CellPress

Trends in
Endocrinology & Metabolism

Review

Gut and vaginal microbiomes on steroids: implications for women's health

Madeline E. Graham,[1,4] William G. Herbert,[2,3,4] Stephanie D. Song,[2] Harshini N. Raman,[1] Jade E. Zhu,[1] Paulina E. Gonzalez,[1] Marina R.S. Walther-António,[2] and Marc J. Tetel[1,*]

This review discusses the interactions of steroids with the gut and vaginal microbiomes within each life phase of adult women and the implications for women's health. Each phase of a woman's life is characterized by distinct hormonal states which drive overall physiology of both host and commensal microbes. These host–microbiome interactions underlie disease pathology in disorders that affect women across their lifetime, including bacterial vaginosis, gestational diabetes, polycystic ovary syndrome (PCOS), anxiety, depression, and obesity. Although many associations between host health and microbiome composition are well defined, the mechanistic role of the microbiome in women's health outcomes is largely unknown. This review addresses potential mechanisms by which the microbiota influences women's health and highlights gaps in current knowledge.

Highlights

Sex steroids modulate the gut and vaginal microbiota, linking their composition and function.

The estrobolome and the glycogen-estrogen hypothesis provide a potential pathway linking the gut and vaginal microbiomes through estrogen signaling.

The gut and vaginal microbiomes are implicated in a wide range of disorders and disease states affecting women across their lifespan, including polycystic ovary syndrome (PCOS), unexplained infertility, obesity, and endometrial cancer. For example, PCOS is characterized by reduced richness and lower relative abundance of short-chain fatty acid (SCFA)-producing microbes in the gut microbiota, and increased alpha diversity and lower *Lactobacillus* spp. abundance in the vaginal microbiota.

Mounting evidence suggests that steroids and gut microbiota acting via the gut–brain axis influence mental health changes that can occur throughout women's life phases, including depression, postpartum depression, and anxiety.

It is well established that the human microbiome has a dramatic impact on health and disease. In this review we discuss the interaction of steroids with the gut and vaginal microbiomes and the implications of these interactions on women's health across three of the major developmental transitions: early adulthood, pregnancy, and menopause.

Microbiota refers to the microorganisms that inhabit the body, including bacteria, viruses, archaea, protozoa, and fungi [1]. These microorganisms, their genomes, and the metabolites they produce comprise the microbiome. The bacterial composition of the human gut is plastic in infants and becomes adult-like around the age of three [2]. Although sex differences in the **gut microbiota** (**GM**, see Glossary) exist before puberty, these differences are greater after puberty [3] and persist into adulthood [4], suggesting that gonadal steroids influence gut microbial composition [5]. Although the plasticity and development of the vaginal microbiome in early years remains unclear, the sudden surge in sex steroid levels during puberty is observed to be associated with lower diversity of the **vaginal microbiota (VM)** [6]. Taken together, these findings suggest that the increase in endogenous steroid hormones at puberty creates a new environment and shapes the adult female gut and vaginal microbiomes. This new adult female hormonal milieu establishes a novel equilibrium between host and commensal microbial physiology, which is central to women's health (Figure 1).

Sites of estrogen modification and response by the microbiota

Estrobolome and the GM

The estrobolome describes the functional collection of all enteric bacterial gene products capable of metabolizing estrogens (Figure 2). In the process of conjugation, estrogens are metabolized in the liver and marked for excretion via urine and feces by the addition of a glucuronic acid moiety [7]. Removal of the glucuronic acid group via deconjugation allows estrogens to remain in the body and exert their effects. Enteric bacteria (e.g., the genera *Bifidobacterium*, *Clostridium*, and *Lactobacillus* [8]) produce β-glucuronidases and β-glucuronides that can determine the fate of

[1]Neuroscience Department, Wellesley College, Wellesley, MA 02481, USA
[2]Department of Surgery, Department of Obstetrics and Gynecology, and Microbiome Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905, USA
[3]Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN 55905, USA
[4]Equal contributions

*Correspondence: mtetel@wellesley.edu (M.J. Tetel).

 https://doi.org/10.1016/j.tem.2021.04.014

estrogens in the gut via deconjugation and conjugation, respectively [7]. The relative ratios of these bacterial enzymes, β-glucuronidases, and β-glucuronides, in the gut directly impact on the amount of circulating estrogens. Some bacteria involved in the estrobolome produce enzymes that differentially deconjugate **estradiol (E2)** and estrone [9]. The selective deconjugation of some estrogens by the GM could alter the circulating hormonal profile, in turn affecting host physiology. Although a direct link between the capacity of the GM to metabolize estrogens and the metabolic changes taking place in pregnancy and at menopause has yet to be shown, there is potential that they are functionally linked through the estrobolome.

Glycogen-estrogen hypothesis and the VM

The hypothesized mechanism by which estrogens establish a high abundance of *Lactobacillus* spp. in the human VM is through driving an increase in glycogen production by vaginal epithelial cells, which promotes *Lactobacillus* growth [6,10,11] (Figure 1A,B). In fact, one evolutionary explanation for how humans developed *Lactobacillus* dominance suggests that the high starch content of human diets led to high glycogen content in the vaginal tract, creating an ideal environment for lactobacilli [12]. Interestingly, intravaginal estrogen treatment in transgender men appears to increase their typically low vaginal *Lactobacillus* abundance [13].

Menstrual cycle

E2 and progesterone levels fluctuate across different phases of the menstrual cycle (Figure 1A). The follicular phase is characterized by rising estrogen levels, in particular E2. During the ovulatory phase, E2 peaks when progesterone levels also begin to rise. During the luteal phase the follicle transforms into the corpus which secretes progesterone for stimulation of the secretory endometrium. When pregnancy does not occur, the corpus luteum degenerates and estrogen and progesterone decline to basal levels, which triggers shedding of the secretory endometrium [14].

Menstrual cycle and the GM

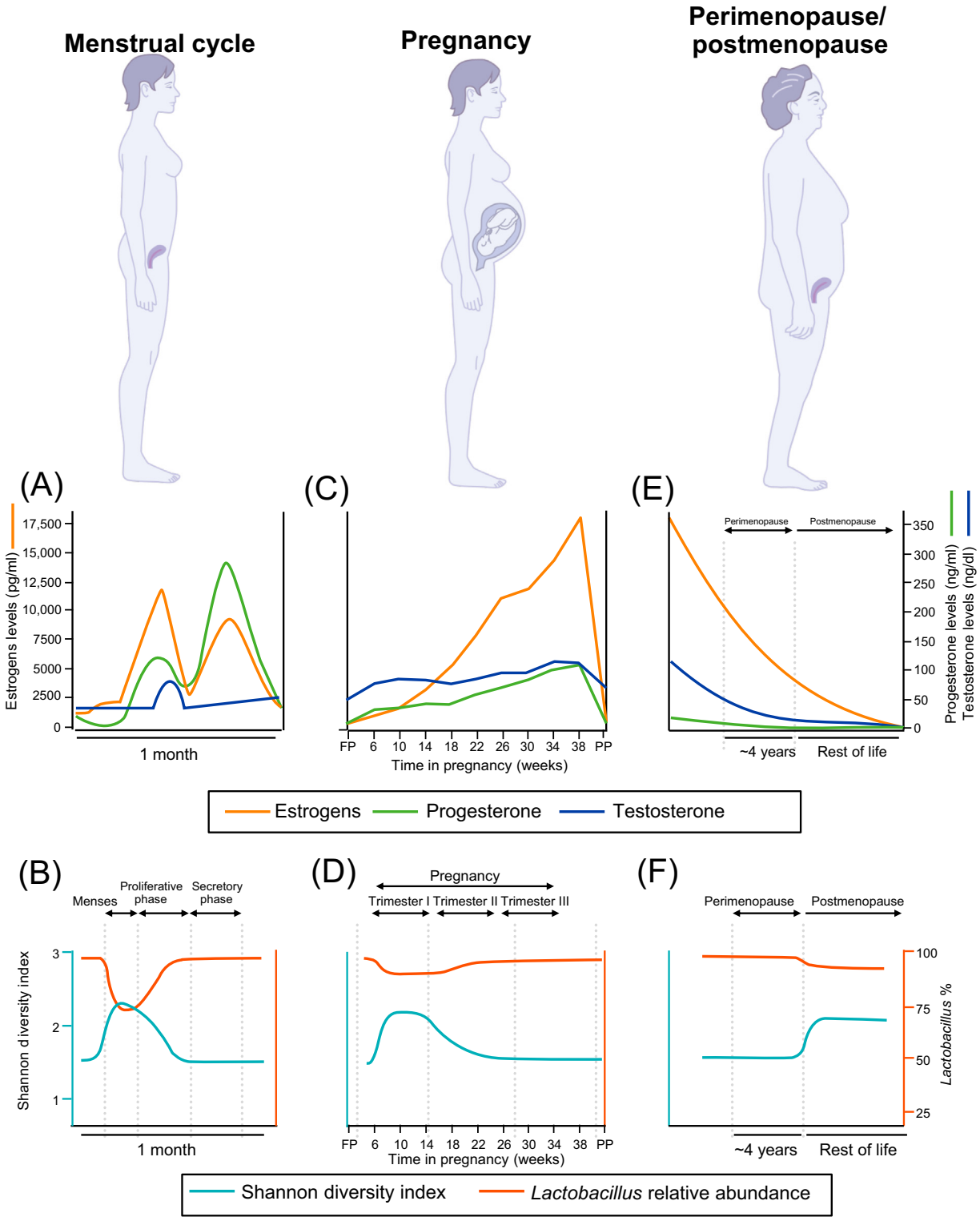
Describing the standard GM in healthy adults is challenging given that each individual's signature is unique owing to a wide variety of factors that influence the GM composition, including sex, age, diet, and body mass index (BMI) [15]. However, across most populations the *Firmicutes* and *Bacteroides* phyla are the most abundant. In support, *Eubacterium rectale-Clostridium coccoides*, *Bacteroides-Prevotella*, and *Faecalibacterium prausnitzii* are the most prevalent gut microbes across all groups of European adults [16]. It appears that women have lower levels of *Bacteroidetes* than men [15,16]. Although a variety of rodent studies suggest that ovarian steroids alter the GM [5,17–19], we are only beginning to understand the impact of the menstrual cycle on the GM [20]. Given that the menstrual cycle affects a variety of gastrointestinal disorders, including irritable bowel syndrome [21], it will be important for future studies to investigate the effects of the menstrual cycle on the GM and the implications for women's health.

Menstrual cycle and the VM

In the healthy adult vaginal microbiome, *Lactobacillus* dominance protects the vaginal environment by producing lactic acid [22], creating an exceptionally low pH environment [23]. In addition to creating an unsuitable environment for pathogenic bacterial growth, which can cause **bacterial vaginosis (BV)**, *Lactobacillus* dominance and low pH contribute to the normal immune response of the vaginal epithelium [24]. A healthy vaginal environment may also be achieved with non-*Lactobacillus* species because many women with low or no *Lactobacillus* have a similar resistance to BV [24]. Moreover, one study reports that transgender men taking testosterone exhibit lower *Lactobacillus* abundances and higher alpha diversity than cisgender women, but these men present as asymptomatic for BV [13].

Glossary

Bacterial vaginosis (BV): a state of perturbed bacterial composition in the vagina, often leading to inflammation.
Estradiol (E2): a major ovarian estrogen steroid hormone.
Gestational diabetes (GD): the onset of diabetes in pregnancy.
Gut microbiota (GM): commensal microbes in the gastrointestinal tract, whereas the gut microbiome constitutes all microbes and their associated gene products.
Hypothalamus–pituitary–adrenal (HPA) axis: the primary neuroendocrine pathway comprising the hypothalamus, anterior pituitary gland, and adrenal glands which regulates homeostasis and the stress response.
***In vitro* fertilization (IVF):** a procedure in which an egg is fertilized by a sperm outside the body.
Polycystic ovary syndrome (PCOS): a common hormonal disorder affecting women of reproductive age, causing elevated androgen levels, irregular menstrual cycles, and often small follicular cysts on the ovaries.
Postpartum depression (PPD): the appearance of depressive symptoms after childbirth.
Sex hormone-binding globulin (SHBG): a glycoprotein that binds estrogens and androgens and modulates their bioavailability.
Short-chain fatty acids (SCFAs): fatty acids with one to six carbons that are produced by bacterial fermentation of non-digestible dietary fibers, and that have a variety of physiological roles.
Unexplained infertility (UI): diagnosis of infertility in the absence of any underlying conditions.
Vaginal microbiota (VM): commensal microbes in the vagina, whereas the vaginal microbiome consists of all microbes and their gene products.



Trends in Endocrinology & Metabolism

(See figure legend at the bottom of the next page.)

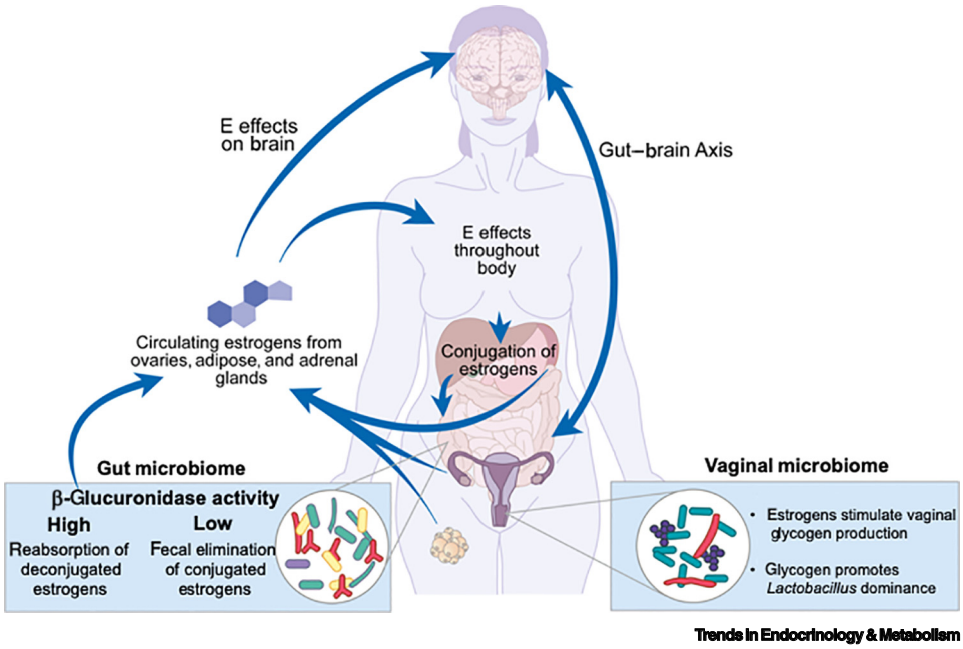


Figure 2. Systemic effects and modulation of estrogens. Estrogens (E) have widespread effects throughout the body and are central to many aspects of women's health. In addition to their central effects, estrogens act on, and are acted on by, the human microbiome. The gut microbiota (GM) consists of microbes that produce β-glucuronidase. This enzyme can deconjugate estrogens that were destined for excretion, causing them to reenter the body and remain active [7]. The composition of the vaginal microbiota (VM) is influenced by estrogens in the body via its effects on the vaginal epithelium [12]. Vaginal glycogen increases in response to estrogens and drives *Lactobacillus* spp. dominance in the VM [11].

The composition and stability of the VM is dynamic throughout the menstrual cycle, with a notable increase in alpha diversity, a decrease in the abundance of *Lactobacillus* spp., and a decrease in community stability during menses [6,25–27]. Transient and permanent changes in the vaginal microbial community state are also more likely to occur during menses [27]. A variety of changes during menses, including the presence of blood, tampon usage, variations in hygiene and sexual activity, and fluctuations in estrogens and progestins, may contribute to these vaginal microbial alterations [26,28]. Alpha diversity, the relative abundance of *Lactobacillus* spp., and community stability correlate with changes in E2 levels across the menstrual cycle (Figures 1A,B) [25,27], triggering the proliferation of squamous epithelial cells that produce more glycogen to fuel *Lactobacillus* spp. growth [11]. Finally, preliminary evidence suggests that progestin-only contraceptives disrupt the usual predictable decline in *Lactobacillus* during menses [27], and contraceptives containing estrogens promote *Lactobacillus* proliferation and reduce the risk of BV [29]. However, more work must be done to better understand the effects of contraceptives on VM and the implications for women's health.

Disorders affecting reproductive age women

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects ~10% of reproductive age women and is characterized by hyperandrogenism, ovarian dysfunction, and metabolic

Figure 1. Women's life phases are characterized by distinct changes in the hormonal milieu and the vaginal microbiota (VM). Estrogens and progesterone follow a cyclical pattern of release during the menstrual cycle (A) which influence the composition of the VM, where the Shannon index is a measure of diversity (B). Pregnancy (C) is characterized by a lack of hormonal cyclicity and steadily increasing sex steroids to maintain pregnancy. The initial change in hormone levels relative to the follicular phase (FP) alters the composition of the VM during pregnancy (D), which later returns to a state similar to that of the secretory phase of the menstrual cycle. Hormone levels return to pre-pregnancy values postpartum (PP). At menopause (E), ovarian function declines and sex steroid levels drop precipitously. The endocrine changes of menopause coincide with drastic changes in the VM (F), including a decrease in the relative abundance of *Lactobacillus* spp.

syndrome [30]. Although there are several diagnostic criteria, the main symptoms include signs of hyperandrogenism such as hirsutism, irregular menstrual periods, and ovarian cysts [31]. Women with severe symptoms of PCOS have elevated androgen levels as well as increased triglycerides, low-density lipoprotein cholesterol, fasting insulin, and insulin resistance indices [32]. PCOS is also associated with decreased GM richness and a lower relative abundance of bacteria that produce **short-chain fatty acids (SCFAs)**, including *Roseburia* and *Odoribacter*, as well as with increased markers of intestinal dysbiosis and permeability [32,33]. SCFAs, a product of fiber fermentation by GM, are associated with gut health and have immunomodulating effects [34]. Specifically, the SCFA butyrate promotes gut barrier function by providing an energy source for epithelial cells, inducing tight-junction protein expression, and by increasing the expression of antimicrobial peptides, which protect against infection by pathogens [35,36]. SCFAs reduce inflammation by altering cytokine expression in intestinal epithelial cells and coordinating the recruitment of immune cells, including neutrophils, monocytes, and macrophages [35].

Because of the inherent challenges in translating the VM of mouse models to humans, fewer studies have explored the relationship between PCOS and the VM. However, one case–control study found that women with PCOS have higher VM alpha diversity, lower abundances of *L. crispatus*, and elevated levels of *Mycoplasma* and *Prevotella*, after adjusting for BMI [37]. In contrast to the VM and PCOS, well-established rodent models of PCOS that recapitulate the endocrine, metabolic, and reproductive hallmarks of the disorder have been used to demonstrate a potentially causal role of the GM in PCOS. Administration of the aromatase inhibitor, letrozole, to female rodents induces a PCOS-like phenotype [38]. Female mice treated with letrozole have an altered GM, including a shift in the Firmicutes/Bacteroidetes ratio, similar to that reported in mouse models of metabolic syndrome [39]. These GM changes in PCOS mice have a causal role in the metabolic and reproductive dysfunction. Letrozole-treated mice cohoused with healthy female mice, and thus exposed to their GM by coprophagy, had marked improvements in metabolic and reproductive phenotype, presumably due to the distinct changes in the GM of the PCOS mice [40].

Other reproductive system disorders are associated with VM composition. *Lactobacillus*-dominated vaginal environments are considered 'healthy' because they confer a lower incidence of BV [28,41]. BV recurrence is most likely to occur during or immediately after menses [26], which is a period of decreased *Lactobacillus* and VM stability, and increased alpha diversity [6,25,27]. Black and Latina women, who are more likely to have low levels of lactobacilli [42], also have a higher BV prevalence (>30%) than white (23%) or Asian (11%) women [43]. Studies suggest that estrogen-containing contraceptives are associated with lower risk of BV [29,44], although a recent randomized controlled trial indicated that combined oral contraceptives did not reduce the risk of BV recurrence [45]. Meanwhile, depot medroxyprogesterone acetate, a progestin-only contraceptive injection, may increase BV risk in some individuals [46].

A wide range of brain disorders, including anxiety, depression, and Alzheimer's disease, involve the gut–brain axis, which is an important communication pathway between the gut microbiome and the brain [5,47]. Anxiety disorders peak during adolescence, and females have a higher risk and degree of severity than males [48]. This sex difference may be due to the interactions between estrogens and the **hypothalamic–pituitary–adrenal (HPA) axis** [49]. Although the exact cause is poorly understood, hormonal fluctuations throughout the menstrual cycle do influence mood, particularly anxiety and depression, such as in premenstrual syndrome and premenstrual dysphoric disorder [50]. Like humans, rodents exhibit sex differences in anxiety [51], likely due to sex hormones such as E2 [52].

Sex differences in GM may also contribute to sex differences in anxiety disorders [53]. Recent studies demonstrate that the gut–brain axis is sexually dimorphic in regard to GM composition, resulting in differences in downstream targets (including the immune and neuroendocrine systems) and susceptibility to a variety of disorders [54]. In support of a role for the GM in anxiety in females, conventionally raised female mice had greater anxiety-like behavior than germ-free female mice lacking a normal GM [55]. Dietary supplementation with the long-chain fatty acid docosahexaenoic acid alters neurobehavior in mice. Male mice challenged with social isolation and treated with docosahexaenoic acid had improvements in anxiety-like behavior and different changes in the GM compared to females – which did not exhibit improvements in behavior [56]. These findings in mice, taken together with the observations of sex differences in the GM and anxiety in humans, support the notion that the GM plays a causal role in brain disorders in women.

Pregnancy

Endometrial receptivity is guided by progesterone production during the luteal phase. During implantation, progesterone and estrogens regulate important physiologic mechanisms in preparation for embryonic development [57]. Estrogen and progesterone levels increase throughout pregnancy, peak during the third trimester [58], and return to pre-pregnancy values by 5 days postpartum [59] (Figure 1C).

Pregnancy and the GM

The hormonal and physiological changes that occur over the course of pregnancy are accompanied by changes in the GM. Between the first and third trimesters of pregnancy, women gain adiposity, have increased insulin levels, and exhibit insulin resistance. The changes in energy metabolism coincide with increases in the relative abundances of the phyla Proteobacteria and Actinobacteria, and an overall decrease in GM richness [60]. Germ-free mice inoculated with fecal microbiota collected from pregnant women in their third trimester gained fat and also had increased inflammatory markers and insulin resistance, suggesting that GM alterations can cause the metabolic changes occurring late in pregnancy [60]. Although the effects of specific hormonal changes in pregnancy on the GM are poorly understood, an increase in progesterone in late pregnancy mediates an increase in the relative abundance of *Bifidobacterium* spp. [61].

Changes in metabolism in late pregnancy can set the stage for the development of glucose intolerance and **gestational diabetes (GD)**. GD is highly prevalent worldwide and is increasing [62]. In addition to the deleterious effects during pregnancy, GD can cause obstetric complications later in pregnancy and increases the risk of type 2 diabetes in mother and baby after delivery [62]. Distinct changes in the GM during pregnancy are associated with GD, namely increased abundances of *Parabacteroides distasonis* and *Klebsiella variicola*, and decreased abundances of *Methanobrevibacter smithii* and *Bidfidobacteria* spp. [63]. In another study, the GM of women with GD resembled that of non-pregnant individuals with type 2 diabetes, and this persisted for 8 months postpartum [64].

Pregnancy and the VM

The normal, healthy VM is distinct during each trimester of pregnancy, and the onset of pregnancy is marked by drastic changes in the VM (Figure 1D) [6]. Microbial diversity decreases and the abundance of *Lactobacillus* spp. increases as pregnancy progresses [65,66]. However, there are opposing findings on whether VM diversity increases or decreases in pregnant women relative to non-pregnant women [6,65,67,68]. Stratifying the pregnant VM across geographical location and ethnic group reveals differences in species abundance [69], perhaps explaining this controversy. Although the prevailing understanding of the role of microbes in the womb posits

that the embryonic environment is free of culturable bacteria, recent research indicates that the womb and fallopian tubes are not sterile and microbes may function in fetal health [70,71]. The postpartum vaginal microbiome is less dominated by *Lactobacillus* spp. and shows increased alpha diversity [69].

Disorders affecting conception, pregnancy, and postpartum health

Unexplained infertility (UI) affects 10% of women undergoing ***in vitro* fertilization (IVF)** in the USA where assisted reproduction techniques are insufficient for successful pregnancy [72]. This lack of success prompts exploration of the role of the VM in IVF [73]. Although the vaginal and cervical microbiomes do not differ significantly between fertile and infertile women, the endometrial microbiome differs in community composition [74], and the VM may be a predictive tool for IVF outcome [75]. Furthermore, women with UI have a cervico-vaginal microbiota that is distinct from that of non-idiopathic infertile women [76]. Further work must be done to determine the composition of the endometrial microbiota at the time of implantation and its effect on both the embryo and endometrium morphology specifically in women with UI.

Preterm birth affected one in ten infants in the USA in 2018 [77]. The preterm birth vaginal microbiome shifts away from the typical increase in *Lactobacillus* prevalence [78]. However, increased levels of *Lactobacillus iners*, in particular, are a risk factor for preterm birth after a gestational age of 16 weeks [79]. Interestingly, translocation of gut microbes into the amniotic fluid is associated with preterm rupture of membranes, suggesting that GM composition and its effect on gut integrity influence the intrauterine environment [80]. Furthermore, women receiving vaginal progesterone treatment to mediate risk factors for preterm birth have unchanged microbial community composition [79]. Preterm rupture of membranes and the accompanying dysbiotic state require temporal examination to deduce why and how these shifts occur and their role in preterm birth.

Although the gut microbiome–brain axis has been implicated in depression [81], its role in **postpartum depression (PPD)** is poorly understood. Antibiotic exposure during pregnancy was a predictive factor for PPD within 1 month post-birth [82]. Although hormone levels decrease sharply following birth, relative estrogen levels do not correlate with PPD [83]. The role of progesterone is less clear because progesterone supplementation worsens depression scores, but decreases recurrent PPD [84]. The modulation of steroid metabolism by the GM discussed in the previous text [7] could play an important role in PPD and needs to be explored. The VM has not yet been implicated in depression, but, given its role in pregnancy and female reproductive health, it might contribute to the interactions between steroid hormones, the microbiome, and the brain.

Across all stages of pregnancy – from before conception to postpartum – the microbiome is a potential mediator of a healthy pregnancy. The distinct challenge in examining the role of both the GM and the VM is the temporal nature of the experiments required and the sensitivity of embryo-endometrial synchrony to temporal disruptions. Novel *in vitro* microfluidic culture systems could potentially offer insight into these temporal changes in the reproductive tract and examine interactions between embryo and endometrium while also supporting microbial coculture [85–87]. Although pregnancy is difficult to examine clinically, there are gaps in knowledge that require the development of novel techniques and approaches to more fully understand and treat reproductive disorders in the future.

Menopause

Menopause is defined by the permanent cessation of ovarian follicle activity and the lack of a menstrual cycle for 1 year, marking the end of natural reproductive life in women [88]. The period

of decline in ovarian function is termed perimenopause and is characterized by increases in follicle-stimulating hormone owing to the lack of follicles and negative feedback from the ovaries [88]. As ovarian activity continues to decrease, the production of E2 and progesterone ceases (Figure 1E). At menopause, estrogens and progestins have declined considerably and women's E2 levels drop to those similar of age-matched men [89]. Endogenous androgen levels also fluctuate throughout menopause, increasing in some women during the menopause transition, and declining thereafter [89]. **Sex hormone-binding globulin (SHBG)** begins to increase after the menopause transition and is associated with the levels of the adipokines leptin and adiponectin in postmenopausal women [90]. As discussed in the following text, these hormonal changes have widespread effects on the health of postmenopausal women.

Menopause and the GM

In addition to changes in the hormonal milieu, menopause also involves alterations in the microbiota [4,91]. Although young adult women have a more diverse GM than their male counterparts, this sex difference is not observed in older adults, suggesting that the lack of ovarian steroids after menopause impacts on the GM [4]. In support of menopause affecting the GM, postmenopausal women had greater abundances of the phylum Firmicutes and the genera *Lachnospira* and *Roseburia* than premenopausal women. A higher Firmicutes to Bacteroidetes ratio is associated with obesity in adults [92]. Both *Lachnospira* and *Roseburia* produce SCFAs, which exert beneficial effects on host health and metabolism [93]. In addition, postmenopausal women, similarly to men, had lower levels of plasma glucagon-like peptide-1 and higher levels of the inflammatory cytokines interleukin-6 and monocyte chemoattractant protein-1 than premenopausal women [91]. Taken together, these findings suggest that menopausal women are more susceptible to metabolic syndrome owing to increased inflammation, reduced satiety, and nutrient uptake signaling.

Menopause and the VM

The vaginal microbiome changes in tandem with hormonal fluctuations as women enter their menopausal years (Figure 1F). Decreased levels of estrogens can cause vulvovaginal atrophy and decreased vaginal secretions that contain nutrients to support bacterial growth [11,94,95]. Consequentially, increased vaginal pH is observed universally across postmenopausal women [94]. However, differences in postmenopausal women's previous usage of contraceptives and reproductive history can contribute to variation in specific microbial makeup and diversity [11]. Lactobacilli remain prominent in the vaginal microbiomes of healthy Chinese postmenopausal women [94], although other studies suggest that strictly anaerobic bacteria codominate vaginal communities [11]. Therefore, the inconsistencies in the correlation between levels of serum estrone, vaginal glycogen, lactobacilli [96], and genitourinary symptoms reported in healthy postmenopausal women may imply that *Lactobacillus* dominance or low pH are not necessarily indicative of health in postmenopausal women [94,96].

Disorders affecting menopausal women

The unopposed estrogen hypothesis posits that exposure to higher circulating levels of estrogens unopposed by the antagonistic effects of progestins is associated with a higher risk of endometrial cancer owing to the proliferative effects of estrogens on the endometrium [97]. In support, unopposed exogenous estrogens from oral contraceptives or hormone treatment increase the risk of endometrial cancer, but not when paired with progesterone [98]. Progestins increase dehydrogenase activity in endometrial cells, reduce estrogen receptor concentrations, and induce endometrial cell differentiation into a secretory state [99]. The specific reproductive tract microbiota in postmenopausal women has a significant overlap with the microbiota associated with endometrial cancer [100]. Commonly, *Lactobacillus* spp. no longer dominate the VM postmenopause, leaving the reproductive tract more susceptible to dysbiosis, and thus putting women at

greater risk for atrophic vaginitis, BV, and endometrial cancer [94,100]. Hormone treatment is commonly administered to restore *Lactobacillus* dominance and reduce symptoms of atrophy [11,94,95].

Because estrogens are crucial in energy homeostasis, the lack thereof results in an increased risk of obesity, metabolic syndrome, diabetes, cardiovascular disease, and cancer [101]. Hormone treatment reduces the risk and severity of type 2 diabetes and metabolic syndrome in menopausal women by improving insulin signaling, but it is not a suitable option for all women because of its risks, including blood clots and steroid-responsive cancers [102]. Interestingly, administration of the probiotic *Lactobacillus plantarum* from fermented milk improves markers of inflammation and cardiovascular health in postmenopausal women with metabolic syndrome [103]. In addition, obese postmenopausal women treated with flaxseed mucilage, which is rich in prebiotic fibers, altered GM composition and improved insulin sensitivity [104]. Finally, SCFAs from GM metabolism of dietary fibers play a role in protection from dysregulated lipid metabolism and inflammatory cardiovascular damage [105].

The drastic decline in ovarian steroids during perimenopause affects mental health, most likely due to the interaction between estrogens and the HPA axis [106]. Across menopausal status, perimenopause is associated with a higher risk of depression symptoms, whereas postmenopause is associated with greater risk of anxiety-type symptoms [107]**.** In support of a role of estrogens, a mouse model of menopause suggests that obesity and diabetes during menopause increase the risk of depression-like symptoms, which are alleviated by estrogen treatment [108]. Furthermore, in another mouse model of menopause, the GM was directly linked to depressive symptoms which were alleviated by progesterone supplementation and were associated with distinct changes in the GM [109]. The impact of the GM on depression in postmenopausal women is poorly understood, but the early results discussed previously highlight its importance in future research.

Concluding remarks

The interaction between sex steroids and the vaginal and gut microbiomes is a bidirectional axis that profoundly impacts on women's health across all life stages. The GM modulates circulating estrogens in the estrobolome, and in turn these circulating estrogens help to shape the VM, driving reproductive tract health. The gut and vaginal microbiomes appear to have crucial overlapping functions in various disease states in women's health. However, there are glaring gaps in our understanding of the mechanisms through which women's health is influenced by the interactions between steroids, the GM, and the VM (see Outstanding questions). In addition, although it is well established that the GM affects brain health and disease through the gut microbiome–brain axis, it is not known whether the VM has similar influences. As highlighted in this review, it will be crucial for future research to explore how these interactions, and the mechanisms involved, impact on women's health.

Acknowledgments

This work was supported, in part, by Clinical and Translational Science Award (CTSA) grant KL2 TR002379 from the National Center for Advancing Translational Science and a career enhancement award from the National Institutes of Health (NIH; grant P50 CA136393) to M.R.S.W-A., and by Wellesley College Jenkins Distinguished Chair in Neuroscience Funds (to M.J.T.).

Declaration of interests

The Mayo Foundation for Medical Education and Research (inventor M.R.S.W-A.) has been issued a patent 'Methods and Materials for Treating Endometrial Cancer', US10072303B2. The content of the patent relates to the use of the microbiome to address endometrial cancer. M.R.S.W-A. is a member of the scientific advisory board of LUCA Biologics Inc. on research related to urinary tract infections, preterm birth, and reproductive medicine. The other authors declare no conflicts of interest.

Outstanding questions

What effects do hormonal contraceptives have on the hormonal milieu, and how might they affect the GM and VM?

What is the mechanistic role of hormones and the reproductive tract microbiome in embryonic implantation and development?

What is the role of the GM in mediating the effects of estrogens on mood and energy metabolism in pregnancy and menopause? Could dietary interventions or supplementation of prebiotics alleviate metabolic dysfunction caused by hormonal shifts?

How do the reproductive tract microbiota and hormonal landscape in menopause drive the pathogenesis of endometrial cancer individually and in concert?

Because of the cyclical nature of female hormones, how can the temporal resolution of GM and VM sampling be improved to reflect these changes? How should women's life phases and/or menstrual phases be taken into account when considering pro- and prebiotic treatments?

If in fact the womb is not sterile, how should treatment of idiopathic infertility, preterm birth, and fetal surgery evolve? Would it be possible to identify new populations at risk for preterm birth or birth complications?

Does the vaginal microbiome play a role in mental health? Could pro- and prebiotic supplementation of both the GM and VM improve mental health as an alternative to steroid hormone-based therapies in premenstrual dysphoric disorder, PPD, or menopause?

Given that gut microbiota can influence the levels of steroid hormones, what is the causal role of aberrant gut microbiota in disorders such as PCOS and in endometrial and other cancers?

References

1. Sender, R. *et al.* (2016) Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* 14, e1002533
2. Yatsunenko, T. *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature* 486, 222–227
3. Yuan, X. *et al.* (2020) Sexual dimorphism of gut microbiota at different pubertal status. *Microb. Cell Factories* 19, 152
4. de la Cuesta-Zuluaga, J. *et al.* (2019) Age- and sex-dependent patterns of gut microbial diversity in human adults. *mSystems* 4, e00261-19
5. Tetel, M.J. *et al.* (2018) Steroids, stress and the gut microbiome–brain axis. *J. Neuroendocrinol.* 30, e12548
6. Kaur, H. *et al.* (2020) Crosstalk between female gonadal hormones and vaginal microbiota across various phases of women's gynecological lifecycle. *Front. Microbiol.* 11, 551
7. Plottel, C.S. and Blaser, M.J. (2011) Microbiome and malignancy. *Cell Host Microbe* 10, 324–335
8. Kwa, M. *et al.* (2016) The intestinal microbiome and estrogen receptor-positive female breast cancer. *J. Natl. Cancer Inst.* 108, djw029
9. Ervin, S.M. *et al.* (2019) Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J. Biol. Chem.* 294, 18586–18599
10. Nunn, K.L. and Forney, L.J. (2016) Unraveling the dynamics of the human vaginal microbiome. *Yale J. Biol. Med.* 89, 331–337
11. Gliniewicz, K. *et al.* (2019) Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. *Front. Microbiol.* 10, 193
12. Miller, E.A. *et al.* (2016) Lactobacilli dominance and vaginal pH: why is the human vaginal microbiome unique? *Front. Microbiol.* 7, 1936
13. Winston-McPherson, G. *et al.* (2019) The vaginal microbiome of transgender men. *Clin. Chem.* 65, 199–207
14. Knudtson, J.M. and McLaughlin, J.E. (2019) *Female Reproductive Endocrinology*, MSD Manual Professional Edition
15. Dominianni, C. *et al.* (2015) Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PLoS One* 10, e0124599
16. Mueller, S. *et al.* (2006) Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl. Environ. Microbiol.* 72, 1027–1033
17. Acharya, K.D. *et al.* (2019) Estradiol and high fat diet associate with changes in gut microbiota in female ob/ob mice. *Sci. Rep.* 9, 20192
18. Kaliannan, K. *et al.* (2018) Estrogen-mediated gut microbiome alterations influence sexual dimorphism in metabolic syndrome in mice. *Microbiome* 6, 205
19. Markle, J.G. *et al.* (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339, 1084–1088
20. Sinha, T. *et al.* (2019) Analysis of 1135 gut metagenomes identifies sex-specific resistome profiles. *Gut Microbes* 10, 358–366
21. Mulak, A. *et al.* (2014) Sex hormones in the modulation of irritable bowel syndrome. *World J. Gastroenterol.* 20, 2433–2448
22. Boskey, E.R. *et al.* (2001) Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum. Reprod.* 16, 1809–1813
23. O'Hanlon, D.E. *et al.* (2019) Vaginal pH measured in vivo: lactobacilli determine pH and lactic acid concentration. *BMC Microbiol.* 19, 13
24. Anahtar, M.N. *et al.* (2015) Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity* 42, 965–976
25. Gajer, P. *et al.* (2012) Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.* 4, 132ra52
26. Sobel, J.D. *et al.* (2019) Conventional oral and secondary high dose vaginal metronidazole therapy for recurrent bacterial vaginosis: clinical outcomes, impacts of sex and menses. *Infect. Drug Resist.* 12, 2297–2307
27. Song, S.D. *et al.* (2020) Daily vaginal microbiota fluctuations associated with natural hormonal cycle, contraceptives, diet, and exercise. *mSphere* 5, e00593-20
28. Noyes, N. *et al.* (2018) Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS One* 13, e0191625
29. Bradshaw, C.S. *et al.* (2013) Recurrence of bacterial vaginosis is significantly associated with posttreatment sexual activities and hormonal contraceptive use. *Clin. Infect. Dis.* 56, 777–786
30. Escobar-Morreale, H.F. (2018) Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat. Rev. Endocrinol.* 14, 270–284
31. Boyle, J.A. and Teede, H.J. (2016) Refining diagnostic features in PCOS to optimize health outcomes. *Nat. Rev. Endocrinol.* 12, 630–631
32. Qi, X. *et al.* (2019) Gut microbiota–bile acid–interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat. Med.* 25, 1225–1233
33. Torres, P.J. *et al.* (2018) Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J. Clin. Endocrinol. Metab.* 103, 1502–1511
34. Donohoe, D.R. *et al.* (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* 13, 517–526
35. Corrêa-Oliveira, R. *et al.* (2016) Regulation of immune cell function by short-chain fatty acids. *Clin. Transl. Immunol.* 5, e73
36. Parada Venegas, D. *et al.* (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* 10, 277
37. Hong, X. *et al.* (2020) Association between polycystic ovary syndrome and the vaginal microbiome: a case–control study. *Clin. Endocrinol.* 93, 52–60
38. Kauffman, A.S. *et al.* (2015) A novel letrozole model recapitulates both the reproductive and metabolic phenotypes of polycystic ovary syndrome in female mice. *Biol. Reprod.* 93, 69
39. Kelley, S.T. *et al.* (2016) The gut microbiome is altered in a letrozole-induced mouse model of polycystic ovary syndrome. *PLoS One* 11, e0146509
40. Torres, P.J. *et al.* (2019) Exposure to a healthy gut microbiome protects against reproductive and metabolic dysregulation in a PCOS mouse model. *Endocrinology* 160, 1193–1204
41. Onderdonk, A.B. *et al.* (2016) The human microbiome during bacterial vaginosis. *Clin. Microbiol. Rev.* 29, 223–238
42. Ravel, J. *et al.* (2011) Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4680–4687
43. Peebles, K. *et al.* (2019) High global burden and costs of bacterial vaginosis: a systematic review and meta-analysis. *Sex. Transm. Dis.* 46, 304–311
44. Crucitti, T. *et al.* (2018) Contraceptive rings promote vaginal lactobacilli in a high bacterial vaginosis prevalence population: a randomised, open-label longitudinal study in Rwandan women. *PLoS One* 13, e0201003
45. Vodstrcil, L.A. *et al.* (2019) Combined oral contraceptive pill-exposure alone does not reduce the risk of bacterial vaginosis recurrence in a pilot randomised controlled trial. *Sci. Rep.* 9, 3555
46. Yang, L. *et al.* (2019) Differential effects of depot medroxyprogesterone acetate administration on vaginal microbiome in Hispanic White and Black women. *Emerg. Microbes Infect.* 8, 197–210
47. Jaggar, M. *et al.* (2020) You've got male: sex and the microbiota–gut–brain axis across the lifespan. *Front. Neuroendocrinol.* 56, 100815
48. Altemus, M. *et al.* (2014) Sex differences in anxiety and depression clinical perspectives. *Front. Neuroendocrinol.* 35, 320–330
49. Maeng, L.Y. and Milad, M.R. (2015) Sex differences in anxiety disorders: interactions between fear, stress, and gonadal hormones. *Horm. Behav.* 76, 106–117
50. Lete, I. and Lapuente, O. (2016) Contraceptive options for women with premenstrual dysphoric disorder: current insights and a narrative review. *Open Access J. Contracept.* 7, 117–125
51. Scholl, J.L. *et al.* (2019) Sex differences in anxiety-like behaviors in rats. *Physiol. Behav.* 211, 112670
52. Heck, A.L. and Handa, R.J. (2019) Sex differences in the hypothalamic–pituitary–adrenal axis' response to stress: an important role for gonadal hormones. *Neuropsychopharmacology* 44, 45–58
53. Foster, J.A. *et al.* (2017) Stress & the gut–brain axis: regulation by the microbiome. *Neurobiol. Stress* 7, 124–136

54. Jašarević, E. *et al.* (2016) Sex differences in the gut microbiome–brain axis across the lifespan. *Philos. Trans. R. Soc. B Biol. Sci.* 371, 20150122
55. Neufeld, K.M. *et al.* (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol. Motil.* 23, 255–e119
56. Davis, D.J. *et al.* (2017) Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. *Brain Behav. Immun.* 59, 38–48
57. Tal, R. *et al.* (2015) *Endocrinology of Pregnancy*, Endotext
58. Schock, H. *et al.* (2016) Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. *BMC Pregnancy Childbirth* 16, 146
59. Hendrick, V. *et al.* (1998) Hormonal changes in the postpartum and implications for postpartum depression. *Psychosomatics* 39, 93–101
60. Koren, O. *et al.* (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 150, 470–480
61. Nuriel-Ohayon, M. *et al.* (2019) Progesterone increases *Bifidobacterium* relative abundance during late pregnancy. *Cell Rep.* 27, 730–736
62. Zhu, Y. and Zhang, C. (2016) Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. *Curr. Diab. Rep.* 16, 7
63. Kuang, Y.-S. *et al.* (2017) Connections between the human gut microbiome and gestational diabetes mellitus. *GigaScience* 6, 1–12
64. Crusell, M.K.W. *et al.* (2018) Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. *Microbiome* 6, 89
65. Walther-Antonio, M.R. *et al.* (2014) Pregnancy's stronghold on the vaginal microbiome. *PLoS One* 9, e98514
66. Romero, R. *et al.* (2014) The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2, 4
67. Freitas, A.C. *et al.* (2017) The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci. Rep.* 7, 9212
68. Chen, Y. *et al.* (2019) Association between the vaginal microbiome and high-risk human papillomavirus infection in pregnant Chinese women. *BMC Infect. Dis.* 19, 677
69. MacIntyre, D.A. *et al.* (2015) The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci. Rep.* 5, 8988
70. Pelzer, E.S. *et al.* (2018) The fallopian tube microbiome: implications for reproductive health. *Oncotarget* 9, 21541–21551
71. Perez-Muñoz, M.E. *et al.* (2017) A critical assessment of the 'sterile womb' and 'in utero colonization' hypotheses: implications for research on the pioneer infant microbiome. *Microbiome* 5, 48
72. Centers for Disease Control and Prevention (2018) *Assisted Reproductive Technology (ART): Fertility Clinic Success Rates Report*, CDC
73. Schoenmakers, S. and Laven, J. (2020) The vaginal microbiome as a tool to predict IVF success. *Curr. Opin. Obstet. Gynecol.* 32, 169–178
74. Wee, B.A. *et al.* (2018) A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Aust. N. Z. J. Obstet. Gynaecol.* 58, 341–348
75. Haahr, T. *et al.* (2019) Vaginal microbiota and in vitro fertilization outcomes: development of a simple diagnostic tool to predict patients at risk of a poor reproductive outcome. *J. Infect. Dis.* 219, 1809–1817
76. Campisciano, G. *et al.* (2017) Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. *J. Cell. Physiol.* 232, 1681–1688
77. Martin, J.A. *et al.* (2019) Births: final data for 2018. *Natl. Vital Stat. Rep.* 68, 1–47
78. Baldwin, E.A. *et al.* (2015) Persistent microbial dysbiosis in preterm premature rupture of membranes from onset until delivery. *PeerJ* 3, e1398
79. Kindinger, L.M. *et al.* (2017) The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. *Microbiome* 5, 6
80. DiGiulio, D.B. *et al.* (2010) Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *Am. J. Reprod. Immunol.* 64, 38–57
81. Foster, J.A. and Neufeld, K-A.M. (2013) Gut–brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* 36, 305–312
82. Murphy, J.R. *et al.* (2018) Maternal peripartum antibiotic exposure and the risk of postpartum depression. *Res. Nurs. Health* 41, 369–377
83. Mehta, D. *et al.* (2014) Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. *Psychol. Med.* 44, 2309
84. Payne, J.L. and Maguire, J. (2019) Pathophysiological mechanisms implicated in postpartum depression. *Front. Neuroendocrinol.* 52, 165–180
85. Xiao, S. *et al.* (2017) A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. *Nat. Commun.* 8, 14584
86. Ferraz, M.A.M.M. *et al.* (2018) An oviduct-on-a-chip provides an enhanced in vitro environment for zygote genome reprogramming. *Nat. Commun.* 9, 4934
87. Wang, C. *et al.* (2021) A novel standalone microfluidic device for local control of oxygen tension for intestinal-bacteria interactions. *FASEB J.* 35, e21291
88. Burger, H.G. *et al.* (2007) A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. *Hum. Reprod. Update* 13, 559–565
89. Yasui, T. *et al.* (2012) Androgen in postmenopausal women. *J. Med. Investig.* 59, 12–27
90. Karim, R. *et al.* (2015) Association of endogenous sex hormones with adipokines and ghrelin in postmenopausal women. *J. Clin. Endocrinol. Metab.* 100, 508–515
91. Santos-Marcos, J.A. *et al.* (2018) Influence of gender and menopausal status on gut microbiota. *Maturitas* 116, 43–53
92. Koliada, A. *et al.* (2017) Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol.* 17, 120
93. LeBlanc, J.G. *et al.* (2017) Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Factories* 16, 79
94. Shen, J. *et al.* (2016) Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. *Sci. Rep.* 6, 24380
95. Muhleisen, A.L. and Herbst-Kralovetz, M.M. (2016) Menopause and the vaginal microbiome. *Maturitas* 91, 42–50
96. Mitchell, C.M. *et al.* (2017) Vaginal microbiota and genitourinary menopausal symptoms: a cross-sectional analysis. *Menopause (New York, N.Y.)* 24, 1160–1166
97. Key, T.J. and Pike, M.C. (1988) The dose-effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br. J. Cancer* 57, 205–212
98. Chlebowski, R.T. *et al.* (2015) Continuous combined estrogen plus progestin and endometrial cancer: the women's health initiative randomized trial. *J. Natl. Cancer Inst.* 108, djv350
99. Lukanova, A. *et al.* (2004) Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. *Int. J. Cancer* 108, 425–432
100. Walsh, D.M. *et al.* (2019) Postmenopause as a key factor in the composition of the endometrial cancer microbiome (ECbiome). *Sci. Rep.* 9, 19213
101. Greendale, G.A. *et al.* (2019) Changes in body composition and weight during the menopause transition. *JCI Insight* 4, e124865
102. Mauvais-Jarvis, F. *et al.* (2017) Menopausal hormone therapy and type 2 diabetes prevention: evidence, mechanisms, and clinical implications. *Endocr. Rev.* 38, 173–188
103. Barreto, F.M. *et al.* (2014) Beneficial effects of *Lactobacillus plantarum* on glycemia and homocysteine levels in postmenopausal women with metabolic syndrome. *Nutrition* 30, 939–942
104. Brahe, L.K. *et al.* (2015) Dietary modulation of the gut microbiota – a randomised controlled trial in obese postmenopausal women. *Br. J. Nutr.* 114, 406–417

105. Tan, J. *et al.* (2014) The role of short-chain fatty acids in health and disease. *Adv. Immunol.* 121, 91–119
106. Riecher-Rössler, A. (2020) Menopause and mental health. In *Mental Health and Illness of Women* (Chandra, P. *et al.*, eds), pp. 147–173, Springer
107. Mulhall, S. *et al.* (2018) Variation in symptoms of depression and anxiety in midlife women by menopausal status. *Maturitas* 108, 7–12
108. Wada, T. *et al.* (2018) Impact of central and peripheral estrogen treatment on anxiety and depression phenotypes in a mouse model of postmenopausal obesity. *PLoS One* 13, e0209859
109. Sovijit, W.N. *et al.* (2019) Ovarian progesterone suppresses depression and anxiety-like behaviors by increasing the *Lactobacillus* population of gut microbiota in ovariectomized mice. *Neurosci. Res.* Published online April 22, 2019. https://doi.org/10.1016/j.neures.2019.04.005