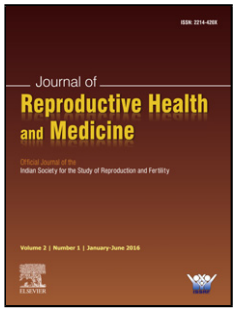

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Female reproductive tract microbiome in gynecological health and problems

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Abstract

Microbes are intimately associated with human existence and health. Gut, oral cavity, skin, respiratory and urinogenical tracts are the major body sites inhabited by large consortia of microorganisms; each with specific composition. Application of high throughput genomic technologies has paved ways to gain an improved knowledge about the composition of the resident microbes and the ecosystem homeostasis and underscores the concept that dysbiosis of the flora may lead to predisposition to infection and diseases. Successful human reproduction

owes an immense debt to this microbial community. Microbial communities exist throughout the entire length of the female reproductive tract at variable composition and density and play a role in gametogenesis, reproductive cyclicity, pregnancy and successful delivery of newborns. This review focuses on the recent studies from all over the globe on the composition of microflora in the female reproductive tract, their spatio-temporal diversity across the age of women and how the host-microbe collaboration is pursued to maintain reproductive efficiency. A special emphasis has been placed on the disruption of the stable flora and it's association with the microbial imbalance and infections in bacterial vaginosis, endometriosis and pre-term birth. Finally, this article highlights that the restoration of normal microbial flora might provide a long-term therapeutic measure for the reproductive failures and endow with solutions to the global problem of reproductive failure, preterm birth and neonatal deaths.

Keywords

Bacterial vaginosis; Human microbiome; Preterm birth; Probiotics; Spontaneous abortion

1. Introduction

The extra-ordinary power of genomic technologies has convincingly demonstrated that under natural conditions, microbes do occur in a "consortium" where the interactions among different members cumulatively result in a system-level community behavior which is more than a mere addition of their activities together [1]. Presence of an incompatible member or intrusion by a pathogen can disturb the compactness of the organization and bring about a loss of homeostasis. Microbe-microbe interaction includes physical contact, chemical and metabolic exchanges [2]. Division of labor among the microbes is another attribute of the consortia which maintains the well coordinated functioning of the system. The consortium has an advantage over a single

species in regard to executing a complex function and achieving endurance against the consistently shifting milieu [3]. Studies have also shown that in comparison to monocultures, a community is better suited to resist invasion by other organisms and combat different kinds of stresses [4]. A vast spectrum of research is thus ongoing to uncover the constitution and basis of microbial ecosystem in a wide variety of environmental and biological systems.

The human body is a home to an extraordinarily diverse population of microbes. As unearthed by Human microbiome projects, there exists intriguing patterns of microbial coexistence, breaching of which leads to diseases in many cases. As early as in 17th century, Antonie van Leeuwenhoek first described that the human body harbored bacteria [5]. Today we know that the microbial genes in the human gut alone (3.3 million) outnumber the protein-coding genes (20,000-50,000) of the human genome [6, 7]. Humans have co-evolved with trillions of microorganisms that inhabit the body in a complex habitat-specific manner and are attuned to factors related to host physiology, age, diet, environmental conditions and the history of exposure to microbes [8-10]. Due to its immense impact on host metabolism and immune system, human metagenome is often regarded as the "second genome" [11-13]. Microbial populations differ markedly between different anatomical sites such as skin, oral cavity, gastrointestinal (GI), respiratory and urogenital tracts and within the various micro-niche of a given site. Besides, they regularly show a large degree of interpersonal variation even in the absence of any diseases [14]. It is thus difficult to define a "healthy core human microbiome" [15]. Despite this ambiguity, an overall alteration in the microbial profile of an individual has been well linked with a number of pathogenic states including cardiovascular diseases, type 2 diabetes, obesity and metabolic disorders and infertility [11, 16-21]. The association of microbes with various reproductive complications has been suspected even before the first success of *in vitro* fertilization. However, due to paucity of data, this

hypothesis remained unexplained till the characterization of microbiome from the reproductive tract.

Almost all facets of human reproduction from gametogenesis, to fertilization and embryo migration, to implantation with implications in early pregnancy failure or loss, and poor obstetric outcomes during gestation and parturition in terms of intrauterine infection and preterm birth are affected by the resident microbiota [11]. Researches focusing on microbial communities that inhabit along the male and female reproductive tracts and exhibit symbiotic, mutualistic and pathogenic relations with the host have been intensified [22, 23]. We present herein a synthesis of research findings deposited over the last one decade on female reproductive tract microbiota, native and dysbiotic, to help the readers ask questions that are worthy of exploration in order to solve various complications related to female reproductive health.

1. Indigenous microbiota in female reproductive system

Urogenital tract microbiome makes up 9% of the total human microbiome [23]. Colonization of microbes in the human body commences after the delivery when new born comes in contact with the maternal womb, vaginal, fecal and skin microbes [24, 25]. The main force that triggers the postnatal immunity is derived from this colonization [26]. Certain microbial species (*Escherichia coli*, *Escherichia fecalis* and *Staphylococcus epidermidis*) have been isolated from the meconium of healthy neonates born to healthy mothers within two hours of delivery indicating that the transfer of bacteria from the maternal body initiates through the amniotic fluid to the fetal circulation during gestation [27]. With the advent of genomic technologies, myriad of non-*Lactobacillus sp*. have been identified with definitive roles in maintaining reproductive fitness, in addition to *Lactobacillus sp.* which is historically regarded as the species of dominance in female urinogenital tract [28].

1. Microflora of vagina

Vaginal microenvironment is a dynamic ecosystem with major capability of maintaining a healthy reproductive environment [29]. It is affected by a number of endogenous (e.g. age, physiology, body size) and exogenous (e.g. mating behavior, substrate use and routine-association with microbes) factors. About 250 bacterial taxonomic units have been identified from the vagina of women of various ages, health status and countries of origin [30]. Microbes mainly reside in the vaginal stratum corneum, the cornified epithelium that forms loose glycogen-filled cells without nuclei and thereby fail to recognize the foreign pathogens [31].

Recent next-generation sequencing and metagenomic analyses have revealed that the vaginal microbiome harbors a high proportion of *Firmicutes* and a low percentage of *Proteobacteria*, *Bacteroidetes*, *Fusobacteria* and *Actinobacteria*. Vaginal microbiota belong to seven community types (I-VII) of which majority (types I, II, III and V) predominated by one or more species of *Lactobacillus.* Frequently detected members include *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners* and *Lactobacillus jensenii*. A prevalence of *Lactobacilli* was identified in a cohort of asymptotic North American women from Asian, African, Hispanic and Caucasian ancestries. Four of five clusters of microbiota observed in this study were dominated by *Lactobacillus sp*. whereas the fifth group comprised a higher proportion of obligate anaerobes [32]. A study from Indianapolis, USA, reported that *Lactobacillus sp.* formed a major phylotype in the vagina of 70% of the adolescent girls even before the onset of menarche [33]. A predominance of lactic acid producing bacteria such as *Lactobacillus*, *Streptococcus*, *Aerococcus* and *Facklamia* was also detected in the girls of age thirteen to eighteen from Uganda [34]. According to another study, women of European ancestry harbored a *Lactobacillus*-dominated microbiome while African-American women exhibited a diverse microbial profile [35]. Several studies from India and China demonstrated different strains of *Lactobacillus sp.* cohabit the vagina of the adult women of reproductive age while post-menopausal women formed a consortium composed

mainly of *Escherichia coli*, *Streptococcus sp*., *Prevotella sp*., *Bacteroides fragilis* and lactic acid producing *Veillonella sp*. and *Anaerococcus lactolyticus* in addition to *Lactobacilli* [36-38]. In 20-40% of the women who lacked vaginal *Lactobacillus,* variable proportions of *Acinetobacter*, *Acidovorax*, *Anaerococcus*, *Atopobium*, *Anaerococcus*, *Cloacibacterium, Coriobacter*, *Corynebacterium*, *Diaphorobacter, Eggerthella*, *Finegoldia*, *Gardnerella*, *Megasphaera, Mobiluncus*, *Peptoniphilus*, *Peptostreptococcus, Prevotella*, *Sneathia*, *Staphylococcus*, *Streptococ*cus, *Ureaplasma*, *Veillonella* were detected [39]. This group of non-*Lactobacillus sp*. influences the vaginal health through the production of short-chain fatty acids (SCFA) and other low molecular weight compounds [29]. Interestingly, there is a remarkable difference in the genetic and metabolic potentials of the vaginal and non-vaginal *Lactobacilli* in humans [30]. Vaginal species have small genomes, high GC content and produce many differentially induced proteins in comparison to their non-vaginal counterparts [40].

Among the vaginal *Lactobacilli*, *Lactobacillus crispatus* and *Lactobacillus iners* are the main producers of *d*-lactic acid and responsible for maintaining an acidic environment (pH – 4.5-5.0) [30, 32]. Estrogen has a role in *Lactobacillus* dominance. It converts columnar epithelium into a thick layer of squamous stratified epithelium and increases the glycogen content for the growth of *Lactobacilli*. Maternal estrogen transferred to newborn increases the availability of glycogen in the vagina of an infant which is metabolized to lactic acid by *Lactobacillus sp.,* within one day of birth. In addition to *Lactobacilli*, *Corynebacteria, Staphylococci, Streptococci* and *Escherichia coli* are capable of degrading glycogen to lactic acid in the newborn within two hours of delivery [41]. A sharp contrast in the vaginal flora is noted between babies delivered through vagina, who inherit microbes from maternal vagina, and the caesarean baby whose microbial composition resembles that observed in the adult skin. Although the initial colonization by microbes in the vagina of an individual depends on the mode of her birth,

microbial composition is eventually regulated by the level of estrogen which varies as a function of her age. Vaginal pH gradually decreases as the level of estrogen rises from puberty and this low pH is maintained throughout the reproductive age along with abundance of *Lactobacillus sp.* This correlation was reflected even in small time scale of menstrual cycles when the population too undergoes a temporal variation [42]. Due to lower dominance of *Lactobacillus sp.,* juvenile and the pre-menarchal vagina are, in general, neutral or alkaline. Acidic pH prevents the growth of various pathogens including Human Immunodeficiency Virus, yeast, *Neisseria gonorrhoeae*, *Atopobium, Megasphaera, Mobiluncus, Prevotella, Sneathia* and *Gardnerella vaginalis* (*G. vaginalis*), the latter being the causative agent of bacterial vaginosis (BV) and help maintaining host-fitness for the reproductive ability [43-47]. *Lactobacillus sp.* also produces a number of antimicrobial compounds including hydrogen peroxide, lactic acid, acidocin and gassericin (by *Lactobacillus gasseri*) that prevent pathogen-colonization [48]. Decline of *Lactobacillus sp.* after menopause poses a risk for contacting with sexually transmitted (STD) and other endocervical and pelvic inflammatory diseases [PID] [49]. In comparison to non-pregnant women, the pregnant women bear a rich and stable community of *Lactobacillus* species [50]. Diversity and population density of microbial population in different sites of vagina also varies. Diversity is usually high in the proximal third of the vaginal tract (e.g., fornix) compared to other regions, indicating the importance of the sampling sites while defining the comprehensive vaginal microbiome [39]. Nonetheless, an overall commonality in the composition of vaginal microbiome could still be detected irrespective of ethnicities. This variation may be attributed to the difference in hormonal levels and other endogenous factors and disparity in the sexual practices and lifestyle [51]. The importance of vaginal *Lactobacilli* has been summarized in Box 1.

1. Moving beyond vagina

Vulvar microbiota showed closeness to that present in vagina. However there are few additional taxa such as *Fusobacterium*, *Murdochiella* and *Segniliparus*, which together accounts for 1.0 to 3.5% of total microbe population in the vulva. Endometrium and upper endocervix have been evaluated for the presence of bacterial species. The abundance of bacteria is lower in upper genital tract compared to that in vagina. *Bacteroidetes* (*Bacteroides xylanisolvens*, *Bacteroides thetaiotaomicron*, *Bacteroides thetaiotaomicron* and *Bacteroides fragilis)* was reported to be a ubiquitous component of microbiome in the uterine endometrium of non-pregnant women of Caucasian ancestry. *Pelomonas* of class *Betaproteobacteria* was another significant member. Other phylagenera mostly include *Lactobacillus iners* (45% UGT, 61% vagina), *Prevotella sp.* (33% UGT, 76% vagina) and *Lactobacillus crispatus* (33% UGT, 56% vagina) [52]. Endometrial community of only 1% of the women showed predominance of *Lactobacillus crispatus* and/or *Lactobacillus iners* in the *Bacteroidetes* core. Two endometrial communities that lacked a *Bacteroidetes* core was dominated by *Lactobacillus crispatus* and cohabited by a high diverse consortium made of *Prevotella sp*., *Atopobium vaginae* and *Mobiluncus curtisii* [53]. Vertical ascend of microorganisms from vagina leads to the colonization of microbes in the uterus. The haematogenous dissemination is also believed to be a process of transfer of bacteria from the oral cavity and GI and respiratory tracts to the uterus and ovarian follicles [54]. However, data clarifying this hypothesis are still not unequivocal [55, 56]. Placenta appears to harbor a low biomass microbiome composed of nonpathogenic commensal phyla such as *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes* and *Fusobacteria*. 16S-based operational taxonomic unit (OTU) analyses revealed associations of the placental microbiome with a remote history of antenatal infection such as urinary tract infection in the first trimester and PTB [57].

1. Microbial dysbiosis and reproductive health problems

Microbial dysbiosis is often regarded as one of the major factors leading to various types of reproductive diseases and conditions. Dysbiosis is referred to as an alteration of the normal microbiota (bacterial or fungal species) due to the exposure to disruptive factors such as antibiotics, chronic disease, stress, medical procedures or medications [58]. According to World Health Organization (WHO) infertility is regarded as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse." In women, about one quarter of infertility is caused by a problem with ovulation. Other probable reasons include hormonal imbalance due to overactive or underactive hypothalamus, pituitary or thyroid gland or due to the prevalence of diabetes and obesity, polycistic ovary syndrome (PCOS), tubal blockages and endometriosis. Microbial dysbiosis particularly in the vagina is also associated with infertility and PTB [59, 60]. Every year, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation). Across 184 countries, the rate of PTB ranges between 5-18% of babies born. It is more prevalent in countries with low resource setting such as those in Africa and South Asia (WHO, 2015). According to an estimate of 2010, India has the highest record (3,51,9100) of PTB [61]. Recurrent spontaneous abortions (RSAs) have also been associated with chronic or recurrent maternal infections at a rate lower than 4% [62]. An abridged account of cause and effect of bacterial dysbiosis in female gynecological problems has been presented in Box 2.

1. Bacterial vaginosis

Vaginal microbial dysbiosis due to displacement of beneficial *Lactobacilli* by a population of gram negative anaerobic bacteria is the major signature of BV pathogenesis [63, 64]. Outcome of BV includes a wide range of clinical complications including upper reproductive tract infections resulting in abortion and PTB [65, 66]. A number of studies have compared the vaginal microbial population between healthy and women with bacterial vaginosis. In a study conducted in a

Belgian cohort *Lactobacillus crispatus* in the healthy women was replaced by *Prevotella sp.*, *Atopobium sp*. and *Mycoplasma hominis* in the affected group [67]. The difference in the composition of the microbiome was reflected in the metabolic profile of the vaginal fluids between the groups. A high proportion of maltose and kynurenine, the breakdown products of L-tryptophan, was presumed to lead to the accumulation of nicotinamide adenine dinucleotide (NAD+) in the healthy women. On the contrary, SCFA and organic acids including acetate, malonate and nicotinate arising due to anaerobic lifecycle of the flora were identified in the patients suffering from BV [40]. Low level of NAD+ in the vagina of BV patients might be attributed to an increased rate of glycolysis due to anaerobic respiration. SCFAs were presumed to be associated with higher immune activity in the reproductive tract of the BV-infected patients [67, 68]. One common feature observed in patients' suffering from BV is that *Lactobacillus* dominance in the type IV community was replaced by *G. vaginalis*. The ecological relationship of *Lactobacillus* and *Gardnerella* investigated in germ-free mice was remarkably intriguing. Estrogen was detected to be a necessary factor for colonization of *Lactobacillus sp*. Pretreatment of the mice with *Lactobacillus* caused a tenfold decrease of the pathogen in the vagina with concomitant diminution of histological lesion compared to mice with monoassociated pathogen. Interestingly, presence of *G. vaginalis* seemed to provide a synergistic effect for growth of *Lactobacillus johnsonii*; as vaginal colonization by latter was also obtained without hormonal treatment when the mice were challenged with *G.* vaginalis [69].

1. Pre-term birth

Presence of a stable microbiome throughout the pregnancy is thought to protect the fetus [70]. As pregnancy advances, the vaginal microbial diversity is reduced. PTB is considered to be a polymicrobial disease. The proportion of bacteria colonizing in the amniotic fluid from the women who delivered pre-term babies was observed to vary from 15-50%. BV due to the

absence of *Lactobacilli* has been associated with PTB and late miscarriage between 25 and 35 weeks. BV was found to account for approximately 80% of the attributable risk for PTB in a large Indian cohort [71]. In general a disturbed *Lactobacillus* population in the vagina along with an abundance of anaerobes such as *Gardnerella sp.* and urease-producing *Ureaplasma sp.* were associated with PTB. A number of studies have also indicated an association of another *Mollicute* viz *Mycoplasma genitalium* with PTB [72]. At what point an aberrant vaginal microbiome composition specifically induces the cascade leading to PTB still remains elusive. It has been demonstrated in a non-human primate model (monkey) that loss of cytoskeletal structures within the amniotic epithelium due to Group B *Streptococcus* (GBS) infection results in weakening of chorioamnion leading to PTB [73]. PTB and preterm pre-labor rupture of membranes (PPROM) has been associated with an early gestational colonization of *Chlamydia trachomatis* in the genital tract [74]. Activation of pro-inflammatory chemokines and cytokines (IL6, IL8, IL-1β, TNF-α) and monocyte chemotactic protein-1 caused by dysbiosis and infection might lead to the inflammation related to PTB [75]. Activation of matrix metalloproteinases and hyaluronidases by the microbes leading to the breakdown of collagen layer from the cervical epithelium has been identified as one of the causes of PTB [76]. The contamination of microbes from extra-reproductive organs like oral cavity might is another possibility as the rate of PTB is increased two to seven fold in the women with periodontal disease. The microbial shifts in the subgingival biofilm during pregnancy lead to the increase in the pathogenic bacteria, with subsequent transfer of the pathogens and their metabolites in the extra-oral sites [77, 78]. A reduction of bacterial diversity in the placenta due to the displacement of *Bifidobacterium* and *Lactobacillus* by *Proteobacteria* is anobserved in other feature of PTB [79]. However, clinical significance of placental disturbances in normal parturition or PTB is still an open question.

Majority of these findings, nevertheless, are correlative in nature. Establishing of causality is an urgent priority, or else the diagnosis and treatment of PTB will remain a distant goal.

1. Endometriosis

Endometriosis is characterized by the outgrowth of the uterine tissue (endometrium) outside the uterus such as in ovaries, bowel and pelvic tissues. The outcomes are infertility and chronic pelvic pain among 5–15% of women of reproductive age. Although considered as a benign disease; there are marked commonalities between the features of endometriosis and that of malignant progression with respect to histopathological and molecular attributes as well as genetic susceptibility, immune and angiogenic dysregulation. A genome-wide expression analysis of autologous, paired eutopic and ectopic endometrial tissues from fertile women with various stages of endometriosis revealed a dysfunctional expression of immuno-neuro-endocrine behavior in the endometrium [80]. The pathognomonic characteristics in women with stage IV ovarian endometriosis showed marked indication of neoplastic potential [81]. The etiology of endometriosis is nevertheless complex. The activation of innate immune system due to microbial infection/disruption of normal flora leading to inflammation is also believed to play a role. Infection in the eutopic endometrium leads to activation of TLRs through the pathogen associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). This initial inflammatory response eventually triggers iron-induced oxidative stress and sterile inflammation leading to increased cellular proliferation and/or attenuated apoptosis that culminate into outgrowth of endometrium [82]. Keeping with this, the women with stage III-IV endometriosis have been reported to have higher rates of tubo-ovarian abscess (TOA), a polymicrobial PID in which *E. coli*, *Neisseria gonorrhea*, *Chlamydia trachomatis* and other obligate anaerobic bacteria are reported to be associated [83]. A significantly higher colonization of *Gardnerella sp*., α-*Streptococcus sp*., *Staphylococaceae* and *Enterococci sp*. with concomitant

decline in *Lactobacillus sp.* were also noticed in the endometrial smears of women diagnosed with endometriosis. Other bacterial species remarkably associated with endometriosis include *Actinomyces sp.*, *Fusobacterium sp.*, *Staphylococcus sp.*, *Propionibacterium sp.*, *Prevotella sp*., and *Corynebacterium sp*. [84].

Ectopic pregnancy and spontaneous abortion

Ectopic pregnancy (EP) is a major female reproductive problem in the developing countries resulting in much morbidity and mortality. Though the exact etiology is still unclear, a relation of EP with PID is quite common. PID has an associated odds ratio of 7.5 with EP while one-third of EP is associated with PID [85]. *Chlamydia* infection in the fallopian tissues is another risk factor for EP. It is postulated that *Chlamydia* infections may reduce the activity of the cilia in the epithelium of the tubes and subsequently the contraction of the smooth muscles, resulting in EP [85]. Recently, application of PCR has identified presence of *Ureaplasma urealyticum* and *Mycoplasma hominis* in addition to *Chlamydia trachomatis* from menstrual tissue samples of patients with EP [86]. In this regard, PCR seems to be a more efficient technique compared to the culture/direct fluorescence antibody (DFA) for the early detection of infections in gynecological problems such as tubal obstruction, pelvic inflammatory disease, ectopic pregnancy, spontaneous abortions and unexplained infertility.

On the contrary, the association between *Chlamydia* with spontaneous abortion is well documented [87]. The disruption of *Lactobacillus sp.* during BV and presence of *Ureaplasma urealyticum* in the genital tract in combination with other microorganisms have also been found to provoke spontaneous abortion and cervical incompetence [88]. Release of proteases due to invasion of *Chlamydia* into the choriodecidual space and subsequent arousal of placental immune response and inflammation (chorioamnionitis) are major factors responsible for premature rupture of the membranes, activation of Arachidonic acid pathway and uterine

contractions in spontaneous abortion [74]. In primates (macaques) *Listeria monocytogenes* was identified to be an endemic agent for abortion. Future studies are, however, necessary to establish that *Listeria monocytogenes* which infect the giant trophoblasts of the placenta results in infection-associated abortion [89]. *Toxoplasma gondii* is an obligate intracellular parasite which may cross the placental barrier and cause spontaneous abortion and preterm labor. A characteristic protein-signature particularly associated with trophoblast invasion and placental development has been identified when placental proteome of *T. gondii*-infected mice was compared with that of uninfected ones [90]. An infection by *Helicobacter pylori* was also reported in implantation/placental failure due to a cross-reaction between placental tissue and the antibodies against the bacteria [91]. Zika virus (ZIKV) infection in pregnant women has recently been identified to cause intrauterine growth restriction, spontaneous abortion, and microcephaly [92]. However the composition and function of microbiome in the genital tract of women with abnormal pregnancy outcomes and spontaneous abortions are still elusive indicating the urgent necessity of further powerful studies.

1. Restoration of the healthy microbial population

The basis of restoration of healthy homeostasis in an individual actually depends on the definition of a "core microbiome" [93]. The identification of a microbiome signature based on the ubiquity and the abundance of 16S rDNA profiles demonstrated the "vaginal microbial strata" to be the most stable ecosystem in all the body niches across populations [94]. Therefore, irrespective of the minor inter and/or –intra variations which might be due to hormonal status of the host, restoration of vaginal microbiome after a disease pathogenesis should not be a distant possibility.

Oral or intravaginal antibiotic is a routine treatment of BV which often relapses and fails to offer a long-term defensive barrier. Application of "probiotics" and cationic antimicrobial peptides

(AMPs) is gaining popularity as an alternative strategy of treatment [95-97]. Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit to the host. Oral or intra-vaginal administration of different species of *Lactobacillus* has shown to increase the number of vaginal *Lactobacilli* which presumably provides a mechanical barrier against *Gardnerella vaginalis* and prevent the adhesion of the pathogens to the vaginal epithelium [98]. A comparison of vaginal microflora from two groups of patients, one treated with probiotics and other with antibiotics, showed that the latter reduce the pathogenic flora while probiotics suppressed the pathogens with subsequent re-establishment of vaginal homeostasis steadily following a prolonged use [99]. Microbiota of neonates delivered by cesarean section (C-section) differs from those delivered vaginally. These neonates are more prone to immune and metabolic disorders. As a remedial strategy, infants delivered by C-section were exposed to maternal vaginal fluids at birth which resulted in partial restoration of microbiota in the C-section delivered babies [100]. However, this practice suffers lack of consensus particularly due to the fact that exposure to maternal vaginal fluids may potentially be associated with risk of infection in the newborns. Furthermore, the association between the mode of delivery and the diversity and colonization pattern of gut microbiota in the infants is only short-lived [101].

Neonates delivered preterm often suffer from different levels of necrotizing enterocolitis (NEC), sepsis, organ immaturity and an abnormal settlement of gut microbiota which may be life threatening or translate into life-long conditions. A predominance of pathogenic organisms and lack of microbial diversity in the GI tract are the key pathological features of NEC in the preterm infants [102]. Probiotics is popularly used for the treatment of these preterm neonates. Prophylactic supplementation of *Saccharomyces boulardii* CNCM I-745 (*S. boulardii*) or *Bifidobacterium breve* M-16V have been found to improve weight gain, improved feeding tolerance in preterm infants with prevent necrotizing enterocolitis without any adverse side

effects [103, 104]. Trial sequential analysis has already shown that the evidence for probiotic supplementation was conclusive and robust. To have a zero tolerance for complications in preterm borns, probiotic prophylaxis is thus increasingly recommended [105]. However, it is also important to note, due to the heterogeneity in the design and execution of clinical studies, the conclusions about the safety and efficacy of probiotics in the preterm population remains debatable [106]. Further large-scale standardized trials are needed to evaluate the role of probiotics in preventing NEC in the low birth weight population born premature.

1. Conclusion

Researches from ages have shown the importance of vaginal microbiota as the first line of defense against infection in females. There is still a dearth of knowledge about the functional mechanisms that maintain a robust and stable ecosystem of microbiome in the female reproductive organs. This offers major challenges to develop therapeutics for the reproductive diseases and infertility that are commonplace in many parts of the world due to various socioeconomic and lifestyle related factors. Since one-solution-fits-all approach has been unequivocally accepted as an unfavorable regimen, there is an increasing demand for personalized treatment taking into account the host genetics, health, immunological and nutritional status and life style. To achieve this, the drivers and principles for intra/inter-variation of microbiome needs to be understood critically in the perspective of patients' age, hormonal activity, dietary and hygienic habits. To this end, it is indeed necessary to catalogue the optimally beneficial microbes and ascertain "who benefits whom". Finally, the communication of vaginal microbiome with the extra-vaginal society needs to be clarified to learn the origin and mechanism of spread of diseases from these sites. Emphasis should be laid on deeper sequencing of the human microbiome across globe to generate longitudinal data over a long period of time to settle "core microbiome" or "microbiome with continuum" debate. This exercise will aid in

generating the microbial sensors of dysbiosis and designing therapeutics that refurbish the indigenous flora. In this regard, it is to be borne in mind that long-term application may lead to the development of therapy-resistance and tendency of resilience of the resident population to reallocate to a new and stable environment. It is hoped that the future research will focus on antibiotic-sparing, individual-centric strategies to combat many of the female reproductive diseases with a goal to restore niche specific structure of microbiome (Highlights).

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References

1. Lindemann SR, Bernstein HC, Song HS, Fredrickson JK, Fields MW, Shou W, et al. Engineering microbial consortia for controllable outputs. *ISME J* 2016.
2. Keller L, Surette MG. Communication in bacteria: an ecological and evolutionary perspective. *Nat Rev Microbiol* 2006; 4(4): 249-58.
3. Brenner K, You L, Arnold FH. Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol* 2008; 26(9): 483-9.
4. Burmolle M, Webb JS, Rao D, Hansen LH, Sorensen SJ, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Appl Environ Microbiol* 2006; 72(6): 3916-23.

5. Dobell C. The Discovery of the Intestinal Protozoa of Man. *Proc R Soc Med* 1920; 13(Sect Hist Med): 1-15.
6. International Human Genome Sequencing C. Finishing the euchromatic sequence of the human genome. *Nature* 2004; 431(7011): 931-45.
7. Yang X, Xie L, Li Y, Wei C. More than 9,000,000 unique genes in human gut bacterial community: estimating gene numbers inside a human body. *PLoS One* 2009; 4(6): e6074.
8. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 486(7402): 207-14.
9. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev* 2012; 70 Suppl 1: S38-44.
10. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature* 2012; 486(7402): 222-7.
11. Franasiak JM, Scott RT, Jr. Introduction: Microbiome in human reproduction. *Fertil Steril* 2015; 104(6): 1341-3.
12. Blaser MJ. Who are we? Indigenous microbes and the ecology of human diseases. *EMBO Rep* 2006; 7(10): 956-60.
13. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* 2009; 7(12): 887-94.
14. Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, et al. Microbial co-occurrence relationships in the human microbiome. *PLoS Comput Biol* 2012; 8(7): e1002606.

15. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; 464(7285): 59-65.

16. Ordovas JM, Mooser V. Metagenomics: the role of the microbiome in cardiovascular diseases. *Curr Opin Lipidol* 2006; 17(2): 157-61.

17. Howitt MR, Garrett WS. A complex microworld in the gut: gut microbiota and cardiovascular disease connectivity. *Nat Med* 2012; 18(8): 1188-9.

18. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. *J Clin Invest* 2014; 124(10): 4204-11.

19. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; 5(2): e9085.

20. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; 490(7418): 55-60.

21. Jayasinghe TN, Chiavaroli V, Holland DJ, Cutfield WS, O'Sullivan JM. The New Era of Treatment for Obesity and Metabolic Disorders: Evidence and expectations for gut microbiome transplantation. *Front Cell Infect Microbiol* 2016; 6: 15.

22. Stumpf RM, Wilson BA, Rivera A, Yildirim S, Yeoman CJ, Polk JD, et al. The primate vaginal microbiome: comparative context and implications for human health and disease. *Am J Phys Anthropol*. 2013; 152 Suppl 57: 119-34.

23. Sirota I, Zarek SM, Segars JH. Potential influence of the microbiome on infertility and assisted reproductive technology. *Semin Reprod Med* 2014; 32(1): 35-42.

24. Collado MC, Cernada M, Bauerl C, Vento M, Perez-Martinez G. Microbial ecology and host-microbiota interactions during early life stages. *Gut Microbes* 2012; 3(4): 352-65.

25. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. Maternal microbiome and pregnancy outcomes that impact infant health: A Review. *Adv Neonatal Care* 2015; 15(6): 377-85.

26. Gomez de Aguero M, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al. The maternal microbiota drives early postnatal innate immune development. *Science* 2016; 351(6279): 1296-302.

27. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008; 159(3): 187-93.

28. Witkin SS, Mendes-Soares H, Linhares IM, Jayaram A, Ledger WJ, Forney LJ. Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase

29. Salas JT, Chang TL. Microbiome in human immunodeficiency virus infection. *Clin Lab Med* 2014; 34(4): 733-45.

30. Hummelen R, Fernandes AD, Macklaim JM, Dickson RJ, Changalucha J, Gloor GB, et al. Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One* 2010; 5(8): e12078.

31. Anderson DJ, Marathe J, Pudney J. The structure of the human vaginal stratum corneum and its role in immune defense. *Am J Reprod Immunol* 2014; 71(6): 618-23.

32. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011; 108 Suppl 1: 4680-7.

33. Hickey RJ, Zhou X, Settles ML, Erb J, Malone K, Hansmann MA, et al. Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *MBio* 2015; 6(2).

34. Thoma ME, Gray RH, Kiwanuka N, Aluma S, Wang MC, Sewankambo N, et al. Longitudinal changes in vaginal microbiota composition assessed by gram stain among never sexually active pre- and postmenarcheal adolescents in Rakai, Uganda. *J Pediatr Adolesc Gynecol* 2011; 24(1): 42-7.

35. Fettweis JM, Brooks JP, Serrano MG, Sheth NU, Girerd PH, Edwards DJ, et al. Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology* 2014; 160(Pt 10): 2272-82.

36. Madhivanan P, Alleyn HN, Raphael E, Krupp K, Ravi K, Nebhrajani R, et al. Identification of culturable vaginal *Lactobacillus* species among reproductive age women in Mysore, India. *J Med Microbiol* 2015; 64(6): 636-41.

37. Garg KB, Ganguli I, Das R, Talwar GP. Spectrum of *Lactobacillus* species present in healthy vagina of Indian women. *Indian J Med Res*. 2009; 129(6): 652-7.

38. Xiao BB, Liao QP. Analysis of diversity of vaginal microbiota in healthy Chinese women by using DNA-fingerprinting. *Beijing Da Xue Xue Bao* 2012; 44(2): 281-7.

39. Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS One* 2013; 8(2): e56111.

40. Mendes-Soares H, Suzuki H, Hickey RJ, Forney LJ. Comparative functional genomics of *Lactobacillus* spp. reveals possible mechanisms for specialization of vaginal *lactobacilli* to their environment. *J Bacteriol* 2014; 196(7): 1458-70.

41. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010; 107(26): 11971-5.

42. Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum Reprod* 2001; 16(9): 1809-13.

43. Donders GG, Van Bulck B, Caudron J, Londers L, Vereecken A, Spitz B. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. *Am J Obstet Gynecol* 2000; 183(2): 431-7.

44. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis* 2003; 37(3): 319-25.

45. Watts DH, Fazzari M, Minkoff H, Hillier SL, Sha B, Glesby M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. *J Infect Dis* 2005; 191(7): 1129-39.

46. Oakley BB, Fiedler TL, Marrazzo JM, Fredricks DN. Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. *Appl Environ Microbiol* 2008; 74(15): 4898-909.

47. Mendling W. Vaginal Microbiota. *Adv Exp Med Biol* 2016; 902: 83-93.

48. Maldonado-Barragan A, Caballero-Guerrero B, Martin V, Ruiz-Barba JL, Rodriguez JM. Purification and genetic characterization of gassericin E, a novel co-culture inducible bacteriocin from *Lactobacillus gasseri* EV1461 isolated from the vagina of a healthy woman. *BMC Microbiol* 2016; 16:37.

49. Moore MS, Golden MR, Scholes D, Kerani RP. Assessing trends in *Chlamydia* positivity and *Gonorrhea* incidence and their associations with the incidence of pelvic inflammatory disease and ectopic pregnancy in Washington State, 1988-2010. *Sex Transm Dis* 2016; 43(1): 2-8.

50. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A* 2015; 112(35): 11060-5.

51. Green KA, Zarek SM, Catherino WH. Gynecologic health and disease in relation to the microbiome of the female reproductive tract. *Fertil Steril* 2015; 104(6): 1351-7.

52. Mitchell CM, Haick A, Nkwopara E, Garcia R, Rendi M, Agnew K, et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am J Obstet Gynecol* 2015; 212(5): 611 e1-9.

53. Verstraelen H, Vilchez-Vargas R, Desimpel F, Jauregui R, Vankeirsbilck N, Weyers S, et al. Characterization of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. *PeerJ*. 2016; 4: e1602.

54. Pelzer ES, Allan JA, Cunningham K, Mengersen K, Allan JM, Launchbury T, et al. Microbial colonization of follicular fluid: alterations in cytokine expression and adverse assisted reproduction technology outcomes. *Hum Reprod* 2011; 26(7): 1799-812.

55. Payne MS, Bayatibojakhi S. Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome. *Front Immunol* 2014; 5:595.

56. Solt I. The human microbiome and the great obstetrical syndromes: a new frontier in maternal-fetal medicine. *Best Pract Res Clin Obstet Gynaecol* 2015; 29(2): 165-75.

57. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med* 2014; 6(237): 237ra65.

58. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. *BMJ Open* 2014; 4(8): e005047.

59. Larsson PG, Fahraeus L, Carlsson B, Jakobsson T, Forsum U. Predisposing factors for bacterial vaginosis, treatment efficacy and pregnancy outcome among term deliveries; results from a preterm delivery study. *BMC Womens Health* 2007; 7: 20.

60. van Oostrum N, De Sutter P, Meys J, Verstraelen H. Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis. *Hum Reprod* 2013; 28(7): 1809-15.

61. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012; 379(9832): 2162-72.

62. Nigro G, Mazzocco M, Mattia E, Di Renzo GC, Carta G, Anceschi MM. Role of the infections in recurrent spontaneous abortion. *J Matern Fetal Neonatal Med* 2011; 24(8): 983-9.

63. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med* 2005; 353(18): 1899-911.

64. Sobel JD. Bacterial vaginosis. *Annu Rev Med* 2000; 51: 349-56.

65. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS* 2008; 22(12): 1493-501.

66. Brotman RM, Klebanoff MA, Nansel TR, Yu KF, Andrews WW, Zhang J, et al. Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *J Infect Dis* 2010; 202(12): 1907-15.

67. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. *Eur J Clin Microbiol Infect Dis* 2015; 34(12): 2367-76.

68. Mirmonsef P, Gilbert D, Zariffard MR, Hamaker BR, Kaur A, Landay AL, et al. The effects of commensal bacteria on innate immune responses in the female genital tract. Am *J Reprod Immunol* 2011; 65(3): 190-5.

69. Teixeira GS, Carvalho FP, Arantes RM, Nunes AC, Moreira JL, Mendonca M, et al. Characteristics of *Lactobacillus* and *Gardnerella vaginalis* from women with or without bacterial vaginosis and their relationships in gnotobiotic mice. *J Med Microbiol* 2012; 61(Pt 8): 1074-81.

70. Fox C, Eichelberger K. Maternal microbiome and pregnancy outcomes. *Fertil Steril* 2015; 104(6): 1358-63.

71. Donders GG, Van Calsteren K, Bellen G, Reybrouck R, Van den Bosch T, Riphagen I, et al. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG* 2009; 116(10): 1315-24.

72. Larsen B, Hwang J. *Mycoplasma*, *Ureaplasma*, and adverse pregnancy outcomes: a fresh look. *Infect Dis Obstet Gynecol* 2010; 2010.

73. Vanderhoeven JP, Bierle CJ, Kapur RP, McAdams RM, Beyer RP, Bammler TK, et al. Group B streptococcal infection of the choriodecidua induces dysfunction of the cytokeratin network in amniotic epithelium: a pathway to membrane weakening. *PLoS Pathog* 2014; 10(3): e1003920.

74. Ahmadi A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Roshani D, Ghaderi E, et al. The Relationship between *Chlamydia trachomatis* genital infection and spontaneous abortion. *J Reprod Infertil* 2016; 17(2): 110-6.

75. Lyon D, Cheng CY, Howland L, Rattican D, Jallo N, Pickler R, et al. Integrated review of cytokines in maternal, cord, and newborn blood: part I--associations with preterm birth. *Biol Res Nurs* 2010; 11(4): 371-6.

76. Lannon SM, Vanderhoeven JP, Eschenbach DA, Gravett MG, Adams Waldorf KM. Synergy and interactions among biological pathways leading to preterm premature rupture of membranes. *Reprod Sci* 2014; 21(10): 1215-27.

77. Bearfield C, Davenport ES, Sivapathasundaram V, Allaker RP. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. *BJOG* 2002; 109(5): 527-33.

78. Zi MY, Longo PL, Bueno-Silva B, Mayer MP. Mechanisms involved in the association between periodontitis and complications in pregnancy. *Front Public Health* 2014; 2: 290.

79. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR, Probiotics in Preterm infants Study Collaborative G. *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. *Lancet* 2016; 387(10019): 649-60.

80. Khan MA, Sengupta J, Mittal S, Ghosh D. Genome-wide expressions in autologous eutopic and ectopic endometrium of fertile women with endometriosis. *Reprod Biol Endocrinol.* 2012;10:84.

81. Ghosh D, Nagpal S, Bhat MA, Anupa G, Srivastava A, Sharma JB, Sengupta J. Gel-free proteomics reveals neoplastic potential in endometrium of infertile patients with stage IVovarian endometriosis. *JRHM I* 2015; 83-95.

82. Kobayashi H, Higashiura Y, Shigetomi H, Kajihara H. Pathogenesis of endometriosis: the role of initial infection and subsequent sterile inflammation (Review*). Mol Med Rep* 2014; 9(1):9-15

83. Chen MJ, Yang JH, Yang YS, Ho HN. Increased occurrence of tubo-ovarian abscesses in women with stage III and IV endometriosis. *Fertil Steril* 2004; 82(2): 498-9.

84. Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis dagger. *Hum Reprod* 2014; 29(11): 2446-56.

85. Li C, Meng CX, Zhao WH, Lu HQ, Shi W, Zhang J. Risk factors for ectopic pregnancy in women with planned pregnancy: a case-control study. *Eur J Obstet Gynecol Reprod Biol* 2014; 181: 176-82.

86. Michou IV, Constantoulakis P, Makarounis K, Georgoulias G, Kapetanios V, Tsilivakos V. Molecular investigation of menstrual tissue for the presence of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* collected by women with a history of infertility. *J Obstet Gynaecol Res*. 2014; 40(1):237-42.

87. Cicinelli E, Matteo M, Tinelli R, Pinto V, Marinaccio M, Indraccolo U, et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent

miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci*. 2014; 21(5): 640-7.

88. Ahmadi A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Nikkhoo B, Soofizade N, et al. Association between *Ureaplasma urealyticum* endocervical infection and spontaneous abortion. *Iran J Microbiol* 2014; 6(6): 392-7.
89. Egal ES, Ardeshir A, Mariano FV, Gondak RO, Montalli VA, dos Santos HT, et al. Contribution of endemic listeriosis to spontaneous abortion and stillbirth in a large outdoor-housed colony of rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci* 2015; 54(4): 399-404.
90. Jiao F, Zhang D, Jiang M, Mi J, Liu X, Zhang H, Hu Z, Xu X, Hu X. Label-free proteomic analysis of placental proteins during *Toxoplasma gondii* infection. *J Proteomics*. 2016;150:31-39.
91. Cardaropoli S, Rolfo A, Todros T. *Helicobacter pylori* and pregnancy-related disorders. *World J Gastroenterol* 2014; 20(3): 654-64.
92. Miner JJ, Cao B, Govero J, Smith AM, Fernandez E, Cabrera OH, Garber C, Noll M, Klein RS, Noguchi KK, Mysorekar IU, Diamond MS. Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* 2016; 165(5):1081-91.
93. Pflughoeft KJ, Versalovic J. Human microbiome in health and disease. *Annu Rev Pathol* 2012; 7: 99-122.
94. ElRakaiby M, Dutilh BE, Rizkallah MR, Boleij A, Cole JN, Aziz RK. Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics. *OMICS* 2014; 18(7): 402-14.

95. Falagas M, Betsi GI, Athanasiou S. Probiotics for the treatment of women with bacterial vaginosis. *Clin Microbiol Infect* 2007; 13(7): 657-64.

96. Sengupta J, Khan M, Huppertz B, Ghosh D. In-vitro effects of the antimicrobial peptide Ala 8,13,18-magainin II amide on isolated human first trimester villous trophoblast cells. *Reproductive Biology and Endocrinology* 2011; 9(49): 1-10.

97. Yasin B, Pang M, Lehrer RI, Wagar EA. Activity of Novispirin G-10, a novel antimicrobial peptide against *Chlamydia trachomatis* and vaginosis-associated bacteria. *Exp Mol Pathol* 2003; 74(2):190-5.

98. Vicariotto F, Mogna L, Del Piano M. Effectiveness of the two microorganisms *Lactobacillus fermentum* LF15 and *Lactobacillus plantarum* LP01, formulated in slow-release vaginal tablets, in women affected by bacterial vaginosis: a pilot study. *J Clin Gastroenterol* 2014; 48 Suppl 1: S106-12.

99. Ling Z, Liu X, Chen W, Luo Y, Yuan L, Xia Y, et al. The restoration of the vaginal microbiota after treatment for bacterial vaginosis with metronidazole or probiotics. *Microb Ecol* 2013; 65(3): 773-80.

100. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med* 2016; 22(3): 250-3.

101. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol*. 2016; 16(1):86.

102. Terrin G, Scipione A, De Curtis M. Update in pathogenesis and prospective in treatment of necrotizing enterocolitis. *Biomed Res Int.* 2014; 2014:543765.

103. Xu L, Wang Y, Wang Y, Fu J, Sun M, Mao Z, et al. A double-blinded randomized trial on growth and feeding tolerance with *Saccharomyces boulardii* CNCM I-745 in formula-fed preterm infants. *J Pediatr (Rio J)* 2016; 92(3): 296-301.

104. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA, Simmer KN. Benefits of *Bifidobacterium breve* M-16V supplementation in preterm neonates - A Retrospective Cohort Study. *PLoS One* 2016; 11(3): e0150775.

105. Ofek Shlomai N, Deshpande G, Rao S, Patole S. Probiotics for preterm neonates: what will it take to change clinical practice? *Neonatology* 2014; 105(1): 64-70.

106. Mihatsch WA, Braegger CP, Decsi T, Kolacek S, Lanzinger H, Mayer B, et al. Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. *Clin Nutr* 2012; 31:6–15.

Box 1

Female reproductive tract is inhabited by microorganisms that begin to colonize in the neonates as they come into contact with maternal body parts. Although the composition of vaginal microbiome varies widely, *Lactobacilli* continue to be the dominant phyla in the female reproductive tract. Species under this genus produces lactic acid and a wide variety of bactericidal molecules that are responsible for maintenance of reproductive health. The abundance and diversity of *Lactobacillus sp*. vary as a function of age, estrogen cycle and lifestyle related factors.

Box 2

Dysbiosis is regarded as one of the major factors leading to infertility, pre-term birth, endometriosis and spontaneous abortion. Bacterial Vaginosis is caused by displacement of *Lactobacilli* by a population of gram negative anaerobes and has been implicated in adverse pregnancy outcomes. Activation of matrix

metalloproteinases and hyaluronidases by the pathogenic microbes in patients with BV leads to breakdown of collagen layer of cervical epithelium and activation of cytokines that cause intra-uterine inflammation, the major causes of pre-term birth and abortion. Ectopic pregnancy and spontaneous abortion have been correlated with *Chlamydia trachomatis* infection that activate arachidonic acid and cause premature uterine contractions in spontaneous abortion. The pathognomonic characteristics in endometriosis showed marked indication of neoplastic potential together with activation of immune and inflammatory pathways due to disruption of normal flora.

Highlights

Administration of oral or intra-vaginal antibiotics is the routine treatment of infection and inflammation of the reproductive tract. Application of probiotics for a prolonged period of time is considered to be an attractive supplementary and/or alternative regimen for re-establishment of vaginal homeostasis. Design of novel antigen-sparing therapy demands a thorough knowledge of resident microbiota in different body niches and the metabolome they exude. Besides, the turnover of microbiome due to different physiological responses needs to be evaluated to develop personalized medicine.