedy PJ, et al. Minireview: gut microbiota: the neglected endocrine organ. Mol Endocrinol 2014;28:1221–38.
39. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015;11:577–91.
40. Knezevic J, Starchl C, Berisha AT, Amrein K. Thyroid-gut-axis: how does the intestinal microbiota influence thyroid function? Nutrients 2020;12:1769.

41. Brotman RM, Shardell MD, Gajer P, et al. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause 2018;25:1321–330.
42. Song SD, Acharya KD, Zhu JE, et al. Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. Microbiome 2020;8:1–20.
43. Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome 2014;2:4.
44. Serrano MG, Brotman RM, Ravel J. The vaginal microbiome: new insights into the pathogenesis of bacterial vaginosis and vaginitis. Curr Opin Infect Dis 2022;35:73–9.
45. Moen MH, Øian P, Mellembakken JR, et al. Vaginal microbiota dynamics in young women—associations with hormones, contraceptives, and sexual behavior. Front Cell Infect Microbiol 2024;14:1314521.
46. Fettweis JM, Serrano MG, Brooks JP, et al. The vaginal microbiome and preterm birth. Nat Med 2019;25:1012–21.
47. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med 2020;382:1244–56.
48. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. Lancet. 2021;397:839–852.
49. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol 2019;15:666–82.
50. Parazzini F, Viganò P, Candiani M, Fedele L. Diet and endometriosis risk: a literature review. Reprod Biomed Online 2013;26:323–36.
51. Nirgianakis K, Egger K, Kalaitzopoulos DR, et al. Effect of omega-3 supplementation in endometriosis-related pain: a randomized controlled trial. Fertil Steril 2020;113:1209–17.
52. Itoh H, Uchida M, Sashihara T, et al. Oral administration of heat-killed *Lactobacillus gasseri* improves symptoms of endometriosis: a randomized trial. J Obstet Gynaecol Res. 2021;47:2761–70.
53. Uchida M, Kobayashi O, Itoh H. Oral administration of heat-killed *L. gasseri* alleviates endometriosis-like lesions in mice. J Med Microbiol 2020;69:1035–44.
54. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol 2018;14:270–84.
55. Lindheim L, Bashir M, Munzker J, et al. Alterations in gut microbiome composition and barrier function in PCOS: a pilot study. PLoS ONE 2017;12:e0168390.
56. Torres PJ, Siakowska M, Banaszewska B, et al. Gut microbial diversity in women with PCOS correlates with hyperandrogenism. J Clin Endocrinol Metab 2018;103:1502–11.
57. Yurtdaş G, Akdevelioğlu Y. A new perspective on PCOS: the gut microbiota. J Turk Ger Gynecol Assoc 2020;21:153–64.
58. Shoaei T, Heidari-Beni M, Tabrizi R, et al. The effects of probiotics on metabolic outcomes in women with PCOS: a systematic review and meta-analysis. Nutrients 2023;15:3184.

59. Chen L, Ma F, Lu W, et al. Effects of synbiotics on hormonal and inflammatory parameters in women with PCOS: a systematic review. Front Endocrinol (Lausanne) 2022;13:869187.
60. Aagaard K, Ma J, Antony KM, et al. The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237ra65.
61. Brown RG, Marchesi JR, Lee YS, et al. Vaginal dysbiosis increases preterm birth risk. Microbiome 2018;6:117.
62. Brown RG, Al-Memar M, Marchesi JR, et al. Preterm birth, bacterial vaginosis, and neonatal outcomes: a cohort study. Am J Obstet Gynecol 2022;226:91.e1–91.e14.
63. Haahr T, Zacho J, Bräuner M, et al. Vaginal microbiota and IVF outcomes: a prospective study. Hum Reprod 2019;34:1369–79.
64. Kyono K, Hashimoto T, Nagai Y, et al. Endometrial microbiota and IVF outcomes: a pilot study. Reprod Med Biol 2018;17:481–6.
65. Fettweis JM, Serrano MG, Brooks JP, et al. Association between vaginal dysbiosis and recurrent pregnancy loss. Reprod Sci 2020;27:132–40.
66. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota has an impact on implantation success or failure. Am J Obstet Gynecol 2016;215:684–703.
67. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. 2010;107:11971–5.
68. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. Nat Med 2016;22:713–22.
69. Vicariotto F, Mogna L, Del Piano M. Clinical evidence for probiotics in preventing perinatal depression. Minerva Ginecol 2020;72:280–9.
70. Slykerman RF, Hood F, Wickens K, et al. Probiotic during pregnancy and postpartum depression: randomized trial. EBioMedicine 2017;24:159–65.
71. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5). 5th ed. Washington, DC; 2013.
72. O'Hara MW, Wisner KL. Perinatal mental illness: definition, description and aetiology. Best Pract Res Clin Obstet Gynaecol 2014;28:3–12.
73. Dennis CL, Falah-Hassani K, Shiri R. Prevalence of antenatal and postnatal anxiety: systematic review and meta-analysis. Br J Psychiatry 2017;210:315–23.
74. Huang H, Song L, Zhao W, et al. The impact of menopause on vaginal microbiota. Front Cell Infect Microbiol 2023;13:1181945.
75. Nappi RE, Palacios S. Impact of vulvovaginal atrophy on sexual health. Maturitas 2014;78:236–40.

76. Gandhi J, Chen A, Dagur G, et al. Genitourinary syndrome of menopause: an overview of clinical manifestations, pathophysiology, etiology, evaluation, and management. Am J Obstet Gynecol 2016;215:704–11.
77. Chen J, Geng S, Li Z, et al. Effect of *Lactobacillus rhamnosus* GR-1 and *L. brevis* on genitourinary syndrome of menopause: randomized controlled trial. Menopause 2020;27:914–23.
78. Kim H, Kim H, Park H, et al. Oral probiotics for postmenopausal women: effects on vaginal health and systemic inflammation. Nutrients 2022;14:1589.
79. Kim MJ, Chung J, Kim DH, Kwon OJ. The effect of *Lactobacillus acidophilus* YT1 (MENOLACTO) on improving menopausal symptoms: a randomized, double-blinded, placebo-controlled clinical trial. J Clin Med 2020;9:2173.
80. Palma F, Volpe A, Villa P, Cagnacci A. Vaginal *Lactobacillus acidophilus* plus low-dose estriol for postmenopausal atrophy: randomized controlled study. Climacteric 2016;19:444–50.
81. Marschalek J, Worda C, Kohlberger P, et al. Influence of oral and vaginal Lactobacilli supplementation on the bacterial vaginosis-related vaginal microbiome in perimenopausal women undergoing chemotherapy for breast cancer. Front Oncol 2022;12:785856.
82. Li J, Wu J, Zhou Y, et al. Effects of probiotics on bone metabolism in postmenopausal women: systematic review and meta-analysis. Front Endocrinol (Lausanne) 2024;15:1367914.
83. Chen J, Kannan S, Tan Y, et al. Human vaginal organoids: a new platform to study host–microbe interactions. Cell Rep 2022;38:110300.
84. Das Neves J, Sarmento B. Vaginal mucosa-on-a-chip: future directions for drug and microbiome research. Adv Drug Deliv Rev 2021;175:113818.
85. Li X, Zhou T, Zhu Y, et al. Vaginal epithelium–microbiome crosstalk model reveals metabolic regulation of dysbiosis. Nat Commun. 2023;14:2294.
86. Hill C, Guarner F, Reid G, et al. Expert consensus on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506–14.
87. European Food Safety Authority (EFSA). Qualified Presumption of Safety (QPS) Approach for Assessment of Microorganisms. EFSA J 2020;18:e05968.
88. Marco ML, Heeney D, Binda S, et al. Health benefits of fermented foods: microbiota and beyond. Nat Rev Gastroenterol Hepatol 2021;18:608–23.
89. Romeo M, D'Urso F, Ciccarese G, et al. Exploring oral and vaginal probiotic solutions for women's health from puberty to menopause: a narrative review. Microorganisms 2024;12:1614.
90. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. Diet and lifestyle in the prevention of ovulatory disorder infertility. Obstet Gynecol 2007;110:1050–8.
91. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. Physiol Rev 2019;99:1877–2013.

92. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017;14:491–502.
93. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev 2015;36:E1–E150.
94. Smith RP, Easson C, Lyle SM, et al. Gut microbiome diversity is associated with sleep physiology in humans. PLoS ONE 2019;14:e0222394.
95. Taheri S, Lin L, Austin D, Young T, Mignot E. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 2004;1:e62.
96. D'Urso F, Paladini F, Pollini M, Broccolo F. Use of Artificial Intelligence Chatbots in Interpretation of Clinical Chemistry and Laboratory Medicine Reports: A Standardized Approach. Appl Sci 2025;15:4232.
97. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol. 2021;18:649–67.
98. Mailing LJ, Allen JM, Buford TW, Fields CJ, Woods JA. Exercise and the gut microbiome: a review of the evidence, potential mechanisms and implications for human health. Exerc Sport Sci Rev. 2019;47:75–85.
99. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol. 2011;9:233–43.

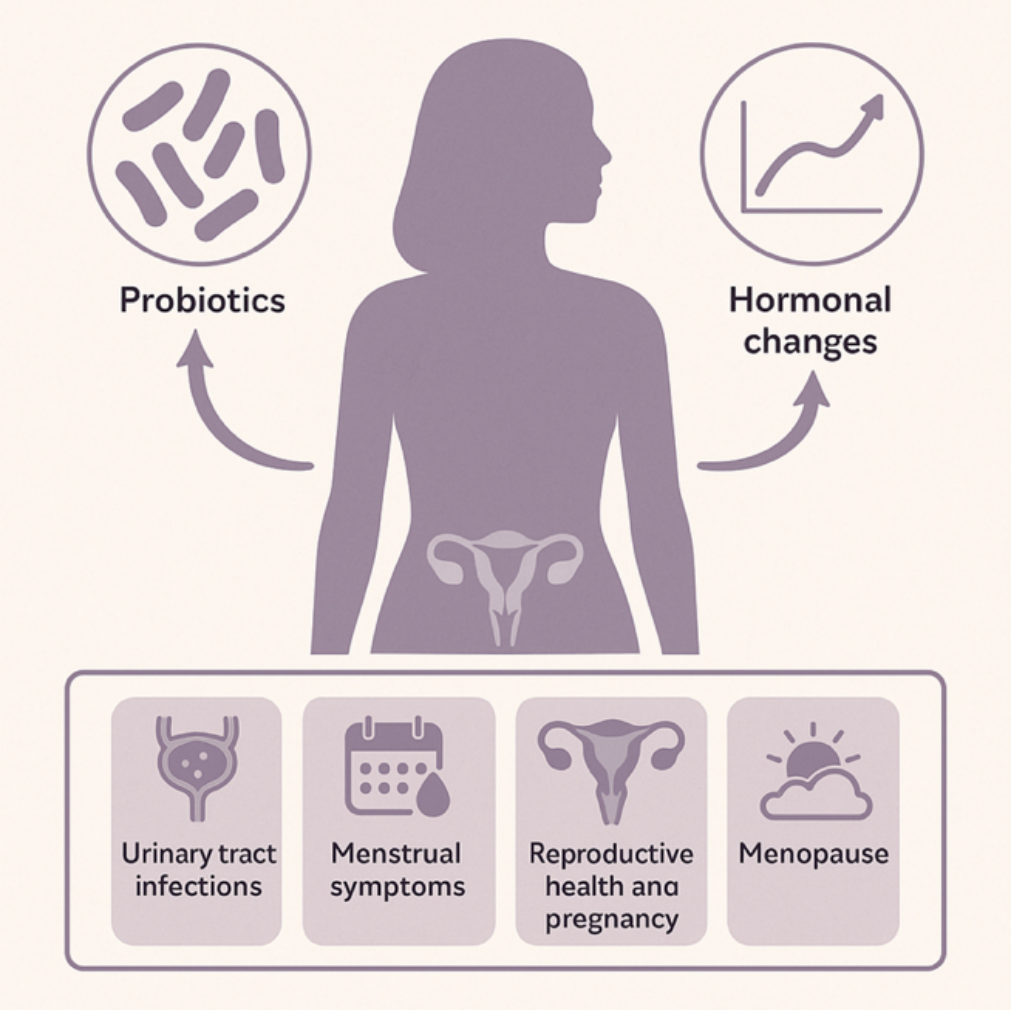

Figure 1. Conceptual model illustrating the interplay between hormonal fluctuations and vaginal microbiota composition across the female lifespan. Probiotic supplementation can help maintain microbial balance during key transitions, such as puberty, menstrual cycles, pregnancy, and menopause, thereby supporting urogenital and systemic wellbeing.

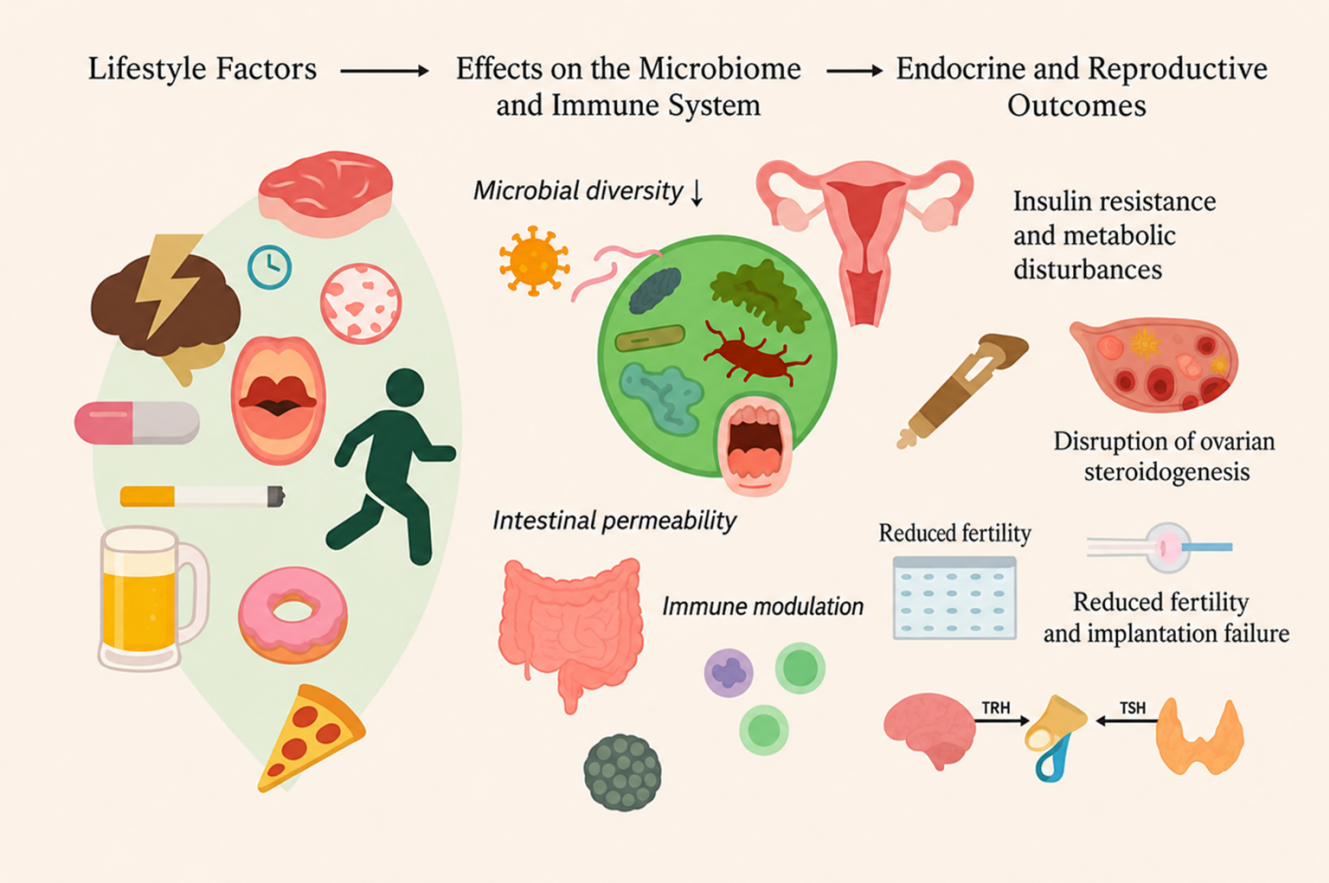

Figure 2. Conceptual overview illustrating hypothetical interactions between lifestyle determinants, the microbiome, and endocrine regulation (arrows denote direction of influence). Daily habits, including dietary patterns (e.g., intake of red meat), physical activity, stress exposure, and sleep quality, modulate the composition of the gut, oral, and reproductive microbiota, leading to shifts between microbial balance (eubiosis) and imbalance (dysbiosis). These microbiota-driven immune dynamics, in turn, communicate bidirectionally with endocrine pathways, particularly the hypothalamic–pituitary–thyroid–gonadal axis, thereby influencing hormonal homeostasis and reproductive outcomes.

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