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An analysis of the Vaginal microbiome and the impact of infections on female infertility, Cervical Intraepithelial Neoplasia (CIN), and Cervical cancer

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ABSTRACT

For healthy reproduction and appropriate vaginal function, keeping the vaginal microbiota in balance is crucial. Both pathogenic and nonpathogenic microbes are present inside the vagina, among them Lactobacillus is the most predominant. Lactobacillus gives protection against a wide range of pathogenic infections by producing lactic acid, hydrogen peroxide, and bacteriocins. A low amount of Lactobacillus strains and a higher amount of facultative anaerobic pathogens inside the vagina consequently lead to vaginal microbiota dysbalance. Female vaginas with a lower concentration of vaginal Lactobacillus are more prone to get upper genital tract infections, sexually transmitted infections, and other anaerobic pathogenic infections. Dysbiosis of the vaginal microbiota is strongly associated with infertility, poor pregnancy rate, pregnancy complications, spontaneous abortion, preterm birth, and frequent abortion. Female infertility is one of the most complex reproductive diseases and there is no effective way to get out of this problem to date. Several infection conditions like bacterial vaginosis, pelvic inflammatory diseases, and endometritis are related to adverse reproductive outcomes and infertility via disturbing normal immunity, normal vaginal microbial composition, regulating pathophysiological pathways, and inducing inflammation. Pathogenic bacteria including Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, herpes simplex, Mycoplasma genitalium are mainly responsible for infertile conditions. The aim of this review is to show the link between vaginal microbial disbalance and female infertility. This review also summarizes the effects of various inflammatory conditions, and infectious diseases of the reproductive system on female infertility. Several pathogenic microbes including sexually transmitted microorganisms and their impacts on female infertility are also reviewed in this paper.

Keywords: Bacterial Vaginosis; Chlamydia trachomatis; Female infertility; Mycoplasma genitalium; Pelvic inflammatory diseases; Vaginal Lactobacillus

INTRODUCTION

Recently female infertility is a serious health issue around the whole world [1]. Infertility, a complicated medical condition can affect an infertile individual's physical, mental, and psychological health. Both female and male infertility can cause varioinfertility-related conditions. Though male fertility is a serious and prevalent factor in infertility studies this review paper only summarizes different bacterial infection-related infertility conditions in women [2]. According to World Health Organization (WHO), infertility is defined as "failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse". At the same time unable to get pregnant after the very first successful pregnancy is called secondary infertility. It may present in both male and female individuals, and that affects a million people across the world. WHO reported that worldwide 186 million people and 48 million couples are suffering from infertility [3]. In the United States near about 6% of married female in the reproductive age group (15 to 44 years) is currently suffering from infertility and 12 % of the total married women population is facing impaired fecundity problems and other pregnancy difficulties. In the United State, behind infertility, the male factor is one of the main reasons. 8% of infertility is because of male partners only over there [4]. In India, a wide range of lifestyle diversity like working patterns, traditions, hygiene patterns, health care facilities, customs, traditions, and external environmental conditions leads to different infertility rates within the different regions and different groups of people. Recently in India prevalence of infertility is between 10 and 14%. The burden is more in urban areas (approx. 1 in 6 couples are suffering from

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infertility). Infertile women are facing psychological, social, and physical ignorance and trauma day by day for the phenomenon. These mental and physical is more severe in India within patriarchal societies. Female infertility can put women into marital insecurity and the consequences can exert massive emotional instability for women. Women use to face lots of disrespect, rejection, teasing, and abusive words at their house as well as in their society. Centers for Disease Control and Prevention (CDC) have started National Public Health Action Plan to prevent, manage and detect infertility. This plan covers some essential points regarding this infertility field like promoting healthy, behaviors to control infertility rate, early diagnosis, preventive measures, and therapeutic remedies to control infertility, and lastly avoiding external exposures including infectious pathogens, environment, hygiene, and iatrogenic agents to secure fertility possibility [5].

A vagina is an appropriate place for microbial growth due to its humid and warm environment, and the presence of nutritional sources. 9% of the total microbiota inside the human body is occupied by vaginal microbiota. Lactobacillus has been found abundantly in the vaginal microbiota and along with lactobacillus many other bacterial species such as *Bifidobacterium*, *Prevotella*, *Gardnerella*, *Megasphaera*, *Atopobium*, *Anaerococcus*, *Sneathia* are also found [6,7]. Normal vaginal microbes can prevent pathogenic growth by using their anti-microbial and anti-inflammatory compounds. *Lactobacillus* use to produce L- lactic acid and D- lactic acid that maintains the normal vaginal acidic pH (4.5) [1]. The normal vaginal pH of a healthy female is near about 4.5 but dysbiosis of the vaginal microbiota may increase the pH value inside the vagina which is directly associated with a higher risk of preterm birth and female infertility.

One descriptive study by Lykke et al. detected an elevation of the pH value in the lower vagina of the female with abnormal vaginal microbiota than the women with normal vaginal microbiota by selecting both pregnant and nonpregnant women for this study. This study also identified some pathogens like *Atopobium vaginae*, *Leptotrichia amnionii*, *Sneathia sanguinegens*, bacterial vaginosis-causing bacteria, *Prevotella* spp., TM7 among women with abnormal vaginal microbiota by using PCR technique [8]. *Lactobacillus* also produces bactericidal peptides bacteriocins which can form pore on the pathogen's cell membrane and consequently rupture it. The balance between normal vaginal microbes and unwanted facultative pathogens in the vaginal microbiome plays an important role in women's reproductive health including fertility chances. Disbalance in the vaginal microbiota can damage the first-line defense mechanisms against pathogens. Bacterial vaginosis, an excess number of facultative anaerobes inside the vaginal microbiota instead of lactobacillus, urinary tract infections, sexually transmitted infections, and preterm birth are some common reasons for vaginal microbial dysbiosis [1]. Infertility-causing pathogenic microbes use to enter the upper genital tract through the vagina. The vaginal microbiota is composed of many anaerobic as well as aerobic bacteria, any kind of external factors including medications, antibiotics, systematic hormones, douching process, contraceptives, frequent sexual intercourse, poor socio-economic status, and stress level can hamper normal vaginal ecosystem after a certain time period. Sometimes, lactobacillus dominant vaginal microbiota is replaced by several harmful anaerobic and aerobic microbes [7,8]. Babu (2017) conducted one cross-sectional study in India by selecting 200 females (84 healthy + 116 infertile) in the age group of 18 to 45 years. The study collected swab samples for microbiological analysis. This study showed a significant amount of lactobacillus present in the vaginal samples among 27.8% (n= 40) of healthy women. The remaining 15.3% (n= 22), 11.1% (n= 16), and 8.3% (n= 12) of healthy women's vaginal samples were dominated by *Micrococcus*, *Enterococcus*, and *Staphylococcus* respectively. At the same time among the infertile patient group 26.5% (n= 30), 23% (n= 26), and 14.1% (n= 16) infertile women showed *Candida*, *Enterococcus*, and *Escherichia coli* dominated vaginal microbiota. A low level of lactobacillus count was detected among infertile female patients. The result of the experiment also identified asymptomatic vaginosis among 27.6% of infertile patients and 7.1 % of healthy females. This study recommended routine screening of the vaginal microbial system during infertility treatment [9]. Some internal factors including age, immune power, hormonal status, and external factors such as infectious microbial exposure, and antibiotic exposure, can facilitate the vaginal microbial disbalance process (dysbiosis). Vaginal microbiota dysbiosis is strongly associated with bacterial vaginosis, which is further directly associated with women's reproductive health disorders, HIV (human immunodeficiency virus), human papillomavirus (HPV), and pelvic inflammatory disease risk. Some common factors like douching, variation in intercourse, stress, race, and regional disparity can also change the vaginal microbiome composition [9]. This review aims to summarize the adverse effect of different bacterial infections on female reproductive health and infertility.

CHLAMYDIA TRACHOMATIS

Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium [10]. Chlamydia *trachomatis* (under the genus *chlamydophila*) is responsible for Chlamydia, one kind of sexually transmitted infectious state. *Chlamydia trachomatis*, mainly serovars A- C are responsible for human blindness, serovars L1- L3 are responsible for lymphatic system infection and for sexually transmitted infections, and *Chlamydia trachomatis* serovars D- K are especially responsible [10]. *Chlamydia trachomatis* can transmit directly through the vagina, oral and anal sex. The fetus can also get this infection during childbirth from the mother. This infection is widely prevalent around the United States as well as around the world. Chlamydia is responsible for cervicitis, proctitis, urethritis, and trachoma, one kind of ocular infection that consequently leads to permanent blindness if remains untreated [11]. Sexually transmitted infections like *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma*, and *Treponema pallidum* can impair normal reproductive functions [10]. Persistent *Chlamydia trachomatis* infection can induce infertility and ectopic pregnancy chance. Young women use to get Chlamydia infection very rapidly that further increases the chance of tubal infertility, pelvic inflammatory diseases, obstetrics consequences, and chronic pelvic pain. 10% to 15% of women infected with Chlamydia

infection show symptomatic pelvic inflammatory disease [11]. Most patients with chlamydial infection are asymptomatic. According to the Centers for Disease Control and Prevention (CDC) in the year of 2018, 4 million people were infected by *Chlamydia trachomatis* [11]. In the United States, *Chlamydia* infection is the most common followed by *Gonorrhea* infection cases. Centers for Disease Control and Prevention estimated 1808703 *Chlamydia* infection cases and 616392 *Gonorrhea* infection cases from fifty states of the United States and the District of Columbia in 2019 [12,13]. Chlamydia infection has also some disadvantages such as antibiotic resistance chance, and side effects on urogenital microbiota [14,15]. Parpillewar & Singh (2021) conducted one cross-sectional study, and the objective of this study was to determine the prevalence of female infertility due to *Chlamydia trachomatis* infection. 75 infertile women (with and without pelvic inflammatory disease) and 75 women with no infertility symptoms were enrolled in this study. The study collected cervical swab samples from recruited women to detect *Chlamydia trachomatis* infection. A Study detected 14 *Chlamydia trachomatis* infections from the infertile women group (42.85 were asymptomatic and 57.14 were symptomatic) and 4 from the control group. This study also agreed that *Chlamydia trachomatis* infection has a negative effect on female fertility processes [16]. Sharaf et al. selected 50 female patients with primary and secondary infertility and 25 healthy pregnant ladies for their prospective randomized clinical study. The study analyzed the anti-chlamydial IgG level of the enrolled females and reported a higher level of IgG in infertile women (46%) compared to the normal pregnant women group (12%). Studies strongly suspect *Chlamydia trachomatis* is one of the major reasons for tubal factor infertility [17]. A retrospective cohort study investigated 253 tubal factor infertile women (who went for tubal flushing) and showed that *Chlamydia trachomatis* infection significantly decreased the pregnancy chance and live birth rate. Therefore, the findings of the study suggest that *Chlamydia trachomatis* infection screening before the tubal flushing procedure [18,19]. One more cross-sectional study in India investigated *Chlamydia trachomatis* infection rate in infertile women. Mania-Pramanik took 896 female patients and performed a PCR technique to determine the presence of *Chlamydia trachomatis* infection. Studies showed a significant negative effect of *Chlamydia trachomatis* infection on female fertility. The result of the study also revealed that *Chlamydia trachomatis* infection rate is much high among women with ectopic pregnancy (25%) and infertility (18.6%) [20]. De Lima Freitas et al. used the polymerase chain reaction technique for *Chlamydia trachomatis* infection identification among 106 infertile women. After analysis study revealed that 52.8% of infertile women were having *Chlamydia trachomatis* infection and out of this 51.8% were above 30 years old. The study also reported that among 56 *Chlamydia trachomatis* infection cases, 55.4% had infertile permanently and 16 % of women faced fetal death during pregnancy [21]. Another prospective study in India by Malik et al. also confirmed the presence of Chlamydia infection among women with secondary infertility. The study selected 40 women with secondary infertility and 30 healthy women as control. Study results indicated that the Chlamydia infection rate was significantly high among secondary infertile women. The study also recommended the immunoglobulin G antibody detection process as a diagnosis tool for Chlamydia infection. The study also indicated the positive advantages of the ELISA technique such as inexpensiveness, ease of measurement, and early process for antibody and antigen diagnosis during chlamydial infection [22]. Srivastava collected vaginal swab samples from 133 infertile female patients to detect various sexually transmitted infections. After analysis study identified 25 patients and 23 patients who had *Chlamydia trachomatis* and *Mycoplasma* infection respectively. Apart from this, the study did not find any *Neisseria gonorrhoeae* and *Treponema pallidum* infection cases. At the end of the experiment, the study concluded that frequently *Chlamydia trachomatis* and *Mycoplasma* infection detection is necessary in case of infertility treatment [12]. An India-based study with 368 female patients identified the effect of chlamydial heat shock proteins (cHSP) 60 and 10 on infertility and ectopic pregnancy. Chlamydial heat shock proteins (cHSP) 60 and 10 could stimulate the production of IL-10, Interferon-gamma, and Tumor Necrosis Factor-alpha from cervical mononuclear cells, and this phenomenon is related to *Chlamydia trachomatis* infection-mediated infertility and ectopic pregnancy [12]. One retrospective study by Siemer et al., 439 (191 with primary and secondary infertility, 248 healthy pregnant women) women did not show any significant association between *Chlamydia trachomatis* infection and infertility rate but this study observed infection-specific IgG and IgA antibodies within both primary and secondary infertile women [23]. Al-Ramahi et al. conducted one Jordan-based prospective controlled study. The aim of the study was to check the burden of *Chlamydia trachomatis* infection among infertile women over a specific area. The study recruited 146 healthy women as control and 152 infertile patients and collected endocervical swab samples for infection detection by polymerase chain reaction technique. No significant effect of *Chlamydia trachomatis* infection on infertility rate was reported [24].

Malik et al. ducted one study to observe the effect of *Chlamydia trachomatis* on female infertility. The study recruited 110 women with primary and secondary infertility and 30 healthy pregnant women as control. The findings of the study stated that 28.1% of the infertile women and 3.3% of the control population had Chlamydia infection (detection of *Chlamydia trachomatis* infection). The study detected a greater number of *Chlamydia trachomatis* infection cases from the infertile women group, especially from asymptomatic cases. The study recommended an early screening process for *Chlamydia trachomatis* infection to establish preventive therapeutic measures against this infection as soon as possible [25]. Lucisano et al. isolated *Chlamydia trachomatis* from 105 women who underwent laparoscopy. The study collected urethral, cervical, endometrial, and peritoneal samples for *Chlamydia trachomatis* isolation. Out of 42 tubal infertilities, 41 were unexplained infertility, 4 were salpingitis and 18 were endometriosis cases 13, 5, 1, and 1 female patient were infected with *Chlamydia trachomatis* infection respectively. However, the study confirmed the association of *Chlamydia trachomatis* infection with tubal damage [26].

MYCOPLASMA GENITALIUM

Mycoplasma comes under the Mollicutes class [27]. Genus *Mycoplasma* consists of more than 100 different strains [28]. *Mycoplasma genitalium* infection can lead to abnormal inflammatory conditions like cervicitis, urethritis, salpingitis, pelvic inflammatory disease [29], and endometritis and ultimately increase the chances of getting female infertility. *Mycoplasma hominis* infection is associated with bacterial vaginosis [30], pyelonephritis, cervicitis, tubal inflammation, pelvic inflammatory disease, endometritis, and postpartum septicemia. Another genital mycoplasma, *Ureaplasma urealyticum* infection can induce the chances of preterm delivery, bacterial vaginosis, cervicitis, urethritis, and chorioamnionitis [29]. Women's reproductive health can be altered due to a wide range of genital mycoplasma infections. Some species like *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, *Mycoplasma spermatophilum*, *Mycoplasma primatum*, *Mycoplasma penetrans*, *Ureaplasma urealyticum* are commonly known as genital mycoplasma. Ma et al. wanted to investigate the potential role of genital mycoplasmas on female infertility and pregnancy outcome. Based on the previous 35 electronic databases study concluded that genital mycoplasma, *Mycoplasma genitalium* infection was one of the significant causative factors for preterm delivery and female infertility, but there was no role of *Mycoplasma genitalium* infection in spontaneous abortion. Whereas, *Mycoplasma hominis* increased female infertility, stillbirth, and preterm membrane rupture chances. The study did not find any potential role of *Ureaplasma urealyticum* on female infertility [31]. Peipert et al. used proportional hazards models and found that age, low socioeconomic status, black race, and previous record of *Mycoplasma genitalium* infection could lower the chances of conception. The study confirmed the impact of *Mycoplasma genitalium* infection on fertility and conception rate (delayed conception) by performing a serological analysis process on 461 participants [32]. Tantengco et al. performed a meta-analysis to confirm the impact of genital mycoplasma on female infertility status. This meta-analysis also selected *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, and *Ureaplasma urealyticum* as potent genital mycoplasmas. This meta-analysis confirmed the potential effect of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* infection on female reproductive health (female infertility) and suggested *Mycoplasma* diagnosis during infertility management [33].

Doroftei et al. proved the negative impact of *Mycoplasma genitalium* infection on women's fertility process. This study recruited 51 infertile patients and 23 females with normal fertility. 19.6 % and 4.4% of infertile and fertile women had *Mycoplasma genitalium* infection in their cervical canal respectively. The study also identified *Mycoplasma genitalium* infection in the abdominal cavity of 5.8% of infertile women [34]. One more related study also checked the burden *Ureaplasma urealyticum* and *Mycoplasma hominis* infection within 411 registered infertile women. As per the study 28.46%, 2.91%, and 0.48% of the infertile women had *Ureaplasma urealyticum*, coinfection, and *Mycoplasma hominis* infection respectively [34]. One Iran-based descriptive study investigated the infection rate of the *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhea* within infertile women. The study collected 65 infertile women to collect vaginal swab samples from them. Both PCR and DNA extraction techniques were performed and ultimately found that out of a total of 65 female samples, 23 patients were infected with bacterial infection

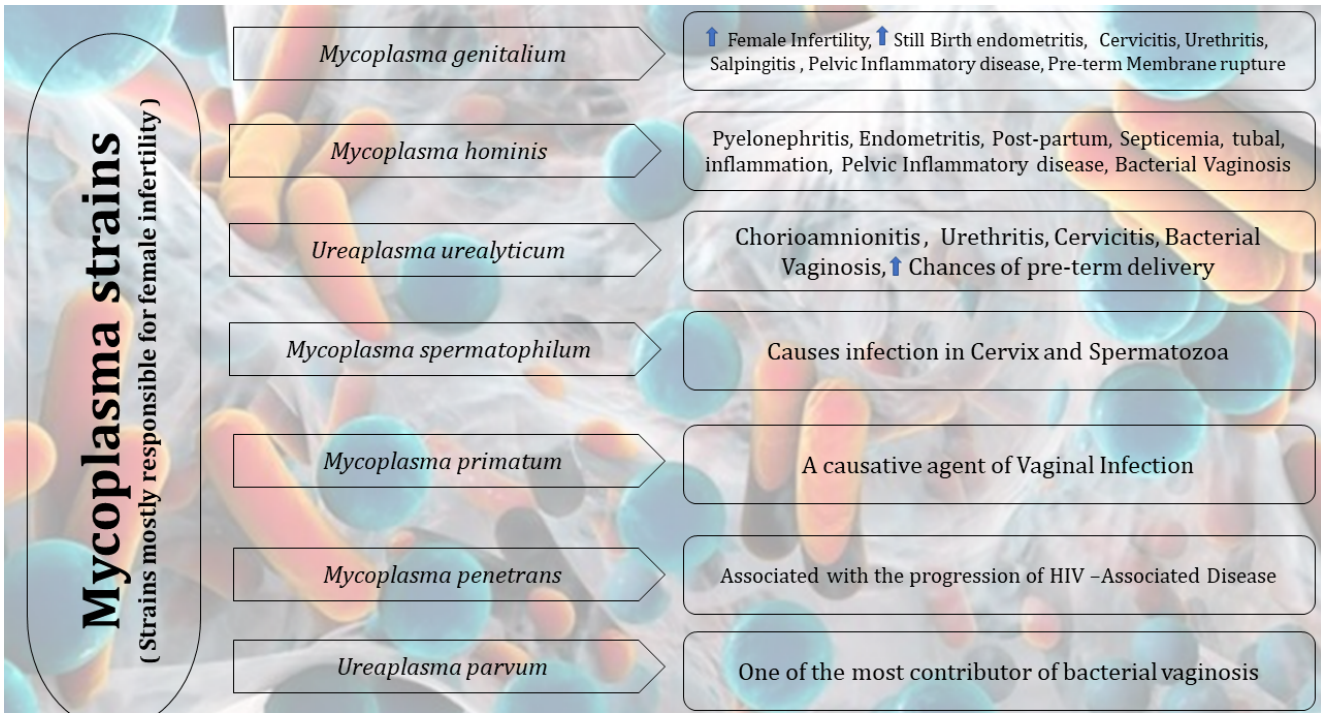

Figure 1. Different strains of Mycoplasma and their pathological role

(2 patients had mixed infection). 16.9%, 13.8%, and 6.2% of infertile patients had *Mycoplasma genitalium*, *Chlamydia trachomatis*, and *Neisseria gonorrhea* infection respectively. The study confirmed the significant presence of *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhea* infections among women with infertility [35]. Another experimental study was conducted to determine *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* infection prevalence among infertile women candidates. The study recruited 104 infertile women and collected their cervical swab samples for further analysis. Multiplex-PCR techniques detected *Ureaplasma urealyticum* infection among 37.5% of the selected females. Only 2.9% had *Mycoplasma genitalium*, *Mycoplasma hominis* infection. The study did not find any association between infertility and the patient's education, age, employment nature, first intercourse age, or abortion history [29]. Baczynska et al. conducted in vitro experiment and detected the moderate effect of *Mycoplasma genitalium* infection on normal human fallopian tubes but they observed massive alteration of the epithelium of normal human fallopian tubes after exposure to *Chlamydia trachomatis*, and *Neisseria gonorrhea* infection [36]. Baczynska et al. suspected *Mycoplasma hominis* infection as one of the major risk factors for infertility due to damaged fallopian tubes. In their study, they analyzed sera for *Mycoplasma hominis* specific antibody detection by selecting 304 infertile women. This study identified *Mycoplasma hominis*-specific antibodies in the sera sample of 97 infertile women. The result of the study indicated potent relation between insufficient fallopian tube passage-related infertility and *Mycoplasma hominis* infection rate [37]. Figure 01 lists seven significant Mycoplasma strains together with each one's harmful characteristics.

BACTERIAL VAGINOSIS

During bacterial vaginosis, lactobacillus-dominated vaginal microbiota is replaced by a wide range of harmful pathogens like *Gardenella vaginalis*, *Mobiluncus*, *Atopobium*, *Prevotella*, *Dialister*, *Mycoplasma*, *Streptococcus*, *Ureaplasma*, *Bacteroides* and many more. These anaerobes decrease antimicrobial peptides amount and normal vaginal pH by inhibiting lactic acid concentration and exhibit the level of short-chain fatty acids (butyrate, acetate, succinate, propionate) and immune mediators (interferon, IL- 2, IL- 6, IL- 8, IL- 10, IL-1β, TNFα RANTES) [6,7]. Vaginal amylase can split complex carbohydrates into glycogen that serves as bacterial food for normal vaginal bacterial growth and survival. During bacterial vaginosis, the vaginal amylase content is getting low, and therefore normal bacteria cannot grow and survive. Additionally, during bacterial vaginosis, the human body become deprived of antimicrobial peptides. Bacterial vaginosis can trigger female infertility by elevating inflammatory responses, hampering immune system potentiality, damaging normal vaginal cells, and sperm, damaging the production of cervical mucus at the time of ovulation, and clogging the fallopian tube by infections mediated scar tissues, decreasing sperm and egg meeting process in a fallopian tube. [38]. Near about 67 species are available that cause bacterial vaginosis. Some common bacterial vaginosis-causing bacteria are *Gardnerella vaginalis*, Dialister spp., Megasphaera spp., *Atopobium vaginae*, *Sneathia amnii*, *Sneathia sanguinegens*, Porphyromonas spp., Prevotella spp., Mobiluncus spp. [39]. Many studies proved the association between idiopathic infertility and bacterial vaginosis, elevated vaginal pro-inflammatory cytokines (IL- 8, IL- 1 β) levels. Bacterial vaginosis is directly associated with poor reproductive health, tubal factor infertility, implantation failure, pregnancy loss, and other sexually transmitted infection's chance [40]. At the same time, some infectious conditions in the female reproductive organs like pelvic inflammatory disease, Bacterial vaginosis, [20] and endometritis can put a woman into various reproductive health-related issues including infertility [41].

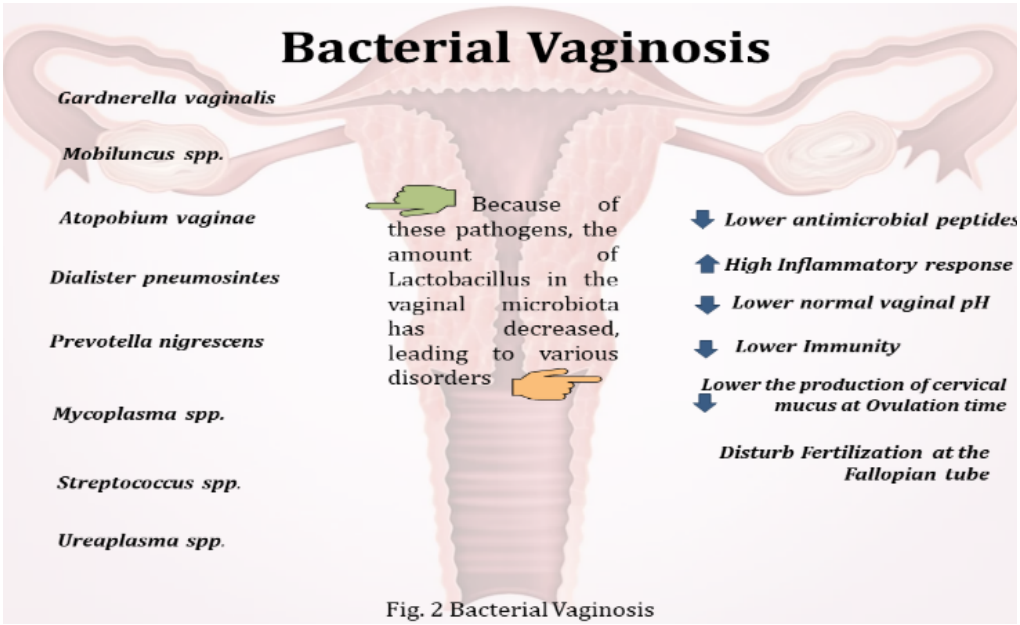

Figure 2. Bacterial vaginosis

A common symptom of bacterial vaginosis is whitish or grey vaginal discharge with a fishy odor. Other symptoms are dyspareunia, dysuria, vaginal pruritus, etc. Patients with bacterial vaginosis are more prone to get other sexually transmitted infections like gonorrhea, and chlamydia,

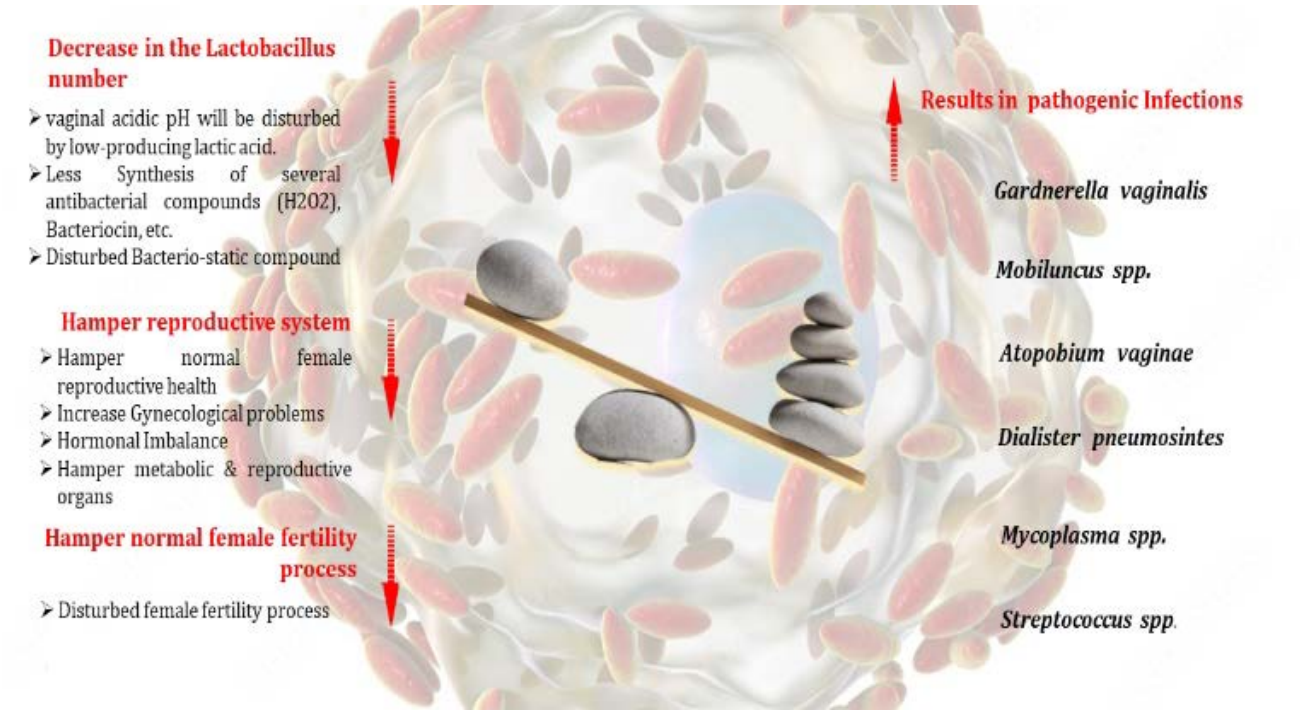

Figure 3. Microbial Dysbiosis in Vagina

even pregnant women with bacterial vaginosis may have a higher risk of preterm delivery. Bacterial vaginosis is not transmitted from person to person. Bacterial vaginosis induces endotoxin secretion, which further triggers prostaglandin and cytokine production in the vagina. Simultaneously bacterial vaginosis inhibits the potentiality of the host leukocytes against infectious diseases. The presence of clue cells, the cervix's epithelial cells is the main indicator of bacterial vaginosis. Douching, antibiotic treatment, multiple sexual partners, frequent use of intrauterine devices, and cigarette smoking are some contributing factors to bacterial vaginosis [42]. Wee et al. performed one initial pilot study (case-control study) to check the vaginal and cervical microbiota composition of women with infertility and normal fertile women. The study selected 31 women (15 infertile and 16 fertile) for sample collection and performed 16 rRNA gene amplicon sequencing techniques for analysis of the collected samples. The study stated that Ureaplasma and Gardenella were most abundantly present in the vaginal and cervical samples of the selected infertile women respectively [43]. Babu checked the difference between the prevalence of bacterial vaginosis in healthy women and infertile women by establishing one cross-sectional study in India. The study considered only 200 women (84 healthy females, 116 infertile females) in the reproductive age group for the study purpose. Studies showed a greater number of Lactobacillus (27.8%) within the vaginal flora of the healthy women group. At the same time, the vaginal flora of the infertile women group was dominated by *Candida* spp. (26.5%), *Enterococcus* (23%), Escherichia coli (14.1%). The study detected the significant presence of bacterial vaginosis and asymptomatic vaginosis among the infertile women population compared to healthy females [9]. Systematic literature reviews from PubMed, CINAHL, EMBASE, ISI Web of Knowledge, and Cochrane Library showed that women with bacterial vaginosis are more prone to get tubal factor infertility. The incidence rate of bacterial vaginosis was higher among tubal factor infertile women and is also often associated with preclinical pregnancy loss [44]. A cohort study once conducted by Salah et al. and the study considered 382 asymptomatic fertile women (control) and 874 infertile women to investigate the presence of bacterial vaginosis in the vaginal samples of the mentioned women and the effect of bacterial vaginosis on fertility and pregnancy rate. The findings of the study showed 45.5% of the infertile women had bacterial vaginosis and whereas only 15.4% of the fertile women had bacterial vaginosis. As per the regression model, bacterial vaginosis is one of the major significant factors for adverse pregnancy outcomes and faulty fertility processes (mainly unexplained infertility) [45,46]. Nwaziri et al. conducted one in vivo study to detect the *Gardenella vaginalis* effects on pregnancy and infertility by using albino rat models. A study found a 20-40% decrease in impregnation and a 70-80% reduction of offspring production ability by rats after being infected with *Gardenella vaginalis* (10^5CFU/ml). This study concluded that *Gardenella vaginalis* have negative effects on rats' normal fertility process and pregnancy outcomes [47]. One experimental study also investigated the effect of vaginal microbiota on early pregnancy failure and conception rate in women who went for in-vitro fertilization (IVF). The study only enrolled 91 female patients for this study. The study analyzed and confirmed that IVF patients who have bacterial vaginosis and a low amount of hydrogen peroxide synthesizing Lactobacillus

within vaginal microbiota are more vulnerable to early pregnancy loss and lower conception chance [48]. One cross-sectional study with 749 confirmed that bacterial vaginosis was more common among tubal infertile women than endometriosis, male factors infertility, and unexplained infertility [49]. Spandorfer et al. conducted one blinded study with 331 IVF patients and the findings of the study showed that bacterial vaginosis was strongly linked with a higher level of IL-1 beta and IL-8 in the cervix. The pro-inflammatory cytokines production by the vaginal microbial community might increase the risk of idiopathic infertility [50]. Figure 02 shows how infections with these bacterial strains result in less lactobacillus and are further harmful. consequences.

CONCLUSION

Microbial dysbiosis may stimulate various kinds of bodily malfunctions including hormonal dysfunction, metabolic dysfunction, reproductive organs dysfunction, and so on. From this review study on the female infertile condition, vaginal microbiota has a major contribution. Vaginal microbiota plays an important role to maintain female reproduction health and prevent several gynecological problems. Generally normal healthy vaginal contain a very minor number of pathogenic microbes. Lactobacillus has a direct role behind this because Lactobacillus maintains vaginal pH around 3.5 to 4.5 by producing lactic acid which helps to reduce pathogens (*Gardnerella vaginalis*, Pepto-streptococci, anaerobic rods, and *mycoplasma* species) growth and multiplication. Antibacterial compounds like H_2O_2 and bacteriocins and bacteriostatic compounds are also produced by Lactobacillus which helps in the bacterial lysis process. Lactobacillus can prevent various pathogenic infection-related gynecological problems such as pelvic inflammatory disease, endometriosis, chronic endometritis, and cancer. Vaginal microbial dysbiosis and/or exposure to any pathogenic microorganisms can lead to infertility. Figure 03 demonstrates how various microbial strain infections cause an imbalance that results in a condition known as microbial dysbiosis in the vagina.

DECLARATIONS

Competing Interest:

There is no conflict of interest.

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Author Contribution

RA: Designed the project, data collection, data analysis, and data interpretation. critical revision of the manuscript and final approval of the version was done by him. **PG**: Literature search, data collection & data interpretation, and drafting of the manuscript were done by her.

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