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Microbiota in Genitourinary System and Infertility

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Abstract

Symbiotic relationship between microbial flora in human body and our body plays a key role in maintaining a healthy life. It has been suggested that microbiota of our body protects us against pathogens, supports our immune system and plays a role in the synthesis of certain necessary micronutrients. Human microbiota is composed of bacteria, fungi and viruses, and their number exceeds 10 trillion in total. This number is 100 times more than the number of other cells in our body. The human gastrointestinal system and the genitourinary system have been showed to have several types of microbes that could have symbiotic functions in several systems including the reproductive system of the females. There have been several developments recently that enabled the researchers to determine the microbial diversity and rates in our body in a surprising accuracy with next-generation sequencing technique (NGS). Such developments have been the turning point in the understanding of relationship between microbiota and human health.

Keywords: Pathogens; *Streptococcus*; Dopamine; Vaginal microbiota

Introduction

Human intestinal microbiota is the most studied microbial flora; it is complex and intensely related to its host (human). Intestinal microbiota is thought to have a role in many pathological conditions in human, and evidence supporting such theory is increasingly improving. Human intestinal microbiota goes through a few developmental processes. First, in the intrauterine environment, the bacteria originating from the mother's intestinal system and vagina invade the fetus. Second, it is subjected to mother's vaginal microbes during vaginal birth [1]. Last but not least, it is known that breast milk is not sterile and contains bacteria such as *Streptococcus, Staphylococcus, Propionibacterium*, and *Bifidobacterium* [2]. In early postnatal period, intestinal microbiota is abundant in *Bifidobacteria*, however, their numbers gradually decrease and by the age of 2, child's intestinal microbiota becomes similar to an adult's intestinal microbiota [3]. Children born by caesarean section has different microbiota that of the vaginally born babies in the first 3-4 months of life; this difference can be explained by the reasons that children born by caesarean section does not contact mother's vaginal microbiota and antibiotic usage of the mother as well as delayed breast feeding [3,4].

The content of intestinal microbiota is affected by factors such as diet, tobacco product and alcohol consumption, age, body-mass index, haemoglobin levels, and antibiotics use [5]. Furthermore, diet is believed to be the reason behind the diversity in microbiota in a given community [6]. Intestinal microbiome is capable of synthesizing certain factors such as; serotonin, gamma-amino butyric acid, histamine, acetylcholine, dopamine, and noradrenaline [7,8]. These factors are permeable and can diffuse from the intestines into general blood circulation that in turn alters the organ systems. Therefore, some researchers refer the intestinal microbiome as an actual endocrine organ [7].

Importance of short-chained fatty acids that are produced as end product due to fermentation of carbohydrates by bacteria in the anaerobic environment of intestines has been emphasized in recent years. These are acetic acid, propionic acid, and butyric acid. Intestines absorb majority of these products and only 5-10% of them are discarded with faeces, as they are highly permeable [8,9]. These short-chained fatty acids have several important functions in our bodies. These fatty acids enable energy storage and utilization that are produced by oxidative phosphorylation. Butyric acid alone provides approximately 60-70% of the energy required for the colon endothelial cells [9]. These fatty acids also inhibit the enzyme called histone deacetylase and consequently decrease inflammation [10]. Last but not least, these fatty acids play some key roles in the growth and functions of microglial and dendritic cells [10].

Intestinal microbiota protects intestines against the pathogenic invasion of bacteria by getting into competition for nutrients or producing antimicrobial proteins or peptides such as bacteriocin [11,12].

The majority of intestine complications related with *Clostridium difficile* are due to alterations in intestinal microbiota. The clinical symptoms accompanied with such complications can vary from mild diarrhea to fatal systemic inflammation syndrome [13]. The bacteria that cause such complications (*C. difficile*) are readily present in the intestine of healthy humans. However, following the use a broad-spectrum antibiotic, the inhibitory effects of the microbiota of the intestines on *C. difficile* are disrupted and thus enabling the colonization. Following increased reproduction and colonization, *C. difficile* produces Rho GTPase toxin that causes the complications aforementioned [13]. There are supporting evidence suggesting the use of orally taken probiotics to treat such complications caused by *C. difficile* both in adults and children [14].

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Literature Review

Microbiota in genitourinary system

It has been long known that microbiota of female reproductive system is very diverse. Traditionally, majority of the research conducted used to be focusing on vaginal microbiota, however, evidence has been collected in the past 1-2 decades on the fact that female reproductive systems including uterus are not sterile [15]. It has been suggested that microbiota extends beyond uterine cavity, and according to some researchers, there may be bacteria found in the fallopian tubes of healthy women. Studies have recently begun on the relationship between reproductive system microbiota and fertilization and successful pregnancy. Some studies have begun to emerge investigating the relationship between mocrobiota and fertility. So far, the relationship between clinically apparent infection and inflammation and impaired reproductive function has been clearly identified. Inflammation due to pathogenic invasion causes proinflammatory cytokines and growth factors to be secreted from immune system cells. Slight changes in microbiome may cause ambiguous changes in tissues, but such changes may be clinically important [16].

Lactobacillus, which has probiotic attributes and prevents reproduction of other bacteria, is prevalent in normal vaginal microbiota [17]. *Lactobacillus* produces high amount of H_2O_2 and prevents other members of microbiota from becoming prevalent in tissue.

Reproductive system's microbiota is not only composed of free bacteria clusters. These bacteria mostly form three-dimensional biofilm structures. These structures function as a protective cover; they involve polysaccharides, nucleic acids and proteins. These biofilm structures prevent immune system from recognizing the pathogens and reduce positive effects of antibiotics [18].

Biofilms usually form in vagina but can extend towards endometrial cavity and even fallopian tubes [18]. Even though the role of biofilms in pathogens of reproductive system is not fully known, what we need to have in mind is that the relationship between microbiome and reproductive system cannot be identified only with presence or absence of certain bacteria.

Microbiome can affect gametogenesis. It has been shown that some bacteria impair follicular development and suppress follicular response to gonadotropins [15].

Vaginal microbiota: Studies on vaginal microbiota of healthy women have been carried out under the management of the human microbiome project [17]. Samples were taken from 3 different areas (introitus, mid-vagina and posterior fornix) of vaginas of 113 healthy, volunteered women, and these samples were analyzed with the 16S rRNA sequencing. The samples taken from the same individual at different times differed scarcely by type of bacteria, and a very little difference was found between the samples from different individuals [17]. The samples taken from different areas of vagina did not differ by type of bacteria, and *Lactobacillus* was prevalent. The fact that samples taken from the same individual at different times showed very little difference is the indicator that vaginal flora is stable. Vaginal microbiota is simpler than other parts of body in terms of content in a healthy woman; therefore, it is safe to assume that even small shifts from "normal" microbiota may cause disease symptoms [18].

It is difficult to identify the bacterial species using traditional approaches. Therefore, a study using 16SrRNA sequencing technique showed that *in vitro* fertilization (IVF) patients with lower diversity in vaginal microbiome have higher chances of pregnancy than that of IVF patients with relatively higher vaginal microbiome diversity [19].

Uterine microbiota: It has been believed that ascending colonization of upper genital system via vagina is associated only with pathological conditions until recently. Uterine cavity has been considered sterile for a long time due to cervical mucus involving high levels of proinflammatory cytokines, immunoglobulins, and antimicrobial peptides and functioning as a protective barrier [20]. However, upward bacteria transport is possible in a healthy reproductive system. One interesting demonstration of this upward transfer phenomenon is, 1-2 ml of radiolabelled human serum albumin is placed in the posterior fornix of the vagina; there was radioactive signal from the uterus within 2 minutes [21].

The initial studies in search to document the uterine microbiome were conducted solely by relying on culture techniques to identify the species of bacteria. One study is particularly interesting as it was conducted on 58 patients undergoing hysterectomy [22]. In this study, quantitative polymerase chain reaction (qPCR) technique was used to identify 12 bacteria species. The vaginal swabs were taken before the operation and the uterine swabs were taken after the operation. It was shown that the upper reproductive tract was dominated by at least one bacterial species in 95% of the study subjects. The most prevalent bacterial species was *Lactobacillus* and *Prevotella*. Another key finding from this study was the fact that there were much less bacteria in the upper reproductive tract than that of the vagina. Therefore, it was concluded that the either the cervix was filtrating the bacteria transport upwards the reproductive tract or the immune system is suppressing the upward bacterial transport (though it is possible that both systems are working together).

Follicular fluid microbiota: As shown in a few studies, human follicular liquid can be cultured and contains bacteria. Follicular liquid was obtained during transvaginal oocyte retrieval in some of these studies, and during laparoscopic interventions in others [23]. However, it has not been clarified whether bacteria are present in the follicles before the oocyte retrieval or the follicular fluid is contaminated during aspiration [24]. Furthermore, it has been found that follicular liquid contains bacteria similar to the ones in vaginal microbiota. Nevertheless, these include *Lactobacillus*, *Bifidobacteria*, *Enterobacteriaceae*, *Streptococcus* and *Staphylococcus*. This finding reinforces the assumption that follicular liquid is not contaminated during oocyte aspiration and is colonized beforehand. This finding suggests that the follicular fluid should be colonized before the oocyte retrival and did not get contaminated during the aspiration [25].

Microbiota and infertility

As aforementioned, parts of the female reproductive tract that was considered to be sterile are in fact found to be not sterile and have a defined "normal" microbiota.

In bacterial vaginitis, reduction of *Lactobacillus* (Döderlein rods) dominance causes increased bacteria diversity, showing increase numbers of *Gardnerella, Mycoplasma*, and *Prevotella* [26]. This increased diversity in bacterial vaginitis increases tendency to gynecological infections such as Chlamydia, Neisseria gonorrhea, Trichomonas, human papilloma virus (HPV), and herpes simplex type-2 [27]. It is known that hydrogen peroxide produced by *Lactobacillus* have virucidal properties, and decreased *Lactobacillus* in bacterial vaginitis increases the tendency to human immunodeficiency virus (HIV) infection [28]. Furthermore, subclinical endometritis was observed in 27% of the patients with vaginal chlamydia infection and 26% of the patients with vaginal gonorrhoea infection [29].

Similarly, to vaginal microbiome, it seems possible that imbalances in uterine microbiome can increase tendency to disorders such as infertility and pregnancy complications. Impaired uterine microbiome can cause early-late abortions, preterm labor and postpartum endometritis, affecting the reproductive system. Follicular fluids were studied during the oocyte retrieval for IVF, and differences were found in the composition of follicular fluid by diseases (polycystic ovary, endometriosis) and with respect to positive or negative implantation [30].

In a study conducted on endometrial microbiota, the patients in which showed *lactobacillus* dominancy (more than 90%) were compared with patients showing decreased levels of endometrial *lactobacillus* (less than 90%). It was found that patients that showed *lactobacillus* dominancy have higher implantation, clinical pregnancy, on-going pregnancy, and live birth rates [31].

If we were to accept the effect of vaginal, uterine, and follicular fluid microbiome on infertility, we would need to investigate possible treatment options. For instance, prophylactic antibiotics usage before embryo transfer in IVF cycles may reduce amount of microbial colonization in upper genital tracts and be effective in increasing the pregnancy rates [32]. Nevertheless, contradictory research results have been reported to date.

Probiotic supplementation on genitourinary system: As aforementioned, the most distinct characteristic of a healthy vaginal microbiome is the relative dominancy of *Lactobacillus* [33]. Beneficial effects of probiotic supplement on human health have been increasingly receiving recognition by physicians. Given the abundance and the effects of microorganisms in reproductive system, it seems reasonable that beneficiary effects of probiotics might be positive on the reproductive system health. Since majority of bacteria in human body is present in the intestines, majority of the studies have investigated oral probiotics intake on intestinal health to date.

For the first time, Hilton et al. reduced the risk of relapse by 7 times by giving 250 grams of yoghurt containing *Lactobacillus acidophilus* a day orally for 6 months to the patients with recurring vulvovaginitis complaint [34]. Later, it was shown that *Lactobacillus rhamnosus* inhibited the reproduction of *Gardnerella vaginalis* and *Candida albicans* through high glycogen metabolism and lactic acid production *in vitro* [35]. In recent years, several studies have been conducted on benefits of probiotic supplement and beneficial effects of probiotics directly on reproductive health are yet to be proven; however, orally-taken *Lactobacillus rhamnosus* and *Lactobacillus fermentum* have been shown to "normalize" vaginal flora among 82% of women with a vaginal dysbiosis history [36]. Supporting the treatment with *Lactobacillus rhamnosus* and *Lactobacillus reuteri* following the antibiotics usage in bacterial vaginitis was reported to increase vagina-specific *Lactobacillus iners* and *Lactobacillus crispatus* [37]. Based on these observations, certain bacterial strains can be utilized to regulate pre-conception vaginal microbiota, but further studies are required to find the ideal combination, dosage and way of application.

In general, a question of "what is the effect of orally taken probiotics?" comes into the mind as probiotic products are becoming more popular in the public. However, to-date, the answer for this question is not straightforward considering the variations in bacterial strains and product formulations of such products. For example, *L. reuteri* SD2112 and *L. reuteri* RC-14 have different genomes as well as different functions. It is documented that *L. reuteri* SD2112 has inhibitory functions on pathogens (through productions of reuterin) in the intestines [38]. On contrast, *L. reuteri* RC-14 produces biosurfactants to block the adhesion of uropathogens [38]. In addition of providing a healthy intestinal microbiota, probiotics are believed to have some degree of support on the immune system [39]. There are several meta-analyses supporting the use of probiotics against infectious diarrhoea, diarrhoea caused by antibiotic use, and irritable bowel syndrome [40].

Discussion and Conclusion

There are numerous studies in the literature concluding negative impact of impaired vaginal microbiota on fertility. However, studies on upper genital systems' (endometrium, fallopian tubes, and follicular fluid) microbiota which has been accepted to be sterile until recently are limited, and no study was found in the literature on the treatment of upper genital system's impaired microbiota with probiotics. On the other hand, based on the assumption that vaginal microbiota can affect upper genital system's microbiota via adjacency, it can be concluded that probiotics used for impaired vaginal microbiota would indirectly affect upper genital system in a positive way.

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